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The Danube River Basin

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The Danube River Basin

Volume Editor: Igor Liska

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geoecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of "pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Volume Preface

The Danube River Basin covers an area of 801,463 km^2, and it is the largest river basin under the EU jurisdiction. It is shared by 19 countries, and this makes it the "most international" river basin in the world. The Danube River Basin is not only characterized by its size and large number of countries but also by its diverse landscapes and the major socio-economic differences that exist. Due to this richness in landscape the Danube River Basin shows a tremendous diversity of habitats through which rivers and streams flow including glaciated high-gradient mountains, forested midland mountains and hills, upland plateaus and through plains and wet lowlands, i.e., the Danube Delta, near sea level.

Given the number of the countries and the diversity of social, political and economic conditions the transboundary river basin management has always been of supreme importance in the Danube River Basin. The Danube River Protection Convention being the legal instrument for transboundary water management was signed in 1994 and it led into establishing the International Commission for the Protection of the Danube River (ICPDR). The ICPDR has been used as a platform for implementing the EU Water Framework Directive and of the EU Floods Directive in the Danube River Basin District. The ICPDR established the Transnational Monitoring Network as an operational tool for water quality monitoring in the Danube River Basin and created a number of permanent expert bodies which have been dealing with the river basin management issues, flood risk management, surface water monitoring and assessment, pressures and measures, hydromorphology, groundwater and other relevant topics. These expert groups are proactive in collecting and evaluating the information necessary for a proper management of an international river basin. Cooperating with other international organizations and institutions active in the basin (e.g., IAD, IAWD, WWF, NORMAN), networking with scientific and regional projects focusing on water management and especially organizing Joint Danube Surveys has further expanded the pool of experts who cooperate on a transboundary level in Danube water research and management. The decades of this successful cooperation resulted not only in collection of an immense amount of data but also in their improved quality and homogeneity.

This book reviews the available knowledge about the chemical, biological and hydromorphological quality elements in the Danube. The first part examines the chemical pollution of surface waters focusing on organic compounds (with a special attention given to EU WFD priority substances and Danube River Basin specific pollutants), heavy metals and nutrients. The attention is, however, also given to pollution of groundwater and drinking water resources by hazardous substances and to the radioactivity in the Danube.

The second part reviews the biology and hydromorphology of the Danube. It focuses on benthic macroinvertebrates, phytobenthos, macrophytes, fish, phytoplankton and microbiology. Separate chapters are dedicated to gaps and uncertainties in the ecological status assessment and to invasive alien species. The chapters on the Danube hydromorphology, sediment management and isotope hydrology contribute to providing a complete picture about the status of the Danube. The comprehensive information provided in the book chapters enables to explore the links between the biology, chemistry and hydromorphology under the conditions of a large river. The backbone of the presented facts is based on the data collected in the frame of the two Joint Danube Surveys organized by the ICPDR in 2001 and 2007 but the overall information provided by the book goes beyond these surveys and has an ambition to reflect the state-of-the-matter in the knowledge about the Danube water quality.

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Managing an International River Basin Towards Water Quality Protection: The Danube Case

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Abstract Nineteen countries share the Danube catchment area, making it the world's most international river basin. Given the number of the countries and the diversity of social, political and economic conditions, the transboundary river basin management is of supreme importance in the Danube River Basin. The Danube River Protection Convention signed in 1994 is the legal instrument for cooperation and transboundary water management, and it led into establishing the International Commission for the Protection of the Danube River (ICPDR). In reaction to the requirements of the EU Water Framework Directive and of the EU Floods Directive, the Contracting Parties of the ICPDR committed themselves to use the ICPDR as a platform for implementing these directives in the Danube River Basin District and for coping on a basin-wide level with the key pressures related to organic pollution, pollution by nutrients and hazardous substances, hydromorphological alterations, flood protection, navigation, hydropower, sediment management and groundwater management. The ICPDR established the Transnational Monitoring Network which regularly monitors water quality in the Danube River Basin as well as the Danube Accident Emergency Warning System which alerts the Danube countries in case of transboundary pollution accidents. The first Danube River Basin Management Plan was published in 2009, and it set the programme of measures with the view of reducing the pressures on the surface and groundwater. At present the first Danube Flood Risk Management Plan is under finalization focusing on flood prevention, protection and preparedness taking into account the environmental objectives of the EU Water Framework Directive. Of high importance is also the cooperation with the other sectors such as navigation and hydropower aiming at sustainable economic development while avoiding the adverse

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effects on the water status. Using a synergy between implementing the Convention and the current EU legislation, a significant progress has been achieved in ensuring the protection and improving water quality in the Danube River Basin.

Keywords Programme of measures, River basin management, The Danube, Water quality monitoring

Contents

Abbreviations

AEWS	Accident Emergency Warning System
BAT	Best available technologies
BOD_5	Biochemical oxygen demand
COD	Chemical oxygen demand
DEFF	Data exchange file format
DRB	Danube River Basin
DRBD	Danube River Basin District
DRBMP	Danube River Basin District Management Plan
DRPC	Danube River Protection Convention
EU MS	Member State of the European Union
FD	EU Floods Directive
ICPDR	International Commission for the Protection of the Danube River

JDS	Joint Danube Survey
NGO	Non-governmental organization
PAH	Polycyclic aromatic hydrocarbons
PRTR	Pollutant Release and Transfer Register
SPM	Suspended particulate material
TNMN	Transnational Monitoring Network
UWWTD	Urban Wastewater Treatment Directive
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

1 Introduction

The Danube River Basin is Europe's second largest river basin, with a total area of 801,463 km^2. More than 80 million people from 19 countries share the Danube catchment area, making it the world's most international river basin [1]. The map of the Danube River Basin District is shown in the Fig. 1.

Given the complexity of the basin, the transboundary river basin management has been considered for decades as a top priority in the Danube River Basin. The official start of the joint cooperation of the Danube countries in water quality protection dates back to 1985 when the Bucharest Declaration was signed giving the way to 'cooperation on questions concerning the water management of the Danube'. However, there was still a need to develop an international strategy for the protection of water resources in the Danube catchment area. Therefore, on the basis of the UNECE Convention on the Protection and Use of Transboundary Waters (Helsinki Convention), a corresponding agreement relating to the international law for the Danube River Basin was developed. The Convention on the Protection and Sustainable Use of the Danube River (Danube River Protection Convention, DRPC) was signed in June 1994 in Sofia [2]. The DRPC was designed to encourage the Contracting Parties to intensify their water management cooperation in the field of water protection and use. With its entry into force on 22 October 1998, the DRPC became the overall legal instrument for cooperation and transboundary water management in the Danube River Basin.

The objectives of the Danube River Protection Convention are as follows:

- Ensuring sustainable and equitable water management
- Conservation, improvement and the rational use of surface waters and groundwater
- Controlling discharge of wastewaters as well as of the inputs of nutrients and hazardous substances from point and non-point emission sources
- Controlling floods and ice hazards
- Controlling hazards originating from accidents (warning and preventive measures)
- Reducing pollution loads entering the Black Sea from sources in the Danube catchment area

Fig. 1 Danube River Basin District

Responding to the obligations of the Convention, the Danube countries have established the International Commission for the Protection of the Danube River (ICPDR) to strengthen regional and transboundary cooperation.

2 Major Pressures to Water Quality in the Danube River Basin

2.1 Organic Pollution

Organic pollution is mainly caused by the emission of partially treated or untreated wastewater from agglomerations, industry and agriculture. Many agglomerations in the Danube River Basin still have no, or insufficient, wastewater treatment and are therefore key contributors to organic pollution. According to the Danube River Basin Management Plan [3], a total of 6,224 agglomerations $\geq$2,000 PE are located in the DRBD, and wastewaters are not collected at all in more than 2,900 agglomerations (12.6% of the total generated load). Approximately 1,000 further agglomerations have collection systems that require more stringent treatment. A preliminary analysis on industrial and food industrial sources of organic pollution identified a total number of 173 facilities emitting directly into the DRBD and 189 facilities with indirect emissions to water through urban sewers [3].

2.2 Nutrient Pollution

Nitrogen and phosphorus emissions cause eutrophication in many surface waters of the Danube River Basin and contribute to eutrophication in the Black Sea North Western shelf. For the period 1988–2005, the Danube, as one of the major rivers discharging into the Black Sea, was estimated to introduce on average about 35,000 tonnes of phosphorus and 400,000 tonnes of inorganic nitrogen into the Black Sea each year [3].The main sources of nutrients in the Danube are agriculture (50%), municipal wastewater (25%) and industry (25%). The legal limit for nutrient content in groundwater is often exceeded throughout the whole basin [1].

Nutrient pollution from point sources is mainly caused by emissions from insufficiently or untreated wastewater into surface waters (from agglomerations, industry and agriculture). Diffuse source pollution is caused by widespread activities such as agriculture. The levels of diffuse pollution are not only dependent on anthropogenic factors such as land use, and land use intensity, but also on natural factors such as climate, flow conditions and soil properties. These factors influence pathways that are significantly different. For nitrogen, the major pathway of diffuse pollution is groundwater, while for phosphorus, it is erosion.

2.3 Hazardous Substances

The major sources of hazardous substances in the Danube River Basin are industrial effluents, storm water overflow, pesticides and other chemicals applied in agriculture as well as discharges from mining operations and accidental pollution.

Information about the emissions of hazardous substances into water in the Danube River Basin was collected through the ICPDR emissions inventories; nowadays, it is brought together in PRTR.

Data on occurrence of hazardous substances in water are collected by the national monitoring programmes; the key attention is given to the priority substances according to the Directive 2008/105/EC [4] amended by the Directive 2013/39/EC [5] and to other specific substances determining the ecological status according to EU WFD [6]. Hazardous substances are also addressed in the ICPDR monitoring activities: few priority substances are annually analyzed within the Transnational Monitoring Network, while a wide range of hazardous substances is monitored during Joint Danube Surveys

2.4 Hydromorphological Alterations

Anthropogenic pressures resulting from various hydro-engineering measures can significantly alter the natural structure of surface waters. This can have negative effects on aquatic populations and result in the deterioration of the status of surface waters. Hydropower generation, navigation and flood protection are the key water uses causing hydromorphological alterations. The Danube River Basin Management Plan identified three key hydromorphological pressures of basin-wide importance: interruption of river and habitat continuity, disconnection of adjacent wetlands and floodplains and hydrological alterations. Attention has to be given also to the planned and ongoing infrastructure projects in the Danube River Basin as they may impact the river hydromorphology adversely.

2.5 Sediment Management

According to the Danube River Basin Management Plan, the sediment balance of most large rivers in the Danube River Basin is considered as disturbed or severely altered. River engineering works, dredging, torrent control, hydropower development and the reduction of adjacent floodplains are the most significant pressures to sediment management. Hydropower plants in the upper Danube catchments trap almost 80–90% of the sediment bed load. In the lower Danube, the transport of suspended load currently reaches only 30% of the original amount recorded, due to abundant anti-erosion and hydro-technical works throughout the entire Danube

River Basin and significant sediment settling in the Iron Gate 1 reservoir. Quality of sediments suffers from emissions of persistent and toxic substances. While recent results for the organochlorinated compounds in sediments and suspended particulate material (SPM) indicated relatively low concentration profiles of these contaminants in the Danube, concentrations of PAHs have been occasionally found at elevated levels. Contamination of sediments by heavy metals (in particular by lead, cadmium, mercury and nickel) is also of concern [3].

2.6 Invasive Alien Species

The Danube River Basin is very vulnerable to invasive species given its direct linkages with other large water bodies. The Danube is a part of the Southern Invasive Corridor (Black Sea–Danube-Main/Danube Canal–Main-Rhine–North Sea waterway), one of Europe's four most important routes for invasive species, and therefore exposed to intensive colonization by invasive species. Results of the second Joint Danube Survey [7] revealed that invasive species have become a major concern for the Danube and that their further classification and analysis are vital for effective river basin management and, especially, for the correct assessment of the ecological status of surface waters.

2.7 Flood Protection

The Danube River Basin suffered from numerous floods in the past; only in the last decades, massive flood events occurred in 2002, 2006, 2010, 2013 and 2014. Despite floods being natural phenomena, the impact of floods has considerable environmental and health consequences. Storage of hazardous substances inside the flood risk areas may result in harmful impacts of water pollution on ecosystems during minor and major floods. Therefore, the Directive 2007/60/EC [8] requires to provide for the establishing of flood hazard maps and flood risk maps including information on potential sources of environmental pollution as a consequence of floods and to develop flood risk management plans listing measures addressing flood-related pollution. It is however to be stressed that structural flood protection measures have a potential to affect water quality significantly, and therefore, their implementation has to respect the environmental objectives of Article 4 of the Directive 2000/60/EC. Therefore, EU environmental legislation asks for the evaluation of better, feasible environmental options to the proposed structural changes to rivers if these changes could lead to a deterioration of the status of these waters. The Water Framework Directive sets out such requirements and strives to balance maintaining human needs while protecting the environment with the ultimate goal of achieving a sustainable approach to water management. Natural flood management considers the hydrological processes across the whole catchment of a river to identify where measures can best be applied, with a focus on increasing water retention capacities.

2.8 Navigation

Historically, the Danube and some of its tributaries have formed important trade routes across Europe. The harnessing of these rivers to facilitate navigation has radically changed their physical and ecological characteristics, while pollution from ships and boats is also a problem. Navigation is a pressure which can potentially affect the ecological and chemical status of large river systems. The major pressures resulting from navigation are changes of the natural river structure and to river course (such as the blocking of connections to separate channels, tributaries and wetlands), disruption of natural flow patterns by hydromorphological alterations, hindering fish migration due to sluices, engineering works designed to remove sediments and clear channels, accidental pollution involving oil or hazardous substances, pollution by discharged bilge water and by wastewater from tank washings and sewage from passenger boats and inadvertent introduction of invasive species (http://www.icpdr.org/main/issues/navigation).

Navigation requirements can result in a stabilized, single thread, ecologically uniform river channel, lacking both natural in-stream structures with their gentle gradients and connectivity with the adjacent floodplains. In addition to other hydromorphological alterations, this might lead to the loss of species [9].

2.9 Hydropower

The increased use of energy from renewable sources, together with energy savings and increased energy efficiency, is an important step towards reduction of greenhouse gas emissions to comply with international climate protection agreements. This development represents a significant driver for the future development of hydropower generation in the countries of the Danube River Basin. The most serious problems resulting from the construction of hydropower facilities are the disruption of the longitudinal continuity of the rivers and dramatic changes in the rivers' hydrological characteristics.

2.10 Groundwater Management

Groundwater should not only be viewed as a key source of drinking water, but it has to be protected for its environmental value as well. Due to slow groundwater flows, the impacts of anthropogenic activities may be detected with a substantial delay.

The overall assessment of pressures on the quality of major transboundary groundwater bodies in the Danube River Basin showed that pollution by nitrates from diffuse sources is the key factor affecting the chemical status of these groundwaters. The major sources of this diffuse pollution are agricultural activities, non-sewered population and urban land use. Groundwater quantity in the Danube River Basin is affected by groundwater abstraction for drinking water supply and for industrial and agricultural use.

3 Major Achievements in Protecting and Improving the Water Quality

3.1 Cooperation in Implementing WFD in the Danube River Basin

In 2000, EU adopted the Water Framework Directive (WFD) to bring together and integrate work on water resource management. The basis for the WFD-related activities is the river basin. The directive's environmental objective is to restore every surface and groundwater body across the EU to a 'good status' by 2015. This includes a good ecological and chemical status for surface waters and a good chemical and quantitative status for groundwater. With the coming into the force of the EU Water Framework Directive in December 2000, the countries of the Danube committed to use this legislation to assist in meeting the goals of the Convention. The commitment to use the methods and meet the goals of the Directive was made by all countries, i.e. not only EU Member States but also accession countries and countries not in the EU (such as Serbia or Moldova). The ICPDR plays a coordinating role in ensuring that a river basin management plan for the entire basin is prepared [10]. The key component in the process of the WFD implementation was the preparation of the Danube River Basin District Management Plan (DRBMP). The key elements of the plan are the analysis of significant pressures in the Danube River Basin, description of monitoring networks and overview of the status of water bodies, economic analysis of water uses and Joint Programme of Measures that were planned to meet the WFD environmental objectives. An important issue in preparation of the Plan was the work of the Danube experts towards the evaluation of pressures on the water bodies, including pollution by organic substances, nutrients and hazardous substances. A comprehensive set of emission data that enabled application of models (just to mention the most important one – MONERIS – which was applied for the assessment of diffuse pollution on a basin-wide scale (http://moneris.igb-berlin.de/) provided the necessary data for preparation of scenarios being an essential foundation for setting the measures.

3.2 Joint Programme of Measures

The Danube Joint Programme of Measures outlines specific actions and scenarios at the basin-wide scale and their likely outcomes by 2015 and beyond. It is firmly based on the national programme of measures of each Danube country, which shall be implemented at the latest by 2012. The Plan also indicates where the proposed measures remain insufficient to meet the WFD requirements on a basin-wide scale and proposes additional actions. It indicates where action is needed and also where further monitoring effort is required. The Plan focuses on the main transboundary problems, the Significant Water Management Issues, that can directly or indirectly affect the quality of rivers and lakes as well as transboundary groundwater bodies. For the Danube River Basin, these were identified as pollution by organic substances, pollution by nutrients, pollution by hazardous substances and hydromorphological alterations or changes to the natural character and structure of the water body. Based on the detailed picture we now have of the Danube Basin waters, the DRBM Plan outlines visions for each issue to achieve an improved and sustainable water environment [11].

Measures identified in the Joint Programme of Measures for organic pollution will result in a considerable reduction of BOD_5 and COD loads. However, following the baseline scenario will still not ensure the achievement of the WFD environmental objectives on the basin-wide scale by 2015. Significant further efforts for the next RBM cycles will still be necessary to ensure this. In the long run, the technical implementation of the UWWTD requirements [12] as well as the IPPC Directive [13] by EU MS and an equal level of measures in non-EU MS would be sufficient to solve the problem of organic pollution.

The planned measures will decrease nitrogen and phosphorus emissions to surface waters in 2015 by 12% and 21%, respectively. This will remarkably improve the situation in the Danube River Basin and the Black Sea, but it will still not be enough for achieving the management objectives of the DRBMP and the WFD environmental objectives on the basin-wide scale. Reductions in nutrient pollution will be achieved as soon as more stringent UWWT obligations with N and P removal for agglomerations >10,000 PE are applied for EU MS. The commitment of the ICPDR of banning phosphorus in laundry detergents in 2012 and in dishwasher detergents in 2015 is seen as a cost-effective and necessary measure to complement the efforts of implementing urban wastewater treatment.

The implementation of the Dangerous Substances Directive, the IPPC Directive, the UWWT Directive and the widespread application of BAT will improve but not solve the problem of hazardous substances. It is estimated that the management objectives and WFD environmental objectives will not be achieved in 2015 regarding hazardous substances; however, there is a need for more monitoring data on hazardous substances, as well as information on sources and relevant pathways. Further measures are the appropriate treatment of priority substances from industrial discharges and further strengthening of prevention and safety measures at contaminated sites. In addition, the continued upgrade of WWTPs with biological treatment (which results in some hazardous substances accumulating in the sewage sludge) as well as increases in the number of WWTPs will contribute to reduce the load of hazardous substances. Finally, additional reduction through product-related measures should be considered.

Measures will be taken to improve river continuity, reconnection of floodplains/ wetlands and hydrological alterations by 2015. However, a significant number of respective pressures will still remain in 2015, and good ecological status/ecological potential will not be achieved by 2015. By 2015, it is expected that 108 barriers will be made passable for fish, whereas 824 river and habitat continuity interruptions will remain. This means that the self-sustainability of sturgeon species and other migratory species in the DRB will be enhanced, but impacts will remain. Remaining continuity interruptions will be addressed by 2021 and 2027. By 2015, 62,300 ha of adjacent floodplains/wetlands will be reconnected and/or the hydrological regime improved, and additional restoration efforts will be taken beyond 2015. Although there is a positive cumulative effect of connected wetlands/floodplains and improvement of the water regime to adjacent water bodies, further investigation is required as to the extent that these reconnections will improve the water status at the basin-wide level, in order to better target measures.

3.3 Basin-Wide Monitoring and Assessment of the Water Status

An essential prerequisite of the assessment of the water status was availability of reliable and harmonized information on water quality. The Danube countries have been actively engaged in a long-term process of ensuring mutual understanding and cooperation in water quality monitoring.

This process started in 1985 with the monitoring of transboundary river sections of the Danube under the Bucharest Declaration and has been boosted since 1996 when yearly status of water quality has been published based upon the Transnational Monitoring Network (TNMN) developed by the Danube countries in response to the Danube River Protection Convention. This monitoring activity provides the necessary basis for a harmonized water quality assessment throughout the whole basin, which not only gives an overview of water quality trends in the basin and of loads of substances discharged into the Black Sea but also fosters achieving of compatibility between water assessment approaches in the Danube River Basin.

The TNMN laboratories in the Danube countries have a free choice of analytical methods they use for the analysis of the agreed set of physicochemical quality elements and priority substances, provided they are able to demonstrate that the methods in use meet the required performance criteria. Therefore, the minimum concentrations expected and the tolerance required of actual measurements have been defined for each determinand so that the method compliance can be checked.

Table 1 Number of surface water bodies in the Danube River Basin District failing to achieve the good chemical status due to particular priority substances defined by the Directive 2008/105/EC

	Danube	Tributaries	Lakes	Coastal waters
Heavy metals				
Cadmium	8	33	1	6
Lead	1	25	1	4
Mercury	6	33		
Nickel		15	1	3
Pesticides				
Trifluralin		3		
Atrazine		1		
Diuron		1		
Isoproturon		2		
Hexachlorocyclohexane	1	1		
Industrial pollutants				
Anthracene		1		4
Octylphenol		6		
Tetrachloroethylene		1		
Trichloroethylene		1		
Trichloromethane		3		
Brominated diphenylether		1		
1,2-Dichloroethane		1		
Di(2-ethylhexyl)phthalate		13		
Naphthalene				4
Other pollutants				
Aldrin	3			6
Pentachlorophenol		2		
Benzo(*a*)pyrene				4
Benzo(*b*)fluoranthene		7		4
Benzo(*k*)fluoranthene		7		4
Benzo(*g*,*h*,*i*)perylene	3	2		3
Indeno(1,2,3-cd)pyrene		2		
Tributyltin compounds		1		
Dieldrin				6
Endrin				6
para–para-DDT	4	10		6
Fluoranthene				4
Hexachlorobenzene				5

To ensure the quality of collected data, a basin-wide Analytical Quality Control programme is annually organized by the ICPDR. For storage of TNMN data, a relational database has been developed by the ICPDR. The TNMN data collection is carried out at the national level by the National Information Managers who receive the data from the national laboratories. After collection, the data are checked and converted into an agreed data exchange file format (DEFF). The national DEFF files are submitted to the TNMN data centre in Slovakia for additional checking and final processing. Having obtained the formal approval by the ICPDR, the data are uploaded into the website.

Agreed and organized data is essential in being able to generate the political will to take actions to address problems. The yearly assessment of water quality has been supplemented by periodic Joint Danube Surveys with the view of providing a comprehensive picture of the status of the river ecosystem based on a wide range of monitoring variables covering biology, chemistry, microbiology, hydromorphology, isotope analysis and toxicology. The scientific contribution of these special monitoring exercises was enormous but similarly important were the aspects of training and methodological harmonization as well as public awareness rising.

The first Joint Danube Survey was carried out in 2001. For the first time, comparable data about the entire course of the river have been provided covering over 140 different biological, chemical and bacteriological parameters. These data were used as an essential information source for the first analysis of the Danube River Basin District according to WFD Article 5. Six years later, the second Joint Danube Survey has created a comprehensive and homogeneous database on the status of the aquatic ecosystem of the Danube and its major tributaries. For the first time, the fish survey on the whole Danube was carried out bringing a unique dataset and contributing also to methodological harmonization between EU and non-EU countries. JDS2 also introduced the first ever systematic survey of hydromorphological parameters in the entire navigable longitudinal Danube stretch using a single method. The survey confirmed earlier ICPDR conclusions of a generally improving trend for water quality along the main Danube River. It also reinforced specific problems, especially at a number of tributaries and downstream of large cities. It appeared as well that a number of specific problem areas such as pollution by WFD priority substances as well as the newly emerging contaminants need further more extensive examination, particularly in some tributaries [7]. JDS2 has proved to be a valuable tool for improving the databases for water quality assessments, and it has confirmed the need to carry out such investigative monitoring exercise on a regular basis. Information produced by the two Joint Danube Surveys helped the ICPDR Contracting Parties to implement the Danube River Protection Convention and the EU Water Framework Directive, and the concept of JDS has become an integral part of TNMN. The data from the two surveys are also an essential source of information used in most of the chapters in this book. The sampling stations of JDS2 are shown in Fig. 2.

The general objective of the WFD is to achieve both ‘good ecological status’ and ‘good chemical status’ of surface waters. The first Danube River Basin Management Plan included information on water status in all surface water bodies in catchments larger than 4,000 km^2. Altogether, 681 river water bodies were evaluated. Out of these, 193 achieved good ecological status or ecological potential (28%) and 437 river water bodies achieved good chemical status (64%). Out of a 25,117 rkm network in the DRBD, good ecological status or ecological potential is achieved for 5,494 rkm (22%) and good chemical status for 11,180 rkm (45%).

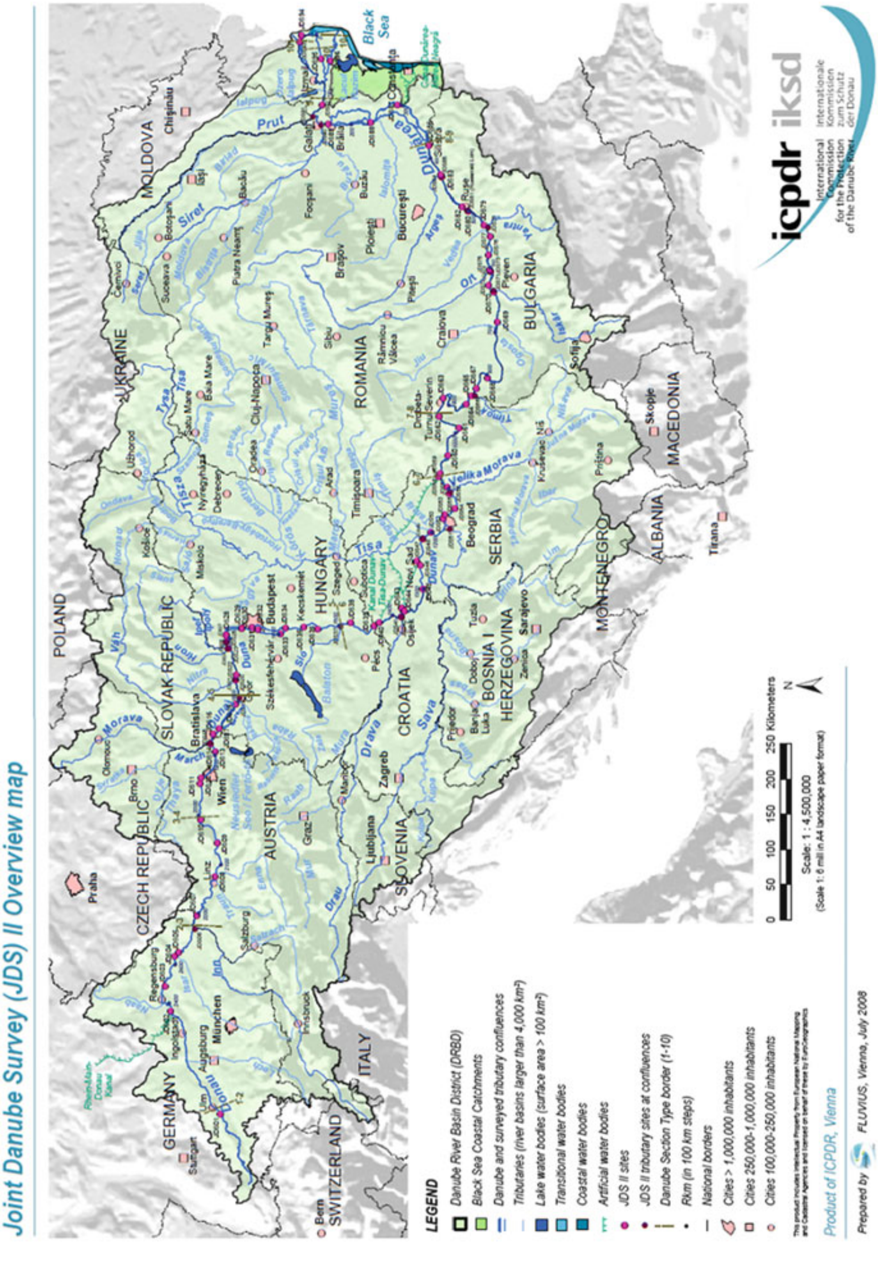

Fig. 2 Sampling sites of the Joint Danube Survey 2

Although many gaps and uncertainties in the assessment of the surface water status still exist, the river basin managers and stakeholders now have a good picture of the condition of the entire Danube Basin for the first time, based on national data, the ICPDR's Transnational Monitoring Network and the two Joint Danube Surveys. Assessment of the chemical status managed to provide the first ever comprehensive overview of contamination of surface waters in the Danube River Basin by WFD priority substances. The priority substances causing poor chemical status in the surface water bodies in catchments larger than 4,000 km^2 are listed in Table 1. From this table, it is apparent that heavy metals, DEHP and *p*,*p*-DDT are priority substances hindering achieving of WFD environmental objectives at most.

At this stage, the status assessment of water bodies is not yet directly linked to the measures and the effects of the measures at the basin-wide scale. A follow-up is therefore needed in order to better understand the linkage between the effects of the measures and the water status at the basin-wide scale [3].

3.4 The Danube Accident Emergency Warning System

The Accident Emergency Warning System (AEWS) of the Danube River Basin is activated whenever there is a risk of transboundary water pollution, or threshold danger levels of certain hazardous substances are exceeded. The AEWS sends out international warning messages to countries downstream to help the authorities put environmental protection and public safety measures into action. Thanks to this system, the adverse consequences of numerous transboundary pollution accidents that occurred during last two decades in the Danube River Basin could be timely and efficiently mitigated.

3.5 Flood Protection

In response to the danger of flooding, the ICPDR adopted the Action Programme for Sustainable Flood Prevention in the Danube River Basin in 2004. The overall goal of this Action Programme is to achieve a long-term and sustainable approach for managing the risks of floods to protect human life and property, while encouraging conservation and improvement of water-related ecosystems. In 2009, in line with the Action Programme, 17 flood action plans for the subbasins of the Danube were adopted by the ICPDR.

At the ICPDR Ministerial Meeting in 2010, the Contracting Parties committed themselves to make all efforts to implement the EU Floods Directive throughout the whole Danube River Basin and to develop an international flood risk management plan. The first milestone in the FD implementation under ICPDR was carrying out a preliminary flood risk assessment and identification of those areas for which it has been concluded that potential significant flood risks exist or might be considered likely to occur. This was followed by the preparation of flood risk and flood hazard maps leading to the elaboration of flood risk management plans. The general objectives of flood maps are to increase public awareness of the areas at risk from flooding, to provide information of areas at risk to give input to spatial planning and to support management and reduction of the risk to people, property and the environment. Flood risk management plans shall address all aspects of flood risk management focusing on prevention, protection and preparedness, including flood forecasts and early warning systems, and taking into account the characteristics of the particular river basin or subbasin. The Danube Flood Risk Management Plan will also include the promotion of sustainable land use practices and improvement of water retention focusing especially on natural water retention measures. These measures aim to safeguard and enhance the water storage potential of landscape, soil and aquifers, by restoring ecosystems, natural features and characteristics of water courses and using natural processes. They support Green Infrastructure by contributing to integrated goals dealing with nature and biodiversity conservation and restoration and provide multiple benefits, including flood protection, water quality and habitat improvement.

Next to developing action programmes and management plans, which create a framework for an effective management of flood risks, the ICPDR elaborated an inventory of contaminated sites in flood-prone areas listing potential pollution threats in case of flood events. This inventory is a basic prerequisite to setting prevention measures minimizing adverse impacts of floods on water quality.

3.6 Navigation

To address the adverse impacts from navigation to water ecology, the ICPDR linked up with the Danube Commission and the International Commission for the Protection of the Sava River to execute in 2007 an intense, cross-sectoral discussion process, which has lead to the adoption of 'Joint Statement on Inland Navigation and Environmental Sustainability in the Danube River Basin'. The Joint Statement provides principles and criteria for environmentally sustainable inland navigation on the Danube and its tributaries, including the maintenance of existing waterways and the development of future waterway infrastructure. All key stakeholders from the basin such as the representatives of navigation authorities, environmental protection authorities, industries and environmental organizations throughout the basin have been involved in this process. The Joint Statement provides also an overview on the legal background regarding both Inland Waterway Transport and environmental issues. The Joint Statement and its practical implementation will ensure the integration of economic development and environmental standards during the planning/implementation of new navigation infrastructure projects. It provides the basis for potential win-win situations for the navigation sector and the environment [9].

To provide further guidance to the Joint Statement, the EU PLATINA project developed a Manual on Good Practices in Sustainable Waterway Planning, which is designed for use in the Danube River Basin. The manual offers general advice on organizing and implementing a balanced and integrated planning process. Thereby, project developers must also consider national, regional and local aspects and requirements when developing an inland waterway transport project. The early integration of stakeholders (including those representing environmental interests) and of environmental objectives and wide communication are essential for successful planning process [14].

3.7 Hydropower

The ICPDR responded to the need of sustainable development of hydropower with minimum effects on the water status by producing in close cooperation with the hydropower sector and all relevant stakeholders the guiding principles on hydropower development. The key element of these principles is a holistic assessment based on a strategic planning approach and being fully in line with the requirements of the WFD, which needs to be carried out for the development of new hydropower plants. The environmentally sound hydropower facilities should fully respect a number of environmental requirements such as minimum ecological flow, upstream and downstream continuity, hydropeaking and sediment/bedload transport. While many Danube countries reported to have environmental requirements in relation to ensuring river continuity and ecological flow requirements included in their existing national legislation, technical guidelines as well as clear criteria, standards and definitions are not always in place yet causing difficulties in the practical implementation. Therefore, the dialogue between water managers and hydropower sector is essential for finding win-win solutions for a sustainable development of hydropower in the Danube River Basin.

Aware of the fact that hydropower plants offer an additional reduction potential for greenhouse gases but recognizing as well their negative impacts on the riverine ecology, the Ministerial Declaration asked in 2010 for the development of Guiding Principles on integrating environmental aspects in the use of hydropower in order to ensure a balanced and integrated development, dealing with the potential conflict of interest from the beginning.

In the frame of a broad participative process launched in 2011, with the involvement of representatives from administrations (energy and environment), the hydropower sector, NGOs and the scientific community, first an 'Assessment Report on Hydropower Generation in the Danube Basin' has been elaborated [15]. The report provides information on a variety of issues, including information on the current situation regarding existing hydropower plants in the DRB. As a second step, the 'Guiding Principles on Sustainable Hydropower Development in the Danube Basin' have been elaborated [16]. Besides outlining background information on the relevant legal framework and statistical data, the Guiding Principles are addressing the following key elements for the sustainability of hydropower:

1. General principles and considerations (the principle of sustainability, holistic approach in the field of energy policies, weighing of public interests, etc.)
2. Technical upgrading of existing hydropower plants and ecological restoration measures
3. Strategic planning approach for new hydropower development
4. Mitigation measures

The Guiding Principles were adopted by the ICPDR in June 2013 and recommended for application by the Danube countries, what is planned to be further facilitated via an exchange of experiences on the application in the frame of a follow-up process.

4 Conclusions

Numerous pressures stemming from anthropogenic activities affect the water quality in the Danube River Basin. To address these pressures, the Danube countries established a platform for cooperation in transboundary river basin management: the International Commission for the Protection of the Danube River founded under the Danube River Protection Convention. Using a synergy between implementing the Convention and the current EU legislation such as WFD and FD, a significant progress has been achieved in ensuring the protection and/or improving water quality in the Danube River Basin. Few examples of actions taken towards water quality protection and of water status-related problems in the basin are provided in this chapter, but they are complemented by additional information in the other chapters of this book highlighting biological, chemical and hydromorphological situation as well as the status of sediments and groundwater.

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Part I
Chemical Pollution

Nutrient Management in the Danube River Basin

Mihaela Popovici

Abstract The EU Water Framework Directive requires that EU Member States implement the necessary measures within their river basin districts to achieve good status of water bodies by 2015. Nutrient pollution is a priority challenge in the Danube River Basin District, interlinking the freshwater with the marine environment – approximately 65% of the Danube River length was categorised as being *at risk* due to nutrient pollution. Eutrophication is of major concern in the Danube Region and especially in the receiving Western Black Sea. The ecological situation in the Black Sea has improved considerably in the last decade (reduced eutrophication, disappearance of anoxic conditions, regeneration of zoo-benthos and phytoplankton); however, the improvement was only partly due to the effect of measures like nutrient removal at wastewater treatment plants (WWTPs) or the ban of P-containing laundry detergents, as it was also to a considerable part due to the economic crises in Danube countries. The nutrient loads are thus still well above the levels of the 1960s; current evidence shows the need to develop newer solutions and to prepare nutrient management strategies to effectively reduce nutrients in the Danube River systems. The assessment of measures related to farming practices and land use management undertaken until end of 2012 provided information on declining trends of nitrogen surplus in all member states in the DRB. The measures related to farming practices and land use management consist most commonly of technical measures to reduce negative impacts caused by agriculture, such as input reduction measures, measures addressing diffuse pollution concerning both fertiliser and pesticide use, livestock farming-oriented measures focusing on the reduction of impacts from animal rearing, the use of manure as a fertiliser, changes in crop production practices as well as improving drainage systems.

Keywords Common agricultural policy, MONERIS, Nitrates directive, Nutrients

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Contents

Abbreviations

AEM	Agri-environmental measures
BAP	Best agricultural practices
BAT	Best available techniques
BSC	Black Sea Commission
CAP	Common Agricultural Policy
CIS	Common Implementation Strategy for the Water Framework Directive
CP	Contracting Party
daNUbs	Nutrient Management in the Danube Basin and its Impact on the Black Sea
DPRP	Danube Pollution Reduction Programme
DRB	Danube River Basin
DRBD	Danube River Basin District
DRBMP	Danube River Basin Management Plan
DRP	Danube Regional Project
DRPC	Danube River Protection Convention
DRS	Danube Region Strategy of the European Commission
EU	European Union
EUSDR	EU Strategy for the Danube Region
FAO	Food and Agricultural Organisation of the United Nations
FAOSTAT	Database of the Food and Agriculture Organisation of the United Nations
GAEC	Good Agricultural and Environmental Condition
GEF	Global Environment Facility
GEP	Good Ecological Potential
GES	Good Ecological Status
GIS	Geographical Information System
HMWB	Heavily Modified Water Body
ICPDR	International Commission for the Protection of the Danube River

MONERIS	Modelling Nutrient Emissions into River Systems
MoU	Memorandum of Understanding
MS	EU Member State
N	Nitrogen
NAP	National Action Plan
ND	Nitrates Directive (Directive 91 /676/EEC)
NSP	National Strategy Plan
NVZ	Nitrate Vulnerable Zone
OM	Ordinary Meeting
P	Phosphorus
POM	Programmes of Measures
RBD	River Basin District
RBM	River Basin Management
RBMP	River Basin Management Plan
RBN	River Basins Network
RDP	Rural Development Programme
RDR	Rural Development Regulation
SMR	Statutory Management Requirement
SWG	Standing Working Group
SWMI	Significant Water Management Issue
UNDP	United Nations Development Programme
UWWTD	Urban Waste Water Treatment Directive (Directive 91/271/EEC)
WFD	Water Framework Directive (Directive 2000/60/EC)
WWTP	Waste Water Treatment Plant

1 Introduction

1.1 Need for Nutrient Management in the Danube River Basin

The Danube River Basin is Europe's second largest river basin, with a total area of 801,463 km^2. It is the world's most international river basin as it borders 19 countries. The ecosystems of the Danube River Basin (DRB) are highly valuable in environmental, economic, historical and social terms, but they are subject to increasing pressure and serious pollution. The Danube River and its catchment provide drinking water, industrial and agricultural water supply, hydroelectric power generation, navigation, tourism, recreational opportunities and fisheries. These intensive uses have created severe problems of water quality and quantity and drastically reduced biodiversity in the basin. The pollution ends up in the Black Sea and affects a very large area.

In order to address these problems, the Danube countries have taken and are taking several actions on the national and international level. A central element in this respect is the implementation of the EU Water Framework Directive (WFD),

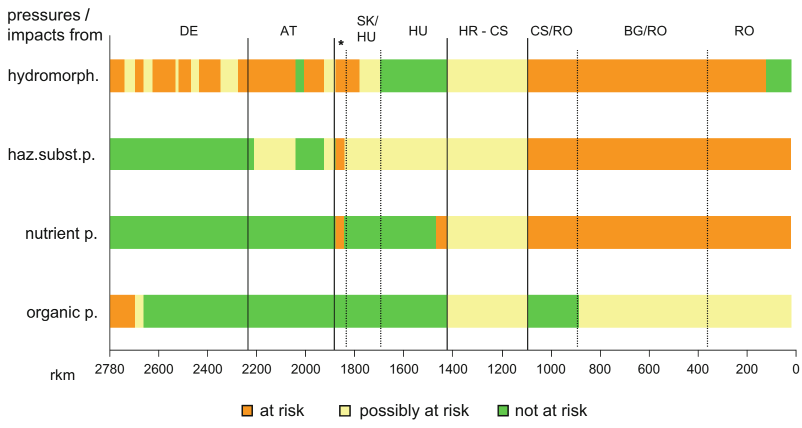


Fig. 1 Results of the risk analysis for the entire Danube River length [1] (*asterisk*: SK territory)

with the Joint Programme of Measures (JPM) incorporated in the Danube River Basin Management Plan. This JPM addresses the Significant Water Management Issues (SWMIs) in the DRB, through several technical measures needed to reduce the negative influence of human activities on the water quality. For each SWMI, visions and operational management objectives have been developed based on shared values with a long-term perspective. Overall, the visions and management objectives reflect the joint approach among all Danube countries and support the achievement of the WFD objectives in the DRB. When addressing pressures at the basin-wide scale, it is clear that cumulative effects may occur, and addressing these issues effectively requires the application of a basin-wide perspective and close cooperation between countries.

Figure 1 illustrates the results of the Danube Basin Analysis (DBA), prepared in line with the requirements of the WFD Art. 5 in 2005, according to the categorised pressures for the entire length of the Danube River itself. 58% of the Danube River length was categorised *at risk* due to organic pollution, 65% due to nutrient pollution and 74% due to hazardous substances. 93% of the Danube River was *at risk* or *possibly at risk* of failing the WFD environmental objectives because of hydromorphological alterations. In conclusion, large parts of the Danube River are subject to multiple pressures. For the entire DRBD, the distribution of pressures is similar.

Nutrient pollution is a priority challenge in the DRBD, interlinking the freshwater with the marine environment – approximately 65% of the Danube River length was categorised *at risk* due to nutrient pollution. While efforts to control nutrient enrichment over the past 30 years yielded some positive results, although the nutrient loads are still well above the levels of the 1960s, current evidence shows the need to develop newer solutions and to prepare nutrient management strategies to effectively reduce nutrients in the Danube River systems.

As a result of considerable investment in upgrades of sewage treatment plants especially in the upper basin, the phosphorus levels have markedly improved throughout most of the river system, although levels remain slightly above the levels of 1960s. Elimination of phosphorus in detergents in some countries and the adoption of best agricultural practices also contributed to reductions in total pollutant load in the Danube River systems and the Black Sea. Nitrogen levels have also improved, but they are still well above the level of 1960s.

Elevated loads of nutrients can enter the river through diffuse sources such as agricultural runoff and urban stormwater and point source discharges from sewage treatment plants. To date, nutrients have been reduced and managed through a range of programmes and initiatives; however much of the river systems remain stressed. Unless well managed, nutrient sources could continue and intensify in the future, with potential increases associated with population growth, agricultural intensification and further urbanisation within the DRBD.

The Danube River Basin Management Plan published in 2009 [2] is a significant first step towards achieving the good water status of water bodies that WFD requires, setting clear and ambitious targets for environmental improvement through the reduction of nutrients pollution in the Danube River systems.

As there is a wide range of factors influenced or affected by nutrient pollution, including the economic considerations, legal requirements or diverse stakeholder interests (such as fishing, drinking water, conservation, forestry and agricultural), the measures set within the Joint Programme of Measures will not be sufficient to achieve the environmental objectives of the WFD at the basin-wide level by end 2015 and need to be further addressed by a basin-wide strategic and coordinated approach.

The ICPDR's basin-wide vision for nutrient pollution is the "balanced management of nutrient emissions via point and diffuses sources in the entire Danube River Basin District that neither the waters of the DRBD nor the Black Sea are threatened or impacted by eutrophication".

Therefore, the countries efforts are focussing on achieving the management objectives related to nutrient pollution agreed in the DRBMP in relation to the Danube impact on the eutrophication of the Black Sea, and thus, the hydrological connection of the Danube River Basin with the Black Sea is of a central consideration.

The Black Sea eutrophication problem can be addressed and benefited by actions taken throughout the Danube River Basin, even in areas not responsible for the largest nutrient inputs to the river system. Actions taken for local reasons unrelated to the Black Sea – to improve water quality upstream in the DRB – will deliver benefits downstream as well.

1.2 Policy Context

Nutrient removal is required to avoid eutrophication in many Danube River Basin surface waters and the Black Sea North Western Shelf, in particular taking into account the character of the receiving coastal waters as a *sensitive area* under the

UWWTD [3]. The nutrient loads discharged from the DRB are an important factor responsible for the deterioration and eutrophication of parts of the Black Sea ecosystem.

The Danube countries committed themselves to implement the Memorandum of Understanding adopted by the International Commission for the Protection of the Black Sea (ICPBS) and the ICPDR in 2001 and agreed that "the long-term goal is to take measures to reduce the loads of nutrients discharged to such levels necessary to permit Black Sea ecosystems to recover to conditions similar to those observed in the 1960s".

The ministers of the Danube countries expressed their commitment in the Danube Declaration adopted at the Ministerial Meeting, February 16, 2010 (Danube Basin: Shared Waters – Joint Responsibilities), with regard to nutrient pollution that – "due to the measures made operational until 2012 – the nitrogen and phosphorus emissions to surface waters in 2015 will be about 12%, respectively 25%, lower compared to the average of the years 2000–2005. The load to the Black Sea will reach a level below the present state but still about 40% above that of the 1960s for nitrogen and about 15% for phosphorus".

The integration of the EU Nitrates Directive [4] with the Water Framework Directive [5] is central to ensure the legal alignment of the National Action Plans and River Basin Management Plans/Programmes of Measures. Furthermore, the integration of environmental concerns in the EU Common Agricultural Policy (CAP) was identified as one of the main priorities of the CAP. Agri-environment, as a key element of this integration, became a compulsory element of the EU Rural Development Programmes from the 2000–2006 programming period. Additionally, the CAP and Rural Development are important in minimum budget allocation for agri-environmental measures that are identified in RBMPs/POM.

The strategic approach to rural development was strengthened in the programming period 2007–2013. Strategic guidelines, which were defined at the EU level, set the overarching priorities of the EU Rural Development policy. Taking the guidelines into account, member states were required to develop a National Strategy Plan, which defined the action of the European Agricultural Fund for Rural Development (EAFRD) Period: 2014–2020 at the MS level. The National Strategy Plan also served as a reference for the development of the national/regional Rural Development Programme, the main instrument through which the rural development strategy is delivered at national or regional level. Agri-environment provides relevant tools to address a wide diversity of farming practices and a broad number of challenges in the EU.

The "Health Check" fine-tunes CAP reform to make the Single Payment Scheme more effective, efficient and simple, to adapt market instruments to meet new market opportunities and to respond to new and ongoing challenges (climate change, bioenergy, water scarcity, biodiversity).

Water plays a central role when it comes to adaptation, as it is vital for several economic sectors. Therefore it is essential to adapt the integrated river basin solutions to extreme events such as floods and drought and manage the resulting impacts on water supply, water quality and ecosystems. In the examination of the implications of measures proposed in the ICPDR Joint Programme of Measures, it is important to identify win–win and no-regret solutions.

To meet the overall binding target for the European Union of 20% renewable energy by 2020 and a 10% minimum target for the market share of biofuels by 2020, the member states are free to decide their preferred "mix" of renewable to take account of their different potentials of bioenergy policy. The national action plans shall also set out "adequate measures to be taken to achieve these targets, including national policies to develop existing biomass resources and mobilise new biomass resources for different uses". This will influence the degree of agricultural intensification, and certain limits on the type and size of biomass production for energy purposes should be imposed.

In view of the Policy Review of the Strategy for Water Scarcity and Droughts, the European Commission has launched several studies (such as GAP analysis, water use in agriculture) which have been integrated in a Blueprint to safeguard European waters published in 2012 [6].

Finally, the EU Strategy for the Danube Region (EUSDR) is the macro-regional development strategy and action plan for the regions and countries located in the catchment area of the Danube River. It targets the sustainable development of the Danube macro-region as well as the protection of its natural areas, landscapes and cultural heritage. The measures related to the Danube Strategy will largely follow the ICPDR's Danube River Basin Management Plan of 2009. The Danube Strategy emphasises the importance of intersectoral collaboration. An active process of cooperation between authorities responsible for agriculture and environment is to be supported to ensure that measures against agricultural pollution are put in place: manure storage facilities, buffer strips, fertiliser and pesticide application limits, for example.

2 Nutrient Pollution in the DRB

Nutrient pollution is mainly caused by emissions from the agglomeration, industrial and agricultural sectors. Furthermore, for agglomerations, the P emissions via household detergents play a significant role. For nutrient pollution, point and diffuse source discharges are to be distinguished. Point source discharges are caused by single activities and are locally confined, whereas diffuse source discharges are caused by widespread activities like agriculture with multiple pathways (erosion, tile drainage, etc.). Agriculture is the major source of diffuse inputs, including fertilisers as well as effluent from huge pig farms and agro-industrial units. Therefore, it is assumed in order to reduce diffuse sources of pollution due to the use of fertilisers that by 2015, the MS will implement the action plans and codes of Good Agricultural Practice on fertilisation under the Nitrates Directive, and the non-MS will apply the ICPDR recommendations on the Best Agricultural Practices (BAP).

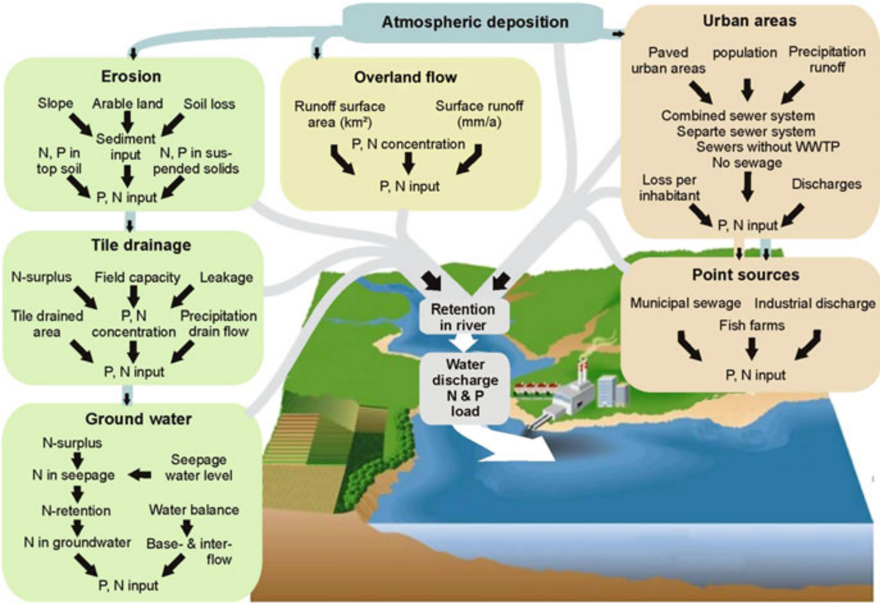


Fig. 2 MONERIS model nutrients inputs into the river systems

Information on the major sources of phosphorus and nitrogen to surface water is important in the assessment of current programmes of measures and future initiatives on abating nutrient pollution. Understanding the transformation and losses of nutrients in the river systems and knowledge about the relative contributions of phosphorus and nitrogen in terms of the total load, the chemical form and input form (continuous vs. climate dependent) are essential in making best choices regarding how to manage nutrient reduction efforts in the basin. Information on source contributions of nutrient loadings has been broken out on the level of an analytical unit, through the application of MONERIS (http://www.icpdr.org/main/activities-projects/moneris-modelling-nutrient-emissions-river-systems) (Fig. 2), which allows regionally differentiated quantification of nutrient emissions into a river system.

N and P emissions cause eutrophication in many DRBD surface waters and contribute to eutrophication in the Black Sea North Western shelf. For the period 1988–2005, the Danube, as one of the major rivers discharging into the Black Sea, was estimated to introduce on average about 35,000 tonnes of P and 400,000 tonnes of inorganic N into the Black Sea each year (Fig. 3).

The present level of the total nutrient load in the Danube River system is considerable higher than in the 1960s, but lower than in the late 1980s. The decrease from the 1990s to the present situation is due to the political as well as economic changes in the middle and lower DRB resulting in (1) the closure of nutrient discharging industries, (2) a significant decrease of the application of mineral fertilisers and (3) the closure of large animal farms (agricultural point sources).

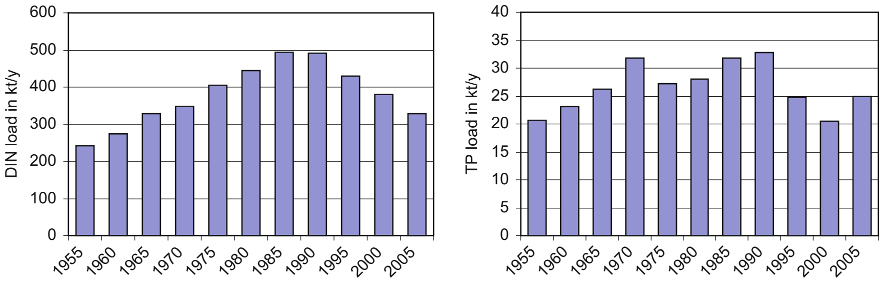


Fig. 3 Long-term discharges of dissolved inorganic nitrogen (DIN) and total phosphorus (TP) (1955–2005)

Furthermore, the application of economic mechanisms in water management (e.g. the *polluter pays principle* also applied in the middle and downstream DRB countries) and the improvement of wastewater treatment (especially in upstream countries) contributed to this decrease.

Whereas point emissions from waste water treatment plants and industrial sources are directly discharged into the rivers, diffuse emissions into surface waters come from different pathways, represented by separate flow components. The direct and diffuse components must be separated, since the underlying processes and the nutrient concentrations are different. The model facilitates the calculations of emissions into surface waters, calculations of nutrient retention in surface waters, and allows a comparison between the calculated and the observed loads.

The N_{tot} and P_{tot} total generated load emissions (point and diffuse) for reference year 2006 emitted from agglomerations $\geq$2,000 PE) were 168.0 kt/a and 28.6 kt/a, respectively.

2.1 *Identification of Point Nutrient Sources*

Nutrient pollution from point sources is mainly caused by emissions from insufficiently treated or untreated wastewater into surface waters (from agglomerations, industry and agriculture). It should be mentioned that the operation of secondary and tertiary treatment levels at wastewater treatment plants (WWTPs) is of particular importance for the respective elimination/reduction of nitrates/phosphates.

Nutrient emissions and the eventual impact from point sources can be measured and expressed in terms of inorganic nitrogen, total nitrogen (N_{tot}), ammonia (NH_4), nitrate (NO_3), nitrite (NO_2) or total phosphorus (P_{tot}) and phosphates (PO_4).

The emission of phosphates via household detergents is significant in the DRB, and it is included in the agglomerations contribution to total emissions. P emissions due to laundry and dishwasher detergents in the DRB are estimated at 9,190 t/a. This is 15.7% of the total P emissions.

The use of mineral fertilisers significantly contributes to nutrient pollution in the DRB, and it is included in the agglomerations contribution to total emissions. The two most important plant nutrients applied as mineral fertilisers are N and P.

2.2 *Diffuse Sources of Nutrients*

Diffuse pollution was highlighted as a major impact on the Danube River systems in the DBA in 2005, as well in the SWMI paper in 2008. Since then, work has continued in the basin to develop measures to address diffuse pollution through a number of routes such as regulation, economic support and catchment management initiatives. The DRBMP published in 2009 set clear and ambitious targets for environmental improvement through the reduction of diffuse pollution in the DRBD. Figures 4 and 5 show the MONERIS results describing that altogether

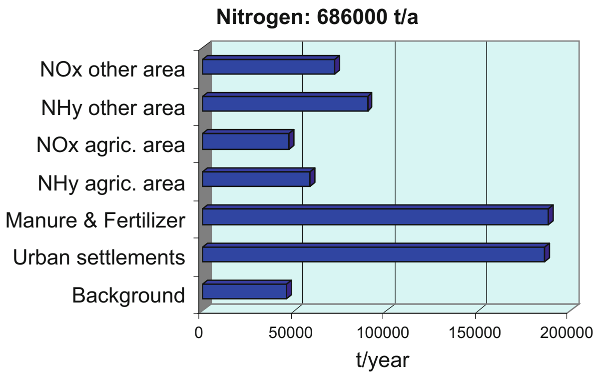


Fig. 4 Sources of nitrogen emissions in the DRBD

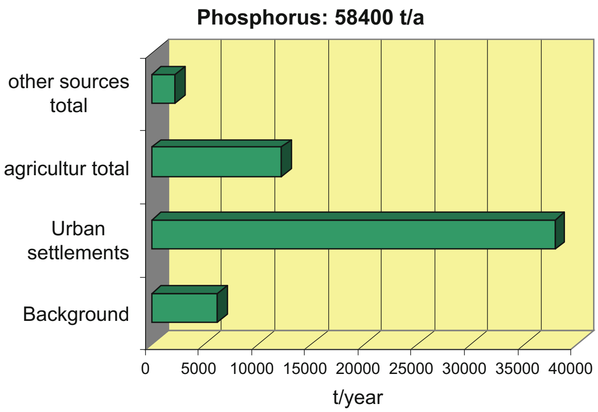
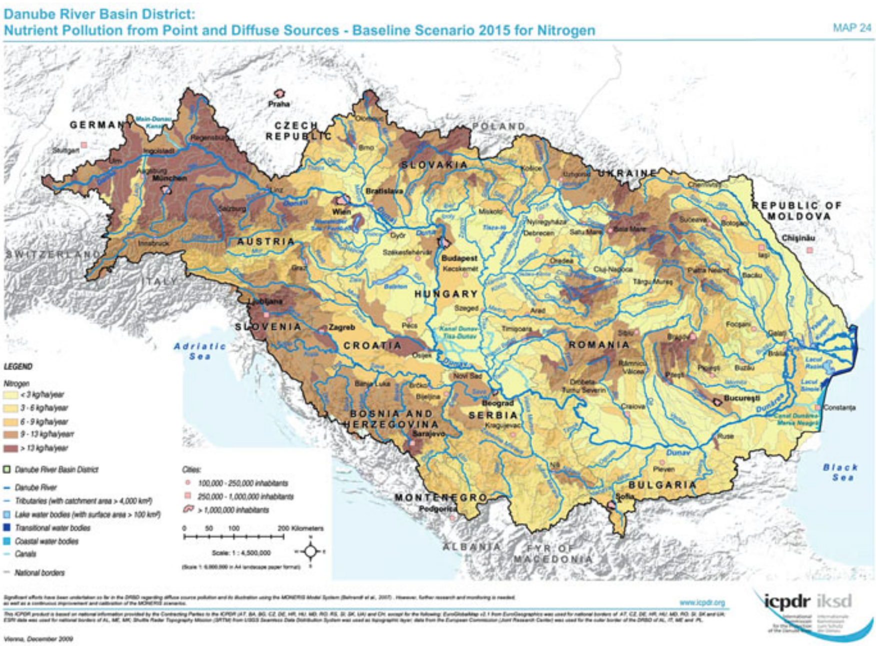


Fig. 5 Sources of phosphorus emissions in the DRBD

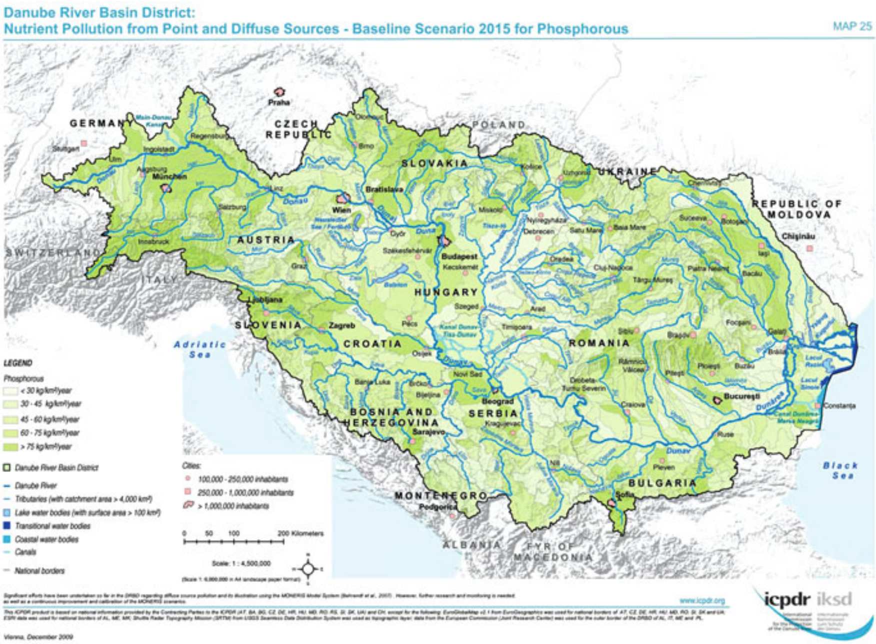
686 kt of N and 58 kt of P in total are annually emitted into the DRB. Values for atmospheric deposition – ammonia nitrogen and nitrogen oxides (NHy and NOx) – are also indicated.

The background conditions presented in MONERIS (7% for N; 9% for P) represent the pre-industrial situation with very limited airborne emissions of reactive N and erosion of soils not yet saturated with P. Consequently, these values are small in comparison with the current emissions in the DRB.

The main contributors for both N and P emissions are agglomerations not served by sewerage collection and wastewater treatment. For N pollution, the input from agriculture (fertilisers, manure, NO_x and NH_y) is the most important (43% of total emissions). For P, emissions from agriculture (area under cultivation, erosion, intensity of production, specific crops and livestock densities) are the second largest source after input from urban settlements. The share of agricultural emissions differs significantly between countries in the DRB (Map 1 and Map 2).



Map 1 Nutrient pollution: Baseline scenario 2015 for nitrogen [2]



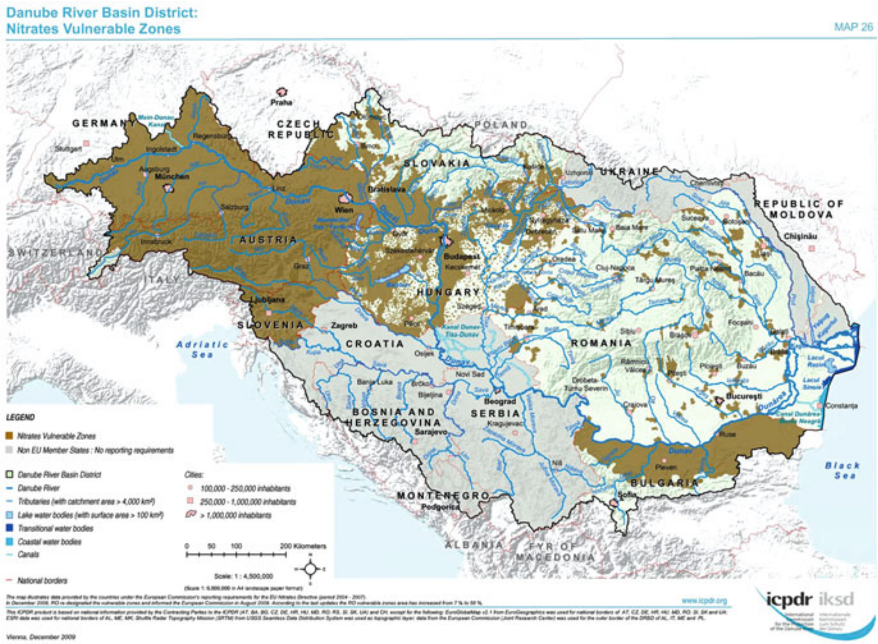
Map 2 Nutrient pollution: baseline scenario 2015 for phosphorus [2]

3 Actions to Manage Nutrients in the DRB

3.1 Implementation of Nutrient Management Legislation

The nutrients management regulatory framework will play an important role in ensuring that visions and the management objectives for nutrient management to meet the WFD objectives are an important consideration in decisions about land use planning and natural resource management. The EU directives have been adopted at the national level, and a number of other regulatory and planning controls are in place to manage point and diffuse sources of nutrients and prevent land degradation.

The EU Nitrate Directive was issued in 1991 [4]. The objectives are to reduce water pollution caused or induced by nitrates from agricultural sources and to prevent such pollution. Member states are required to identify Nitrate Vulnerable Zones (NVZs) on the basis of the results of monitoring requirements. Action Programmes with mandatory measures concerning agricultural practices must be implemented in these areas, and monitoring of water quality according to specific requirements is performed. The action programmes include the maximum amounts of animal manure that can be applied to land every year, which is equivalent to 210 kg N per ha for the first NAPs and 170 kg N per ha for the next ones. Also Codes of Good Agricultural Practice (CGAP) must be elaborated and are



Map 3 Nutrient vulnerable zones in the DRBD [2]

mandatory in the NVZs and voluntary outside the NVZs. To ensure that actions are successfully carried out, an implementation framework has been developed, and responsibilities as well as agreed time frames have been incorporated into specific actions. Different Danube countries have taken different approaches regarding the designation of NVZs (Map 3). The territorial approach was accepted by Austria, Germany and Slovenia, while in Czech Republic, Hungary, Romania and Slovakia and Bulgaria, Nutrient Vulnerable Zones were identified.

3.2 Implementing Authorities, Funding Opportunities and Monitoring of Implementation

The guiding principle and recommendation organising the implementation of the RDPs are established in the relevant set of rural development regulations.

The implementation procedures cover several aspects including the designation of the implementing bodies, definition of their responsibilities and tasks and vertical coordination required to translate in concrete actions on-the-ground the national and regional level rules. At the MS level, the institutional set-up for implementation procedures is based on three bodies, which every MS has to designate according to Article 74 of the RD Regulation, namely:

1. The Managing Authority
2. The Paying Agency
3. The Certifying Body

There are conditions and specific rules for financing expenditure under the common agricultural policy (CAP). Two funds were created: the European Agricultural Guarantee Fund (EAGF) and the European Agricultural Fund for Rural Development (EAFRD) as stipulated by the Council Regulation (EC) No 1290/2005 of 21 June 2005 on the financing of the common agricultural policy. The most predominant approach used to the implementation of Leader projects (http://ec.europa.eu/agriculture/rur/leaderplus/index_en.htm) was "measure by measure". It is essential to encourage appropriate stakeholders to steer projects and also to identify financing means.

Monitoring and evaluation, acceptance by farmers and controllability of the measures are important factors in the implementation of nutrient management policies. Measures need to be reviewed nationally, through jointly organised mechanisms (such as interministerial committees operational in a number of the Danube countries) to ensure the coordination of resources. The evaluation is based on reliable information and evidence base to link nutrient inputs (cause) with the water quality information (effect) and the most cost effective methods of reducing nutrient pollution. The effectiveness of the measures is closely linked with the mechanism of control, and the review of the measures provides evidence that the management of nutrient pollution is effective. To determine the nutrient reductions, the effectiveness of the measures, the transformations in the river systems, the responses of the systems and the lag times, both pre- and post-implementation monitoring must be designed. In addition the anticipation of the nutrient reduction and its trends can be assessed based on monitoring data.

The quantification of achievable nutrient load reductions and implementation costs is useful when assessing the fulfilment of the WFD objectives. The concept of ecosystem services is often used by the Danube countries to provide a better understanding of the costs and benefits of various initiatives.

According to the calculation of scenarios (MONERIS results), a comparison between the 2006 and anticipated reduction by 2015 shows a reduction of both N and P pollution in the Danube River Basin. In 2006, the N emissions to surface waters were 686 kt/a, whereas the calculated values to achieve the management objective by 2015 will be 602 kt/a, which is a reduction of 12% (84 kt/a) (Fig. 6). For phosphorus, (Fig. 7), P emissions to surface waters were in 2006 of 58 kt/a, whereas the calculated values to achieve the management objective by 2015 will be 46 kt/a, which is a reduction of 21% (12 kt/a). This evaluation documented the conclusion that the management objective by 2015 related to reduction of nutrient load to the level of 1960s will be only partially achieved for nitrogen and phosphorus.

For each of the RBMP cycle, a basin-wide integrated assessment will be conducted every 6 years to assess the progress and document the lessons learned through the implementation process. With the determination of what pollution

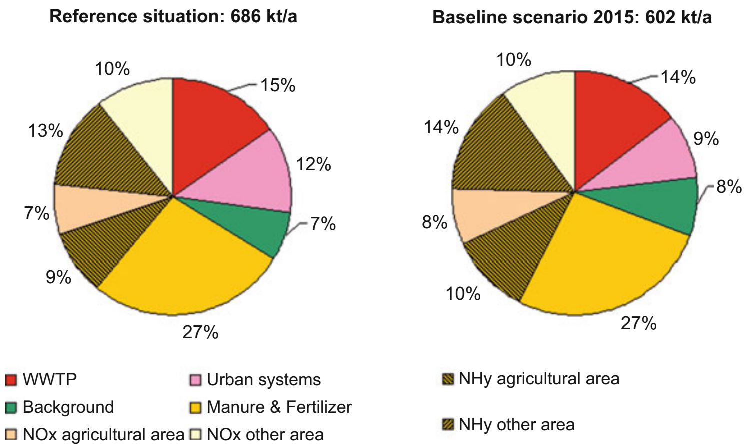


Fig. 6 Sources of nitrogen emissions in the DRB in 2006 and 2015

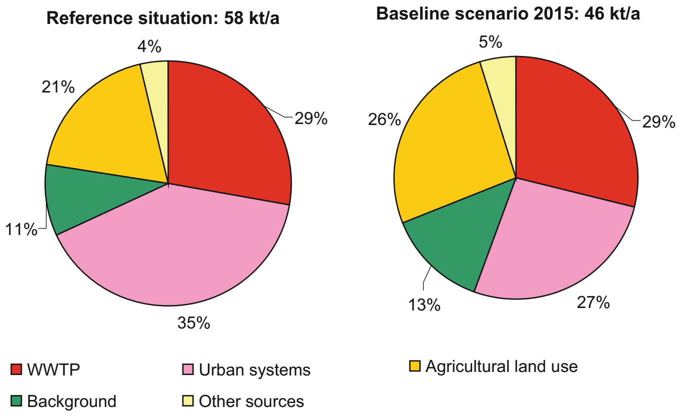


Fig. 7 Sources of phosphorus emissions in the DRB in 2006 and 2015

reductions are achievable, quantitative reduction targets can be established and future progress evaluated in relation to achieving respective WFD targets.

4 Conclusions

Nutrient removal is required to avoid eutrophication in many DRB surface waters and the Black Sea North Western Shelf, in particular taking into account the character of the receiving coastal waters as a sensitive area under the UWWTD.

The nutrient loads discharged from the DRB are an important factor responsible for the deterioration and eutrophication of parts of the Black Sea ecosystem.

The DRBM Plan highlighted that the nitrogen load to the Black Sea will reach a level that is below the present state but still far above (40%) that of the 1960s, and therefore, the management objectives and the WFD environmental objectives on the basin-wide scale will not be achieved by 2015. For phosphorous, the respective management objective and the WFD environmental objectives on the basin-wide scale will not be achieved by 2015, as the level will be still 15% above the level in the 1960s. This requires that further actions are taken beyond 2015. The implementation of the Nitrates Directive in EU Member States, an improved application of the concept of BAT in non-EU Member States, and the reductions in nutrient pollution achieved in wasterwater treatment plants with nitrogen and phosphorus removal for agglomerations >10,000 PE will reduce nutrient pollution considerably.

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Pollution by Nutrients in the Danube Basin

Carmen Hamchevici and Ion Udrea

Abstract The assessment of pollution by nutrients in the Danube River has a long-term history at the basin-wide level, especially for estimating the influx of these substances to the Black Sea. The main aim of this chapter is to provide a general overview of the nutrient levels in the Danube Basin based on the data collected in the frame of long-term Trans-National Monitoring Network (TNMN) of the International Commission for the Protection of the Danube River (ICPDR) during 2001–2009. For selected monitoring sections, the dependence of the nutrient concentrations on corresponding daily discharges is also investigated. A comparative view of the surveillance TNMN data and investigative data obtained during the two monitoring programs known as Joint Danube Surveys 1 and 2 (JDS1 and JDS2) is presented. In order to get a general overview of the nutrient levels over the studied period, the temporal trends were analyzed using nonparametric Spearman correlation coefficient before and after removing the impact of the daily discharge on the measured concentration.

Keywords 90 percentile, Nutrients, Spearman correlation coefficient, Temporal trend

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Contents

1 Introduction

Assessment of nutrient levels in water ecosystems has particular importance due to the fact that the input of nutrients into surface waters (mainly nitrogen and phosphorous), either from natural or anthropogenic sources, leads to the process being known as eutrophication. The direct consequences of eutrophication – increased algal bloom, accelerated biological activity (metabolism and decomposition), widespread reduction in dissolved oxygen concentration, growth of higher plants, changes in aquatic food chain, and eventually a disturbed ecosystem and a deteriorated water quality – make the assessment of nutrient level to be one of the most important issues in assessment of water quality.

1.1 Relevancy of Nutrients in the Danube Basin

The nutrient loads and their consequences have been recognized as one of the most striking issues in the Danube catchment area, the Danube Delta and the Black Sea. In the recent decades, comprehensive studies and projects were dedicated to the nutrient problem in the Danube River Basin [1–3]. In addition, nutrient data were subject for modeling tools that quantified the Danube in-stream loads of nitrogen and phosphorous (Danube Water Quality Model) and estimated the nutrient emissions (MONERIS Model). More details about these models are provided in other chapters of this book [4, 5]. According to the MONERIS results, 686 kt of N and 58 kt of P are annually emitted into the Danube River Basin, figures that are much above the background conditions – 7% for N and 9% for P [5].

1.2 Relevancy of Nutrients in the Water Framework Directive

The EU Water Framework Directive (WFD) significantly changed the water management by shifting the view of water quality from chemical targets to ones based on ecological assessment of natural systems [6, 7]. In Annex V, Section 1.1.1 (Rivers), WFD lists three groups of quality elements to be used in this assessment, among which the third group refers to the “chemical and physico-chemical elements supporting the biological elements.” Within this group, under the “General” category, the following quality elements are listed: thermal conditions, oxygenation conditions, salinity, acidification status, and nutrient conditions. Besides Annex V, WFD explicitly refers to nutrients (Annex VIII. 11) as “substances which contribute to eutrophication (in particular nitrates and phosphates).”

The present chapter aims to provide a general overview of the nutrient levels in the Danube Basin based on the data collected in the frame of long-term Trans-National Monitoring Programme (TNMN) over 9 years (2001–2009). A comparative view of the surveillance TNMN data and investigative data obtained during the two monitoring programs known as Joint Danube Surveys 1 and 2 (JDS1 and JDS2), in 2001 and 2007, respectively, is provided as well.

Additional information on nutrients in the Danube Basin based on TNMN data in previous time period (1996–2000) and during Joint Danube Surveys can be found elsewhere [8–11].

2 Methods

2.1 Data Collection and Processing

The present chapter takes into account measured concentrations (mg.L^{-1}) during 2001–2009 for four dissolved nutrient forms (N-ammonium (N-NH_4), N-nitrites (N-NO_2), N-nitrates (N-NO_3), P-orthophosphates (P-PO4)) and two total forms (total nitrogen (TN) and total phosphorous (TP)); in selected monitoring stations, also corresponding daily discharge data (m^3.s^{-1}) were considered. Data set was produced in the frame of TNMN program and during JDS1 and JDS2. Within TNMN monitoring, data is yearly collected at the national level by the National Data Managers (NDMs) who are in charge with data checking, conversion into an agreed data exchange file format (DEFF), and sending it to the TNMN data management center in the Slovak Hydrometeorological Institute in Bratislava. This center performs an additional data validation and uploads them into the central TNMN database. In cooperation with the ICPDR Secretariat, the TNMN data are uploaded into the ICPDR website (www.icpdr.org) [12].

For each parameter, data processing includes the calculation of basic descriptive statistics (mean, median, minimum, maximum, lower and upper quantiles, 10th and

90th percentile (C10 and C90), skewness, and kurtosis (not shown in the chapter)). When the concentration of a certain parameter was below the limit of detection (LOD) reported by laboratory, the measurement result was set to half of the LOD. Starting with 2008 for stations DE1, DE2, and DE5, data was sent according to the Directive 2009/90/EC, using the limit of quantification (LOQ) instead of LOD. Therefore, values below LOQ were replaced by half of this limit.

2.2 Monitoring Stations

The surface water monitoring network of TNMN has changed over the considered time period, especially after the completion of revision in 2007 in line with the WFD implementation requirements, reaching a number of 116 monitoring sites out of which 44 sites are located on the main course of Danube River and 72 sites on major primary and secondary tributaries [12]. This paper deals with data recorded in 42 monitoring locations on the Danube River, listed in Table 1. (Names and coordinates of the locations listed corresponding to each TNMN code can be found in TNMN Yearbook [12].) For those stations with three sampling locations on profile (left bank, middle, and right bank), only the results recorded in the middle were processed.

The size of the data set differs among the monitoring sites depending on the changes in the network structure: station DE1 was replaced by DE5 in 2007 and stations AT5 and AT6 were included in 2006 and SK5 in 2009; data corresponding to RS9 was available for 2002–2009, to RO18 for 2007–2009, and to UA1 and UA2 for 2004–2009. Stations at the same river km in neighboring countries are located just upstream/downstream of an international border.

For result presentation, the splitting of the Danube River into three main sections was applied: upper Danube, from river km 2,581 to 1,879 (stations DE1 to AT6); middle Danube, from river km 1,869 to 1,077 (stations SK1 to RS6); and lower Danube, from river km 1,071 to 0 (stations RO1 to RO8).

2.3 Sampling and Analysis

The national laboratories involved in the TNMN are fully responsible for sampling, preserving, storage, and analysis of water samples. The analytical methods used are mostly based on ISO standards, or they are home developed and validated according to the required performance criteria. For JDS water samples, dissolved nutrient forms were analyzed on board of the laboratory ship immediately after sampling using well-defined Standard Operating Procedures based on ISO standards; total N and total P were analyzed in an accredited laboratory by international standardized methods. For the entire data set, results for dissolved nutrients refer to water samples filtered by 0.45 μm pore size membranes prior to analysis.

3 Results and Discussion

3.1 Spatial Distribution

The box plots illustrated in Figs. 1a, b, 2a, b, and 3a, b present the descriptive statistics (median, lower and upper quantiles, C10 and C90, as well as outliers and extreme values) for each nutrient form. Also the spatial concentrations profiles along the Danube River are showed.

The basis for the spatial evaluation is 90 percentile (C90) for each considered parameter. C90 method has the advantage that those extreme values caused by exceptional conditions or (unlikely) measuring errors are not taken into account, but it still represents "unfavorable situations" that occur in a monitoring site in a given period of time.

As regards the distribution of **N-NH4**, C90 values show a decreasing line in the upper Danube, from 0.130 mg.L^{-1} at river km 2,581 (DE1) to 0.059 mg.L^{-1}at river km 1,879 (AT6). An increasing profile is noticed in the middle Danube, from 0.240 mg.L^{-1} at river km 1,869 (SK1) to 0.400 mg.L^{-1} at river km 1,155 (RS5), with values below 0.200 mg.L^{-1} in several stations located between river km 1,806 (SK2) and river km 1,429 (HR1). More scattered distribution is present in the lower Danube, where few concentration leaps are visible: from 0.442 mg.L^{-1} at river km 1,071 (RO1) to 0.160 at river km 955 (RS7), in the backwater of the Iron Gates reservoir and from 0.500 mg.L^{-1} at river km 641 (BG2) to 0.160 mg.L^{-1} at river

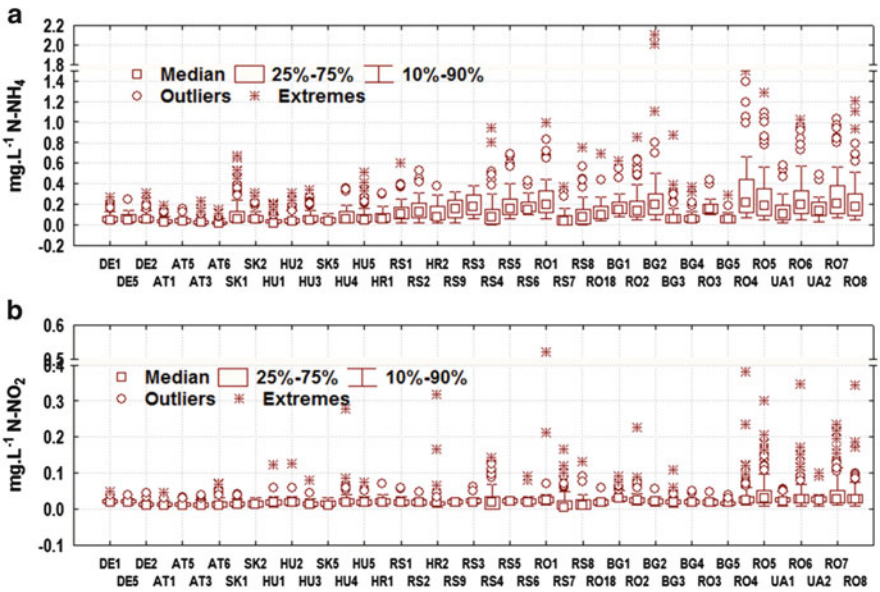

Fig. 1 Box plots of N-NH$_4$ (**a**) and N-NO$_2$ (**b**) concentrations (mg.L^{-1}) in the Danube River (2001–2009)

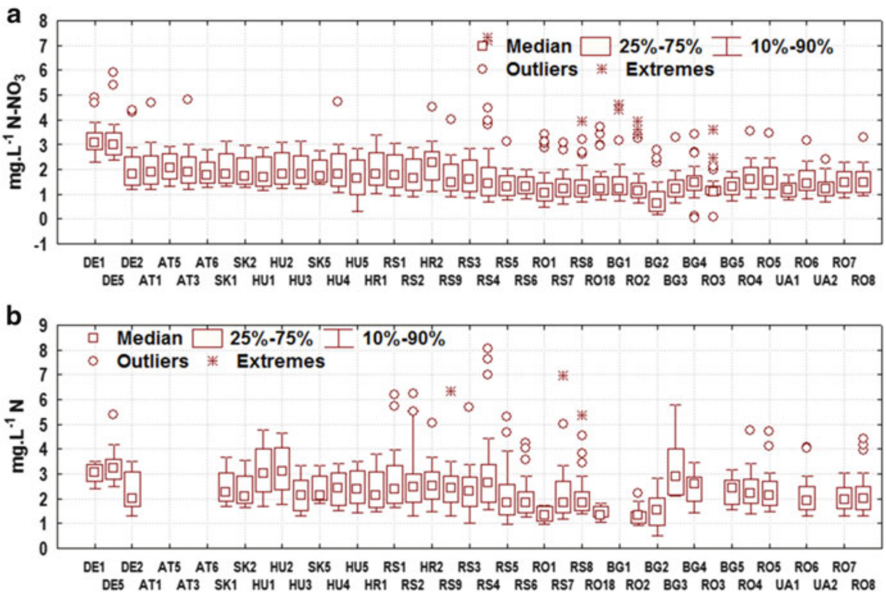

Fig. 2 Box plots of N-NO_3 (**a**) and TN (**b**) concentrations (mg.L^{-1}) in the Danube River (2001–2009)

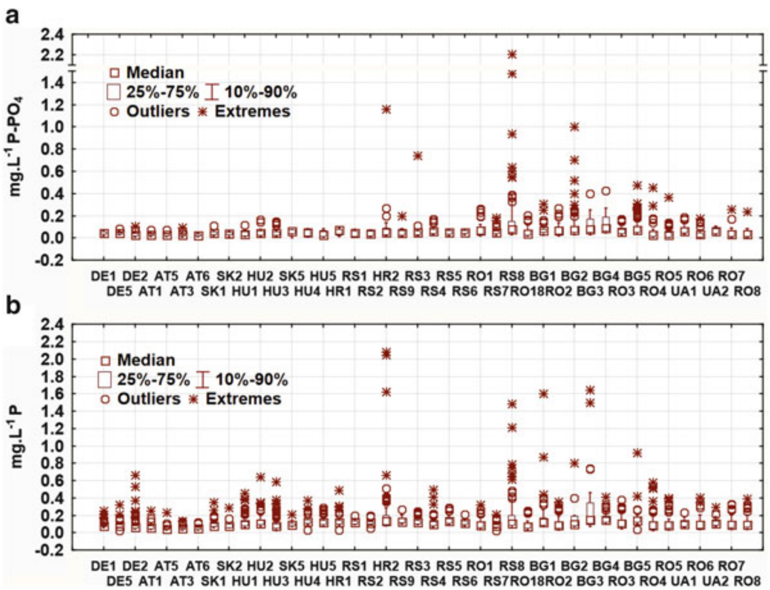

Fig. 3 Box plots of P-PO4 (**a**) and TP (**b**) concentrations (mg.L^{-1}) in the Danube River (2001–2009)

km 554 (BG3). A distinctive situation appears at transboundary sections for which high differences between C90 values occur: at river km 375 (0.121 mg.L^{-1} at BG5 vs. 0.666 mg.L^{-1} at RO4), river km 132 (0.300 mg.L^{-1} at UA1 vs. 0.566 mg.L^{-1} at RO5), and river km 18 (0.270 mg.L^{-1} at UA2 vs.0.570 mg.L^{-1} at RO6).

Relatively similar with N-ammonium, C90 of **N-NO$_2$** concentrations slightly decrease in the upper Danube, from 0.030 mg.L^{-1} at river km 2,581 and river km 2,538 (DE1 and DE5) to 0.025 mg.L^{-1} at river km 1,935 (AT3). In the middle Danube, C90 values range from 0.030 mg.L^{-1} at river km 1,869 (SK1) to 0.070 mg. L^{-1} at river km 1,174 (RS4), while in the lower Danube, more ascending profile is present, from 0.035 mg.L^{-1} at river km 1,077 (RS6) to 0.115 mg.L^{-1} at river km 0 (RO7).

The longitudinal variation of C90 for **N-NO$_3$** concentrations shows a marked decreasing profile from upper to middle and lower Danube, from 3.90 and 3.80 mg. L^{-1} at river km 2,581 (DE1) and 2,548 (DE5), respectively, to 1.50 mg.L^{-1} at river km 641 (BG2). The last stretch of the lower Danube is characterized by C90 values ranging around 2.00 mg.L^{-1}.

Relatively similar to N-nitrates, the spatial profile of **TN** decreases from upper to middle and lower Danube, with several peaks: C90 values above 4.00 mg.L^{-1} were calculated for river km 1,806 (HU1), 1,768 (HU2), and 1,174 (RS4) and above 5.00 mg.L^{-1} at river km 1,367 (RS2) and river km 641 (BG3).

A decreasing line in C90 values for **P-PO$_4$** is noticed in the upper Danube, from 0.065 mg.L^{-1} at river km 2,538 (DE5) to 0.039 mg.L^{-1} at river km 1,879 (AT6). A slight increasing profile appears in the middle Danube, reaching 0.140 mg.L^{-1} at river km 1,337 (HR2), but more pronounced increasing is visible in the first part of lower Danube, from 0.077 mg.L^{-1} at river km 1,077 (RS7) to 0.268 mg.L^{-1} at river km 503 (BG4). In the last 500 km of the river, a decreasing profile is noticed, down to 0.085 mg.L^{-1}. The maximum C90 value is calculated at river km 851, at station RS8 (0.380 mg.L^{-1}).

Similarly to P-orthophosphates, **TP** pattern shows decreasing values in the upper Danube, from 0.130 mg.L^{-1} at river km 2,581 (DE1) to 0.073 at river km 2,113 (AT5). Along the middle stretch, an increasing tendency occurs from 0.130 mg.L^{-1} at river km 1,869 (SK1) to 0.290 mg.L^{-1} at river km 1,337 (HR2). From river km 1,077 (RS6) downstream to river km 554 (BG3), C90 values increase from 0.160 mg.L^{-1} to 0.460 mg.L^{-1}. In the last stretch of the lower Danube, TP values go down to 0.205 mg.L^{-1} at river km 0 (RO8).

3.2 Comparison of the Long-Term Data (TNMN) with the Investigative Measurements (JDS)

Among the specific objectives of the investigative monitoring surveys organized by the ICPDR (JDS1 and JDS2), one refers to comparing the results of the two surveys [10]. Moreover, the comparison of results obtained during both JDSs (2001 and 2007) with the data generated by the long-term surveillance type of monitoring (TNMN) offers the possibility to design a future strategy for the next JDS (JDS3 in 2013) in an optimal way. Figure 4a–e which presents the comparison of the momentary results for five nutrient parameters obtained during JDS1and JDS2 with the corresponding TNMN data (C90 for 2001 and 2007, respectively) concludes the following:

– The spatial profiles of the two data sets are relatively similar.
– For N-ammonium, the C90 of TNMN data are generally higher than the "snapshot" measurements obtained during the two JDSs (especially in the middle and lower Danube, as a possible indication of the influence of insufficiently treated municipal wastewaters).
– For N-nitrates, the C90 of TNMN data are much higher than the ones recorded during JDSs, more pronounced in the upper Danube, most likely due to both point and diffuse sources.
– For the rest of the nutrient forms, except for several locations, the long-term data and the momentary ones are situated at the same concentration levels.

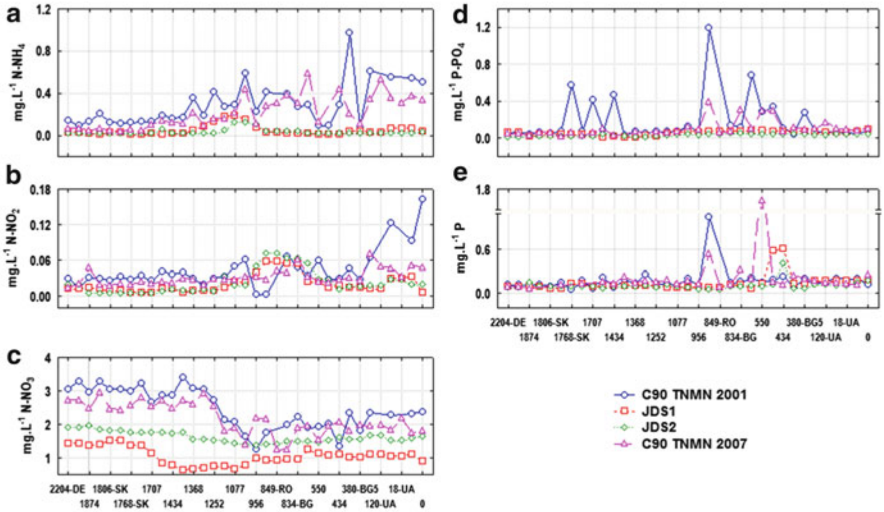

Fig. 4 Comparison between long-term monitoring TNMN data vs. investigative monitoring data – JDS1 and JDS2

As regards the comparison between JDS1 and JDS2, similar profiles of spatial distribution (with specific local variations) are visible for N-NH_4, N-NO_2, and TP. Concentrations measured for N-NO_3 during JDS2 were systematically higher than ones from JDS1, while concentrations from JDS2 were generally lower than ones from JDS1 for P-PO_4.

3.3 Dependence Between Nutrient Concentrations and Flow Discharges

Taking into account that an increased flow discharge of a river can influence the pollutants concentrations in both ways – concentration might decrease as result of dilution or increase due to the surface runoff – the dependence between the nutrient concentrations and the corresponding daily discharges was tested. The data set used for this test comprises 22 monitoring stations (marked stations in Table **1** for which daily discharges data were available) and all six nutrient forms.

As it can be seen in Figs. **1**a, b, **2**a, b, and **3**a, b, the investigated data sets are positively skewed (most of the skewness indexes > 2) with many outlier values. Therefore, prior to statistical computation, the data set was tested for normality by Shapiro-Wilk test. Taking into account the data deviation from normality, in order to test the relationship between the nutrient concentrations and the corresponding discharges, the Spearman *R* correlation coefficient (R_{SP}) was applied, which is a nonparametric measure of the correlation between variables with non-normal distribution. Actually, the R_{SP} is Pearson correlation coefficient when the values

Table 1 List of monitoring locations on the main course of the Danube River

No	TNMN code	River km	No	TNMN code	River km	No	TNMN code	River km
1	DE1[a]	2,581	15	HU5[a]	1,435	29	BG1	834
2	DE5[a]	2,538	16	HR1	1,429	30	RO2[a]	834
3	DE2[a]	2,204	17	RS1	1,427	31	BG2	641
4	AT1[a]	2,204	18	RS2	1,367	32	BG3	554
5	AT5[a]	2,113	19	HR2	1,337	33	BG4	503
6	AT3[a]	1,935	20	RS9	1,287	34	RO3[a]	432
7	AT6[a]	1,879	21	RS3	1,258	35	BG5	375
8	SK1[a]	1,869	22	RS4	1,174	36	RO4[a]	375
9	SK2[a]	1,806	23	RS5	1,155	37	RO5[a]	132
10	HU1[a]	1,806	24	RS6	1,077	38	UA1	132
11	HU2[a]	1,768	25	RO1[a]	1,071	39	RO6[a]	18
12	HU3[a]	1,708	26	RS7	955	40	UA2	18
13	SK5	1,707	27	RS8	851	41	RO7[a]	0
14	HU4[a]	1,560	28	RO18	851	42	RO8[a]	0

[a]For these stations, daily discharges ($m^3.s^{-1}$) were available

Table 2 Results of testing the dependence of N-NH_4 concentrations (mg.L^{-1}) on the corresponding daily discharges ($m^3.s^{-1}$) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	152	0.34	<u>4.22</u>	HU3	211	0.02	0.29
DE2	254	−0.04	−0.60	HU4	182	−0.21	<u>−2.89</u>
DE5	78	0.28	<u>2.46</u>	HU5	207	−0.11	−1.62
AT1	174	−0.15	−1.92	RO1	157	0.09	1.13
AT5	48	−0.26	−1.78	RO2	200	0.39	<u>5.47</u>
AT3	108	−0.01	−0.05	RO3	76	0.28	<u>2.40</u>
AT6	97	0.10	1.00	RO4	215	0.25	<u>3.68</u>
SK1	223	0.02	0.24	RO5	217	0.09	1.25
SK2	108	−0.01	−0.06	RO6	98	0.09	0.88
HU1	181	−0.03	−0.44	RO7	100	−0.06	−0.59
HU2	180	−0.11	−1.48	RO8	86	0.03	0.32

of the variables are replaced by their ranks (the values of the variables are ranked from the smallest to the largest) [11, 13, 14].

The null hypothesis (nutrient concentrations are independent of discharge) is rejected if $|R_{SP}| \times \sqrt{n-1} > u_{1-\alpha/2}$, where $u_{1-\alpha/2}$ denotes the $(1-\alpha/2)$ 100% quantile of a standard normal distribution [11]. At a chosen significance level of $\alpha = 0.05$, the null hypothesis of independence is rejected if the test statistic $t_s = |R_{SP}| \times \sqrt{n-1} > 1.96$ [15, 16].

Tables 2, 3, 4, 5, 6, and 7 present the results of the R_{SP} and the corresponding test statistic obtained for the investigated nutrient forms in selected stations. Marked t_s (underlined values in tables 2–7) are statistically significant at the chosen significance level, which means that a positive t_s value indicates that the larger discharge, the higher nutrient concentration, while negative t_s value indicates an opposite situation (the larger discharge, the smaller concentration).

- **N-NH_4** (Table 2): no specific pattern of R_{SP} in the upper and middle Danube is noticed, but all coefficients are positive in the lower Danube, except for one station (RO7); two positive test statistics in the upper Danube (DE1 and DE5) and three in the lower Danube indicate positive correlation between N-ammonium and daily discharges during the studied time period. In the middle Danube, in one station (HU4) test statistic shows negative dependence between the two variables.
- **N-NO_2** (Table 3): for this intermediate nutrient form, the least relevant R_{SP} values are calculated; therefore, only two positive test statistics (DE1 and DE5) do not indicate clear correlation between N-nitrite concentration and discharge values.
- **N-NO_3** (Table 4): all R_{SP} coefficients in the upper Danube are negative, while in the lower Danube, all are positive, which comes in good agreement with results based on the TNMN data during 1996–2005 [11]. The values of the test statistics

Table 3 Results of testing the dependence of N-NO_2 concentrations (mg.L^{-1}) on the corresponding daily discharges ($m^3.s^{-1}$) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	74	0.25	$\underline{2.12}$	HU3	211	0.13	1.89
DE2	175	−0.08	−1.03	HU4	182	−0.13	−1.74
DE5	77	0.43	$\underline{3.73}$	HU5	207	0.07	0.96
AT1	151	−0.12	−1.42	RO1	157	−0.01	−0.10
AT5	48	−0.10	−0.71	RO2	200	0.01	0.19
AT3	108	0.06	0.59	RO3	77	−0.05	−0.47
AT6	97	0.19	1.85	RO4	215	−0.03	−0.44
SK1	223	0.06	0.94	RO5	218	0.12	1.74
SK2	108	0.11	1.16	RO6	98	0.17	1.68
HU1	181	−0.03	−0.35	RO7	100	0.04	0.42
HU2	180	−0.02	−0.27	RO8	86	−0.12	−1.09

Table 4 Results of testing the dependence of N-NO_3 concentrations (mg.L^{-1}) on the corresponding daily discharges ($m^3.s^{-1}$) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	152	−0.16	$-\underline{1.99}$	HU3	211	0.06	0.90
DE2	254	−0.21	$-\underline{3.40}$	HU4	182	−0.03	−0.39
DE5	78	−0.23	$-\underline{1.99}$	HU5	207	0.19	$\underline{2.70}$
AT1	151	−0.26	$-\underline{3.14}$	RO1	157	0.38	$\underline{4.74}$
AT5	48	−0.37	$-\underline{2.55}$	RO2	200	0.27	$\underline{3.80}$
AT3	108	−0.16	−1.68	RO3	77	0.18	1.53
AT6	97	−0.27	$-\underline{2.64}$	RO4	215	−0.13	−1.92
SK1	223	−0.09	−1.37	RO5	218	0.25	$\underline{3.74}$
SK2	108	0.00	0.05	RO6	98	0.25	$\underline{2.51}$
HU1	181	−0.08	−1.13	RO7	100	0.08	0.77
HU2	181	−0.06	−0.84	RO8	86	0.25	$\underline{2.27}$

in the upper Danube indicate relatively strong negative correlation between N-nitrates and corresponding daily discharges in all stations, except for one (AT3). Only one station in the middle Danube (HU5) and 5 stations (out of eight) in the lower Danube present strong positive correlation between the concentrations and flow values.

- **TN** (Table 5): similarly with N-NO_3 (as expected since the major weight in the TN content is given by N-nitrates), all R_{SP} coefficients in the upper Danube are negative, but only one significant test statistic is calculated at station DE2; in the middle and lower Danube, all are positive, except for two stations (SK1 and RO1). Three positively significant t_s are calculated in the middle stretch at stations HU1, HU2, and HU5 as well as three t_s in the lower stretch (RO5, RO6, and RO8).

Table 5 Results of testing the dependence of TN concentrations (mg.L^{-1}) on the corresponding daily discharges (m^3.s^{-1}) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	24	−0.33	−1.59	HU3	99	0.17	1.67
DE2	73	−0.27	−$\underline{2.25}$	HU4	28	0.06	0.32
DE5	48	−0.04	−0.25	HU5	117	0.20	$\underline{2.11}$
AT1	–	–	–	RO1	11	−0.20	−0.62
AT5	–	–	–	RO2	14	0.25	0.90
AT3	–	–	–	RO3	–	–	–
AT6	–	–	–	RO4	129	0.08	0.89
SK1	172	−0.05	−0.69	RO5	153	0.29	$\underline{3.61}$
SK2	108	0.05	0.55	RO6	64	0.30	$\underline{2.35}$
HU1	102	0.23	$\underline{2.34}$	RO7	65	0.14	1.11
HU2	100	0.26	$\underline{2.55}$	RO8	57	0.30	$\underline{2.21}$

Table 6 Results of testing the dependence of P-PO$_4$ concentrations (mg.L^{-1}) on the corresponding daily discharges (m^3.s^{-1}) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	152	0.19	$\underline{2.39}$	HU3	211	−0.12	−1.79
DE2	254	−0.20	−$\underline{3.15}$	HU4	180	−0.15	−1.95
DE5	78	0.17	1.48	HU5	207	−0.05	−0.72
AT1	175	−0.20	−$\underline{2.63}$	RO1	157	0.13	$\underline{1.60}$
AT5	48	−0.28	−1.92	RO2	201	0.03	0.44
AT3	108	−0.19	−1.92	RO3	77	0.02	0.18
AT6	97	−0.18	−1.81	RO4	215	0.09	1.36
SK1	172	−0.10	−1.29	RO5	218	0.09	1.34
SK2	108	−0.05	−0.57	RO6	98	−0.04	−0.42
HU1	181	−0.05	−0.73	RO7	100	−0.17	−1.73
HU2	181	−0.15	−$\underline{2.04}$	RO8	86	−0.09	−0.86

- **P-PO$_4$** (Table 6): 15 out of 22 R_{SP} coefficients are negative for this nutrient form. In the upper Danube, t_s indicate a positive correlation at station DE1, while in the middle Danube, only one t_s value is negatively significant at HU2; in the lower Danube, no significant correlation is noticed, results confirmed by previous information [11].
- **TP** (Table 7): except for two negative values (at AT5 and HU3), all R_{SP} coefficients are positive and large enough for the test statistics to be significant in half of the investigated stations (the first three stations in the upper Danube, six stations in the middle, and two in the lower Danube, respectively).

Table 7 Results of testing the dependence of TP concentrations ($mg.L^{-1}$) on the corresponding daily discharges ($m^3.s^{-1}$) based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Station	No of obs.	R_{SP}	t_s
DE1	152	0.46	5.61	HU3	189	−0.10	−1.37
DE2	254	0.30	4.77	HU4	182	0.41	5.58
DE5	78	0.57	4.99	HU5	208	0.28	4.01
AT1	174	0.11	1.49	RO1	152	0.10	1.20
AT5	48	−0.08	−0.53	RO2	193	−0.003	−0.04
AT3	108	0.03	0.34	RO3	76	0.04	0.35
AT6	97	0.11	1.04	RO4	193	0.16	2.27
SK1	172	0.46	5.97	RO5	192	0.15	2.02
SK2	93	0.54	5.20	RO6	88	0.15	1.40
HU1	181	0.41	5.55	RO7	90	0.13	1.21
HU2	182	0.45	6.01	RO8	76	0.04	0.36

3.4 *Temporal Trends*

The nonparametric Spearman's criterion was also used in order to investigate whether the TNMN data set for nutrients along the Danube River had a certain temporal trend (increasing or decreasing) over the studied time period. The R_{SP} was calculated between the measured concentration of a given parameter and the number of days corresponding to the interval 2001–2009 (first sampling day in 2001 was set as 0), using the significance level of $\alpha = 0.05$: the null hypothesis of no trend is rejected if the test statistic $t_s = |R_{SP}| \times \sqrt{n-1} > 1.96$ (underlined values in tables 8–13). Therefore, the resulting trend was considered to be significantly positive (marked with ↑) if $t_s > 1.96$ and significantly negative (marked with ↓) if $t_s < -1.96$ [15, 16]. In order to remove the potential influence of the discharge on the measured concentration (tested in the chapter by [5]), further statistical analysis was applied [11]: the resulting residuals of the linear regression between nutrient concentration (dependent variable) and corresponding daily flow (independent variable) and the number of days were used to calculate $R_{SP\text{–}rez}$ and test statistic $t_{s\text{–}rez}$. Tables **8**, **9**, **10**, **11**, **12**, and **13** present the results of this trend analysis. A general good agreement is noticed between the significance of the statistic tests obtained for the trend in which the impact of the daily discharge was not excluded (t_s) and the trend in which this influence was removed ($t_{s\text{–}rez}$), which means that even if the discharge flow affects the measured nutrient concentration in a certain extent, it does not significantly change the temporal trend for the investigated parameter [11]:

Table 8 Results of testing the temporal trends in N-NH_4 concentrations (mg.L^{-1}) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	152	0.14	1.67	–	0.17	$\underline{2.13}$	↑
DE2	254	−0.41	$-\underline{6.58}$	↓	−0.40	$-\underline{6.40}$	↓
DE5	78	0.01	0.07	–	0.04	0.33	–
AT1	174	−0.08	−1.12	–	−0.10	−1.27	–
AT5	48	0.03	0.19	–	0.04	0.25	–
AT3	108	−0.01	−0.09	–	−0.02	−0.19	–
AT6	97	−0.04	−0.36	–	−0.02	−0.22	–
SK1	223	−0.52	$-\underline{7.69}$	↓	−0.51	$-\underline{7.65}$	↓
SK2	108	−0.44	$-\underline{4.52}$	↓	−0.44	$-\underline{4.59}$	↓
HU1	181	−0.45	$-\underline{6.08}$	↓	−0.46	$-\underline{6.19}$	↓
HU2	180	−0.41	$-\underline{5.48}$	↓	−0.40	$-\underline{5.35}$	↓
HU3	211	−0.30	$-\underline{4.35}$	↓	−0.30	$-\underline{4.28}$	↓
HU4	182	−0.06	−0.87	–	−0.07	−1.00	–
HU5	207	−0.04	−0.56	–	−0.05	−0.69	–
RO1	157	−0.11	−1.38	–	−0.10	−1.24	–
RO2	200	−0.23	$-\underline{3.31}$	↓	−0.25	$-\underline{3.53}$	↓
RO3	76	−0.17	−1.47	–	−0.16	−1.41	–
RO4	215	−0.30	$-\underline{4.41}$	↓	−0.37	$-\underline{5.38}$	↓
RO5	217	−0.27	$-\underline{4.01}$	↓	−0.27	$-\underline{3.95}$	↓
RO6	98	−0.30	$-\underline{2.93}$	↓	−0.29	$-\underline{2.87}$	↓
RO7	100	−0.36	$-\underline{3.62}$	↓	−0.38	$-\underline{3.83}$	↓
RO8	86	−0.32	$-\underline{2.99}$	↓	−0.32	$-\underline{2.99}$	↓

- **N-NH_4** (Table 8): in more than half of the stations, significant decreasing trend was obtained, especially in the middle (SK1, SK2, HU1, HU2, HU3) and lower Danube (RO2, RO4, RO5, RO6, RO7, and RO8). In the upper Danube, strong negative trend appeared at station DE2 and slightly positive at DE1 (after the influence of the discharge was excluded).
- **N-NO_2** (Table 9): N-nitrites decreased in the upper Danube (station DE2), more pronounced in the middle Danube (SK1, SK2, HU1, HU2, HU3, HU4, HU5), and moderate in several stations from the lower stretch (RO1, RO2, RO4, RO5, and RO7).

Table 9 Results of testing the temporal trends in N-NO_2 concentrations ($mg.L^{-1}$) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	74	0.21	1.78	–	0.21	1.81	–
DE2	175	−0.16	−2.15	↓	−0.16	−2.18	↓
DE5	77	−0.18	−1.60	–	−0.17	−1.48	–
AT1	151	−0.11	−1.32	–	−0.12	−1.43	–
AT5	48	−0.04	−0.27	–	−0.03	−0.22	–
AT3	108	−0.08	−0.78	–	−0.08	−0.85	–
AT6	97	−0.17	−1.62	–	−0.15	−1.47	–
SK1	223	−0.38	−5.63	↓	−0.38	−5.63	↓
SK2	108	−0.31	−3.26	↓	−0.31	−3.22	↓
HU1	181	−0.30	−4.02	↓	−0.30	−4.04	↓
HU2	180	−0.29	−3.87	↓	−0.29	−3.89	↓
HU3	211	−0.29	−4.25	↓	−0.30	−4.29	↓
HU4	182	−0.26	−3.51	↓	−0.25	−3.39	↓
HU5	207	−0.22	−3.20	↓	−0.18	−2.61	↓
RO1	157	−0.41	−5.17	↓	−0.41	−5.06	↓
RO2	200	−0.47	−6.66	↓	−0.47	−6.66	↓
RO3	77	−0.04	−0.32	–	−0.04	−0.31	–
RO4	215	−0.32	−4.68	↓	−0.33	−4.79	↓
RO5	218	−0.14	−2.05	↓	−0.14	−2.02	↓
RO6	98	−0.13	−1.27	–	−0.12	−1.23	–
RO7	100	−0.37	−3.70	↓	−0.36	−3.60	↓
RO8	86	−0.14	−1.28	–	−0.15	−1.43	–

- **N-NO_3** (Table 10): in twelve stations along the entire course of the Danube, significant negative trend was detected, following the trend calculated for the TNMN data from the previous period of time, 1996–2005 [11]. In the lower Danube, significant positive trend was found at stations RO2 and RO3.

Table 10 Results of testing the temporal trends in N-NO_3 concentrations (mg.L^{-1}) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	152	0.14	1.78	–	0.15	1.89	–
DE2	254	−0.14	−2.17	↓	−0.15	−2.41	↓
DE5	78	−0.15	−1.33	–	−0.17	−1.47	–
AT1	151	−0.29	−3.53	↓	−0.31	−3.84	↓
AT5	48	−0.38	−2.59	↓	−0.41	−2.82	↓
AT3	108	−0.16	−1.68	–	−0.19	−1.93	–
AT6	97	−0.24	−2.31	↓	−0.25	−2.48	↓
SK1	223	−0.13	−1.93	–	−0.13	−1.95	–
SK2	108	−0.15	−1.59	–	−0.15	−1.54	–
HU1	181	−0.22	−2.94	↓	−0.22	−2.96	↓
HU2	181	−0.17	−2.32	↓	−0.18	−2.39	↓
HU3	211	−0.18	−2.61	↓	−0.17	−2.46	↓
HU4	182	−0.08	−1.08	–	−0.08	−1.01	–
HU5	207	−0.18	−2.56	↓	−0.16	−2.28	↓
RO1	157	0.06	0.78	–	0.09	1.13	–
RO2	200	0.16	2.22	↑	0.17	2.33	↑
RO3	77	0.33	2.85	↑	0.32	2.80	↑
RO4	215	−0.19	−2.76	↓	−0.16	−2.28	↓
RO5	218	−0.25	−3.68	↓	−0.24	−3.59	↓
RO6	98	−0.24	−2.37	↓	−0.24	−2.35	↓
RO7	100	−0.25	−2.45	↓	−0.24	−2.37	↓
RO8	86	−0.16	−1.48	–	−0.15	−1.35	–

- **TN** (Table 11): no significant trend along the Danube River, except for decreasing trend in five stations – four in the middle (SK1–HU2) and one in the lower Danube (RO5) (at stations SK2 and RO5, the negative trend was not confirmed after the influence of the discharge was removed).

Table 11 Results of testing the temporal trends in TN concentrations ($mg.L^{-1}$) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	24	−0.23	−1.12	–	−0.15	−0.74	–
DE2	73	−0.11	−0.95	–	−0.10	−0.86	–
DE5	48	−0.03	−0.17	–	−0.02	−0.15	–
AT1	–	–	–	–	–	–	–
AT5	–	–	–	–	–	–	–
AT3	–	–	–	–	–	–	–
AT6	–	–	–	–	–	–	–
SK1	172	−0.19	−2.42	↓	−0.18	−2.36	↓
SK2	108	−0.20	−2.04	↓	−0.17	−1.80	–
HU1	102	−0.50	−5.05	↓	−0.46	−4.64	↓
HU2	100	−0.52	−5.19	↓	−0.48	−4.75	↓
HU3	99	−0.08	−0.77	–	−0.03	−0.26	–
HU4	28	−0.28	−1.44	–	−0.31	−1.59	–
HU5	117	−0.06	−0.60	–	−0.01	−0.08	–
RO1	11	0.18	0.58	–	0.05	0.17	–
RO2	14	−0.17	−0.61	–	−0.16	−0.56	–
RO3	–	–	–	–	–	–	–
RO4	129	−0.16	−1.79	–	−0.15	−1.72	–
RO5	153	−0.17	−2.07	↓	−0.13	−1.60	–
RO6	64	−0.14	−1.11	–	−0.14	−1.09	–
RO7	65	−0.24	−1.89	–	−0.22	−1.76	–
RO8	57	−0.13	−0.98	–	−0.02	−0.13	–

- **P-PO_4** (Table 12): significant negative trend appeared in the upper Danube (AT3, AT6, and AT5 when the influence of the discharge was excluded) and in the lower Danube (RO3). Strong negative trend was detected at stations RO2 and RO3, both before and after removing the discharge influence.

Table 12 Results of testing the temporal trends in P-PO_4 concentrations (mg.L^{-1}) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	152	0.04	0.49	–	0.06	0.68	–
DE2	254	0.02	0.34	–	0.02	0.28	–
DE5	78	0.08	0.70	–	0.11	0.96	–
AT1	175	−0.08	−1.08	–	−0.08	−1.11	–
AT5	48	−0.28	−1.92	–	−0.34	$-\underline{2.31}$	↓
AT3	108	−0.20	$-\underline{2.10}$	↓	−0.21	$-\underline{2.16}$	↓
AT6	97	−0.34	$-\underline{3.35}$	↓	−0.36	$-\underline{3.57}$	↓
SK1	172	0.08	1.11	–	0.09	1.16	–
SK2	108	0.15	1.56	–	0.15	1.56	–
HU1	181	−0.06	−0.85	–	−0.05	−0.71	–
HU2	181	−0.04	−0.53	–	−0.04	−0.57	–
HU3	211	−0.07	−1.08	–	−0.08	−1.16	–
HU4	180	0.06	0.74	–	0.05	0.64	–
HU5	207	−0.05	−0.73	–	−0.05	−0.66	–
RO1	157	−0.28	−3.54	–	−0.29	−3.64	↓
RO2	201	−0.21	$-\underline{2.94}$	↓	−0.21	$-\underline{2.98}$	↓
RO3	77	−0.77	$-\underline{6.71}$	↓	−0.76	$-\underline{6.60}$	↓
RO4	215	0.03	0.49	–	0.06	0.90	–
RO5	218	−0.09	−1.30	–	−0.09	−1.34	–
RO6	98	0.10	0.99	–	0.09	0.93	–
RO7	100	0.05	0.47	–	0.00	0.04	–
RO8	86	0.21	1.89	–	0.21	1.90	–

- **TP** (Table 13): decreasing trend in the upper Danube was found at station AT6. Significant negative trend was also found in five stations from the middle Danube (SK2, HU1, HU2, HU4, and HU5, but for the first three stations, the trend was not confirmed after the influence of the discharge was excluded) and in one station from the lower Danube (RO3). Increasing trend was noticed as well at station RO4, confirmed also after removing the discharge influence.

Table 13 Results of testing the temporal trends in TP concentrations (mg.L^{-1}) in the Danube River based on TNMN data from 2001 to 2009 (underlined values are statistically significant at level 0.05)

Station	No of obs.	R_{SP}	t_s	Trend	R_{SP-rez}	t_{s-rez}	$Trend_{rez}$
DE1	152	0.06	0.74	–	0.09	1.09	–
DE2	254	−0.05	−0.75	–	0.09	1.42	–
DE5	78	0.07	0.58	–	0.14	1.20	–
AT1	174	0.04	0.57	–	0.06	0.81	–
AT5	48	0.00	0.03	–	0.01	0.06	–
AT3	108	−0.30	$-\underline{3.06}$	↓	−0.28	$-\underline{2.87}$	↓
AT6	97	0.09	0.88	–	0.13	1.32	–
SK1	172	−0.14	−1.86	–	0.00	−0.06	–
SK2	93	−0.23	$-\underline{2.18}$	↓	−0.10	−0.91	–
HU1	181	−0.18	$-\underline{2.45}$	↓	−0.13	−1.73	–
HU2	182	−0.15	$-\underline{1.99}$	↓	−0.06	−0.76	–
HU3	189	0.12	1.58	–	0.12	1.70	–
HU4	182	−0.45	$-\underline{6.09}$	↓	−0.47	$-\underline{6.26}$	↓
HU5	208	−0.50	$-\underline{7.16}$	↓	−0.46	$-\underline{6.67}$	↓
RO1	152	−0.12	−1.49	–	−0.12	$-\underline{1.46}$	–
RO2	193	−0.05	$-\underline{0.76}$	–	−0.05	$-\underline{0.67}$	–
RO3	76	−0.71	$-\underline{6.13}$	↓	−0.70	$-\underline{6.06}$	↓
RO4	193	0.15	$\underline{2.09}$	↑	0.14	2.00	↑
RO5	192	−0.09	−1.26	–	−0.08	−1.14	–
RO6	88	−0.03	−0.25	–	−0.01	−0.14	–
RO7	90	−0.03	−0.27	–	−0.01	−0.10	–
RO8	76	−0.05	−0.43	–	−0.02	−0.20	–

4 Conclusions

The spatial distribution of the investigated nutrients based on the long-term monitoring program of the ICPDR (TNMN) during 2001–2009 shows a general decreasing tendency in the upper Danube stretch followed by an increasing line along the middle and lower Danube in the case of N-ammonium, N-nitrites, P-orthophosphates, and total phosphorous. A marked decreasing profile from upper down to middle and lower Danube is noticed for N-nitrates and total nitrogen.

As regards the comparison between the results obtained in JDS1 (2001) and JDS2 (2007) with the corresponding TNMN data, it can be concluded that the "snapshot" measurements obtained in the frame of an investigative monitoring of JDS type are complementary to the surveillance data recorded over a year (C90 of TNMN concentrations) and confirm the quality of results obtained on an annual basis at the basin-wide level by the institutions in the riparian countries.

The Spearman rank correlation coefficients and the resulting test statistics used for investigation of dependence between the nutrient concentrations and the corresponding daily discharges available in 22 selected stations show several significant correlations between the two considered variables, depending on the monitoring station and parameter involved, especially for N-nitrates, total nitrogen, and total phosphorous.

Temporal trends (using the above-mentioned Spearman rank correlation coefficients and the corresponding test statistics) show a general decreasing temporal tendency over the studied period (2001–2009) for all nutrient forms (except for few locations), even after the impact of the corresponding daily discharge on the measured concentration was excluded.

Acknowledgments The authors gratefully acknowledge the International Commission for the Protection of the Danube River in Vienna, Austria, and the JDS1and JDS2 Core Member Teams for their support. Special thanks to Lea Mrafkova, from the Slovak Hydrometeorological Institute (TNMN Data Management Center) in Bratislava, Slovakia, for providing the TNMN data set.

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The Danube Water Quality Model and Its Application in the Danube River Basin

Jos van Gils

Abstract The Danube Water Quality Model (DWQM) was developed in the framework of the GEF project "Danube River Basin Pollution Reduction Programme" (1999) and updated in a large international research project called "Nutrient Management in the Danube Basin and its Impact on the Black Sea" (acronym *daNUbs*, 2001–2005). The DWQM simulates the water quality in the Danube River and its main tributaries as a function of space and time, dependent on the river morphology and hydrology and on emissions calculated by the model MONERIS. The specific goal of the DWQM is to simulate the nutrient loads to the Black Sea in support to the management of the nutrients nitrogen (N) and phosphorus (P) in the Danube River Basin and to distribute them over time and over the different nutrient species. Both distributions are decisive for the assessment of the impact of the Danube outflow on the north-western shelf of the Black Sea. This chapter discusses the set-up of the DWQM and its application to the conditions around the year 2000, which served both to enhance our understanding and to calibrate and validate the DWQM. The validated DWQM has been used to assess five scenarios for future management alternatives, varying from "business as usual" to "deep green". Where appropriate, we refer to the underlying scientific papers and reports.

Keywords Danube River, *daNUbs*, Modelling, Nutrient management scenarios

Contents

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1 Introduction

The Danube Water Quality Model (DWQM) goes back to the late 1990s, when the first version was developed in the framework of the GEF project "Danube River Basin Pollution Reduction Programme" [1, 2]. The experience gained during this project was used to formulate a large international research project called "Nutrient Management in the Danube Basin and its Impact on the Black Sea" (also known by its acronym "*daNUbs*") [3]. This project ran from 2001 to 2005, and it was financed by the EU Fifth Framework Programme and 18 participating European research partners.

Both the GEF project and *daNUbs* addressed the management of the nutrients nitrogen (N) and phosphorus (P) in the Danube River Basin. At the time, nutrient emissions in the preceding decades had led to severe ecological problems: the deterioration of groundwater resources and the eutrophication of rivers, lakes and especially the Black Sea ([4] and references therein). These problems are directly related to social and economic issues (e.g. drinking water supply, tourism and fishery as affected sectors; agriculture, nutrition, industry and wastewater management as drivers). We refer to the chapters by Popovici [5] and by Hamchevici and Udrea [6] in this book for further backgrounds. In order to recommend proper management for the protection of the water system in the Danube Basin and the Black Sea, *daNUbs* provided an interdisciplinary analysis of the Danube catchment area, the Danube River system and the mixing zone of the Danube River in the north-western Black Sea.

One of the cornerstones of the analysis provided by *daNUbs* was the use of mathematical modelling, for two reasons. First, the set-up, calibration and validation of mathematical models help to find out to what degree the available information and knowledge are consistent. It also helps to determine how far our understanding of the system under study reaches and to determine data and knowledge gaps. Next to learning to what degree the researchers understand the behaviour of the Danube River Basin and the north-western Black Sea ("diagnosis"), *daNUbs* also used models to study possible future lines of developments, formulated as scenarios ("prognosis").

The issue of nutrient management in the Danube River Basin shows a high level of complexity, including the natural and socio-economic features of the basin, resulting nutrient emissions to the surface waters, in-stream transformation, storage and losses, conveyance of nutrients towards the Danube Delta and the Black Sea. At the time that the *daNUbs* project was formulated, it was decided to cover this complexity by two connected models, MONERIS and the DWQM, each with their own specific strong points.

MONERIS (MOdelling Nutrient Emissions in RIver Systems) can be characterised as a lumped catchment model, covering the whole basin (land + water) divided in sub-catchments. MONERIS has been developed during the 1980s and 1990s and has been applied and further developed for a wide range of rivers, in Europe and outside Europe. The model is based on data regarding the river flow and the water quality as well as on digital maps and extensive statistical information about the relevant socio-economic drivers, such as population density, wastewater management, livestock, fertiliser use, etc. It uses semiempirical relations to calculate the multi-annually averaged emissions of N and P to the surface waters, distributed over different pathways, as well as the in-stream retention in the small-scale surface water network.

The Danube Water Quality Model (DWQM) covers the Danube River itself and its main tributaries. It is based on generic programmes to calculate the water flow and the water quality. The DWQM calculates the in-stream nutrient loads and the storage and losses in the Danube River and its main tributaries. It is based on data regarding the yearly point and diffuse emissions to the surface water from MONERIS as well as on daily hydrological data for different stations in the Danube basin. Eventually, it calculates the nutrient fluxes towards the Danube Delta.

Below, we will discuss the highlights of the development and the application of the DWQM. A full record is provided by the relevant *daNUbs* reports [7–9].

2 Danube Basin Water Quality Model Set-Up

2.1 Objectives

The envisioned role of the DWQM within *daNUbs* led to the following objectives: (1) the dynamic modelling of the water quality in the modelled river stretches, based on emission estimates generated by MONERIS; (2) the analysis of the in-stream retention processes on a spatially varying basis, in order to study the role of large wetlands and reservoirs (Gabcikovo, Iron Gates, Danube Delta); and (3) the modelling of the outflow to the Black Sea on a day-to-day basis, in terms of the water discharges and the concentrations of the relevant water quality parameters. The modelling of organic pollution and dissolved oxygen was not within the scope of the DWQM.

2.2 General Structure and Model Formulations

The DWQM consists of two modules: the channel flow (CF) module that calculates water levels and water flows as a function of space and time and the water quality (WQ) module that calculates the concentration of relevant water quality variables as a function of space and time. A preprocessor prepares the necessary input data on the basis of hydrological data and the output from MONERIS. Figure 1 provides an overview.

The CF module uses the so-called shallow water equations to calculate the water level and the water flow (in m^3/s) in the river network as a function of time (for an account of the equations, see [10]). The WQ module uses the advection diffusion equation [11] to calculate the concentrations of the relevant water quality variables. These include four nitrogen species (nitrates NO_3^-, ammonium NH_4^+, particular organic nitrogen PON, dissolved organic nitrogen DON), three phosphorus species (*ortho*phosphates PO_4^{3-}, particulate inorganic phosphorus PIP, particulate organic phosphorus POP), two silica species (dissolved silicates, opal silicate), phytoplankton, dead organic carbon and inorganic suspended matter. The terms considered in the water quality model equations are demonstrated in Fig. 2, for a schematic representation of river segment i. They include the longitudinal river fluxes of water and substances, the lateral inflow of water and substances from the Danube sub-catchments linked to the river as well as decay and transformation processes within the river.

The model contains all relevant processes for the modelled variables [11]. Figure 2 shows a schematic representation of the relevant processes for the nitrogen and phosphorus species. Of particular relevance are storage and loss processes, which remove N and P from the water column and prevent or reduce the downstream transport. For N, the most relevant process is denitrification: a loss process which takes place in aquatic sediments. It is driven by the oxidation of organic

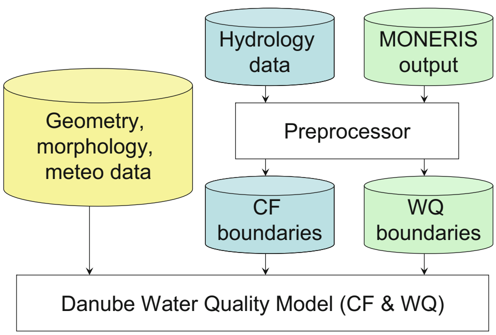
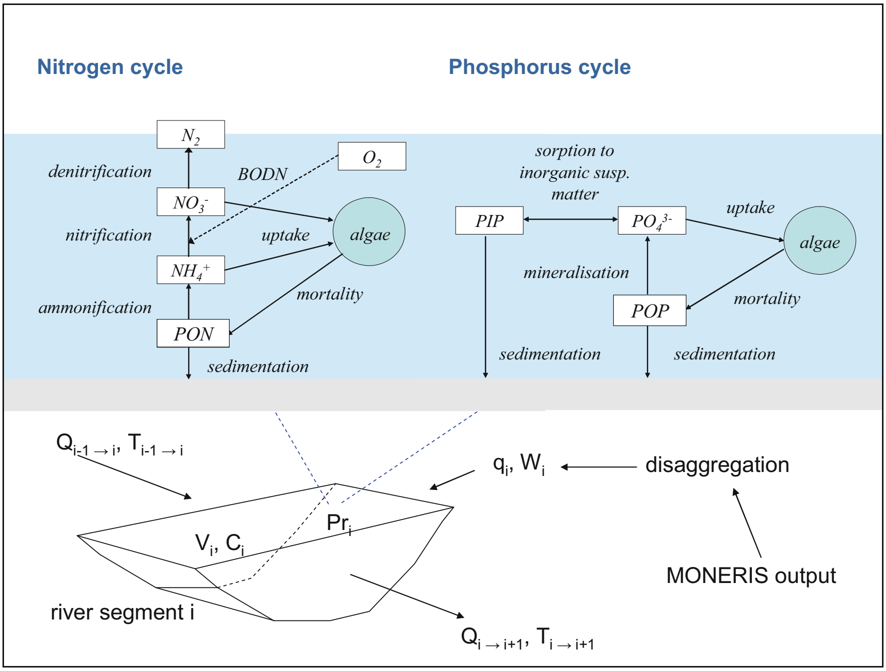


Fig. 1 General structure of the DWQM



N_2	nitrogen gas	DO	dissolved oxygen
NO_3^-	nitrates	PIP	particulate inorganic phosphorus
NH_4^+	ammonium	PO_4^{3-}	ortho-phosphates
PON	particulate organic nitrogen	POP	particulate organic phosphorus

V	water volume (m^3)	C	concentration (g/m3)
Q	river water flux (m^3/s)	T	river substance flux, = QC (g/s)
q	lateral water inflow (m^3/s)	W	lateral substance flux (g/s)
Pr	decay and transformation processes within the river segment (g/s)		
i-1	upstream segment	i+1	downstream segment

Fig. 2 Schematic overview of the water quality model formulations

carbon at a depth where dissolved oxygen is no longer available as an oxidator and nitrates take over this role. As a result, N_2 (and to some extent N_2O) escapes to the atmosphere. For P, the most relevant process is storage of PIP and POP in aquatic sediments in areas of net deposition (e.g. wetlands and floodplains).

2.3 Emission Data

The DWQM relies on MONERIS to calculate the (multi-annually averaged) emissions of nitrogen and phosphorus towards the surface waters. MONERIS calculates these emissions for all sub-catchments in its schematisation. The application to the Danube Basin has about 400 sub-catchments (see Fig. 3) and has been validated on the basis of historical data [12, 13].

The emissions are calculated taking into account seven different pathways to reach the surface waters, in particular: (1) point sources (mostly wastewater treatment plants (WWTPs) and some industry), (2) urban area run-off, (3) atmospheric deposition, (4) tile drainage, (5) groundwater inflows, (6) surface run-off and (7) erosion. Figure 4 shows the relative distribution of the emissions of N and P over these seven pathways. For nitrogen, the most important pathways are the groundwater inflows and the WWTPs. For phosphorus, the most important pathways are the WWTPs and the erosion.

While calculating the emissions of nutrients to the surface water, MONERIS already takes into account the loss of nitrogen in the soil and groundwater mainly due to denitrification. Averaged over larger areas (14 subbasins), the retention in the soil and the groundwater varies between 62% (Sava) and 99% (Delta Liman). MONERIS also addresses the storage and losses of nutrients in the smaller surface waters which are not explicitly included in the DWQM. Averaged over larger areas (14 subbasins), the retention in the smaller surface waters varies between <40% (Germany, Austria, Sava, Drava) and >80% (Delta Liman).

All together, the MONERIS sub-catchments cover the whole catchment. For every sub-catchment we assume that we know at what point the emissions from this

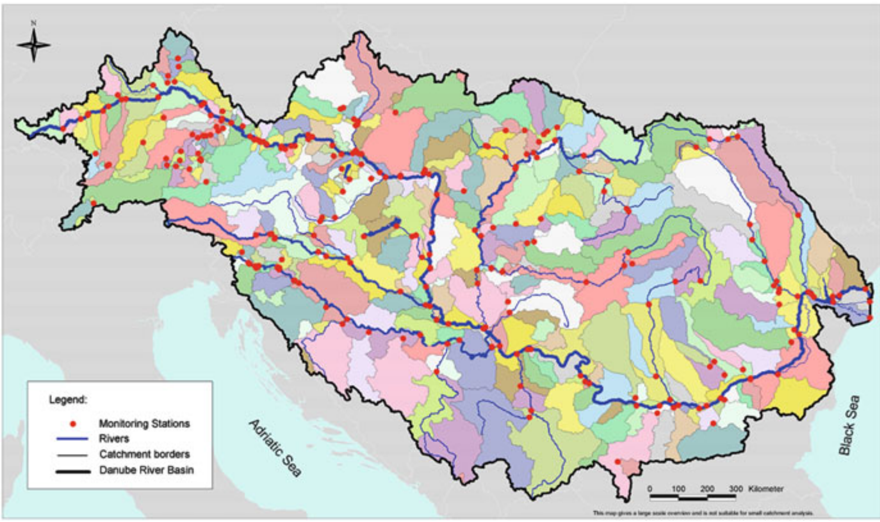


Fig. 3 Overview of the schematisation of the Danube Basin in MONERIS

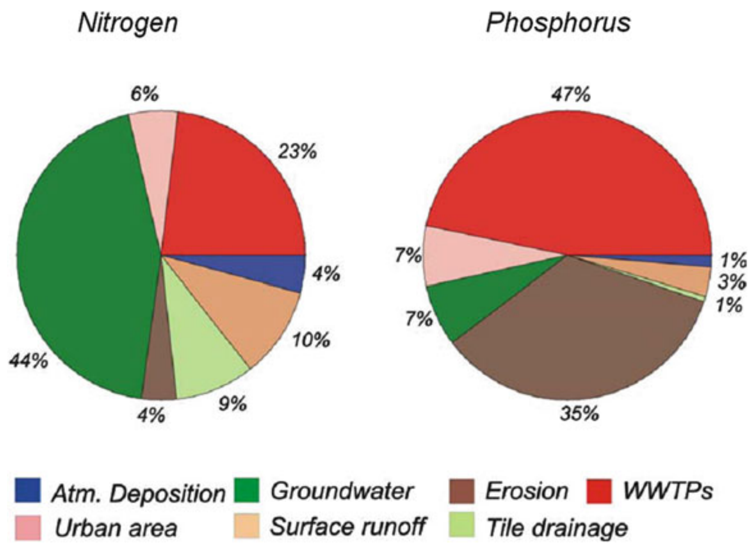


Fig. 4 Relative distribution of nutrient emissions from different pathways for the total Danube Basin (1998–2000)

sub-catchment reach the river network schematised in the DWQM. This point is called the connection point. Every sub-catchment is connected to one connection point, while one connection point can be the recipient of one or more sub-catchments.

2.4 DWQM River Basin Geometry and Morphology

The DWQM derives information about the alignment of the Danube and its main tributaries, the cross sections and the major river structures from the information collected for the set-up of the Danube Basin Alarm Model [14]. Figure 5 provides an overview of the modelled river branches. This figure also shows the connection points to the MONERIS sub-catchments, the major structures and the locations of the defined cross sections.

Fig. 5 The schematisation of the Danube River and its main tributaries in the DWQM, selected hydrological stations

2.5 Creating CF and WQ Boundaries

The output from MONERIS provides the multi-annually averaged inflows of water and substances from the Danube catchment area at the connection points to the Danube River and its main tributaries. A core part of the DWQM is the disaggregation of these inflows over time. To disaggregate the water inflows, the DWQM uses the monitored water discharge time series at selected stations (Wolfsthal, Hercegszanto, Bazias, Tiszasziget and Reni; see Fig. 5). These discharge time series characterise the hydrological regime of the river as well as the spatial variability of this regime. The observed water discharge time series are used to distribute the water inflows at the connection points over time, without changing the average inflows per point. The result is provided to the CF module as input and allows it to accurately simulate the discharge as a function of time throughout the river network.

To disaggregate the inflows of N and P at every individual connection point, the DWQM divided the inflows into three categories:

- All inflows associated with MONERIS pathways with a point source character are disaggregated assuming a constant load in the river.
- All inflows associated with MONERIS pathways with a groundwater/base flow character are disaggregated assuming a constant concentration in the river.
- All inflows associated with MONERIS pathways with a surface run-off/erosion character are disaggregated assuming a concentration proportional to the river flow.

The DWQM uses the disaggregated water inflows to calculate the disaggregated inflows of N and P according to the above assumptions. On top, the DWQM assumes that the retention of nitrogen in the smaller surface waters not included in the DWQM (which is also calculated by MONERIS) varies seasonally with a sinusoidal pattern, with zero retention on 31 January and maximum retention on 31 July. The result is provided to the WQ module as input. The formulas are provided by Constantinescu and van Gils [7] and van Gils [8] respectively.

2.6 Water Quality Monitoring Data

The set-up of the DWQM also relied on the analysis of water quality data from the Danube Basin. The Trans-National Monitoring Network of the International Commission for the Protection of the Danube River (TNMN) proved an extremely valuable data set, because for the years 1996–2002, it provides continuous (>12/year) data for stations throughout the basin (>61). Very useful also were the results from the first Joint Danube Survey (JDS1, August–September 2001), which provides homogeneous data along the whole river satisfying very high quality standards. The model set-up was further supported by data collected during dedicated *daNUbs* surveys and by data from various other sources, compiled by van Gils [9].

3 Modelling the Existing Situation

By simulating different years from the period around 2000 (1997–2003), the developers demonstrated the capabilities of the combined models MONERIS and DWQM to represent the existing situation. Below, we will first present selected results from the analysis of field data, which provide the basis for "checking" the model. Next, we will present some highlights from the model validation.

3.1 *Selected Results from Data Analysis*

The 1997–2001 data from the TNMN have been used to compile overviews of the in-stream transports ("loads") of nitrogen and phosphorus in the Danube and the main tributaries. Figure 6 shows the results for dissolved inorganic nitrogen (DIN, sum of ammonium, nitrites and nitrates). We note that in 1997–2001 the stations within Serbia were not yet participating in the TNMN. Therefore, the load from the Sava had to be calculated from the change in the in-stream load along the relevant Danube section, assuming that the net retention in the Iron Gates area (yellow section in Fig. 6) is negligible. We note that there were insufficient data for organic nitrogen to compile a similar picture for total nitrogen. For total phosphorus (Fig. 7), the available field data provided ambiguous results. Firstly, the results from pairs of stations on both sides of the river at the same longitudinal position

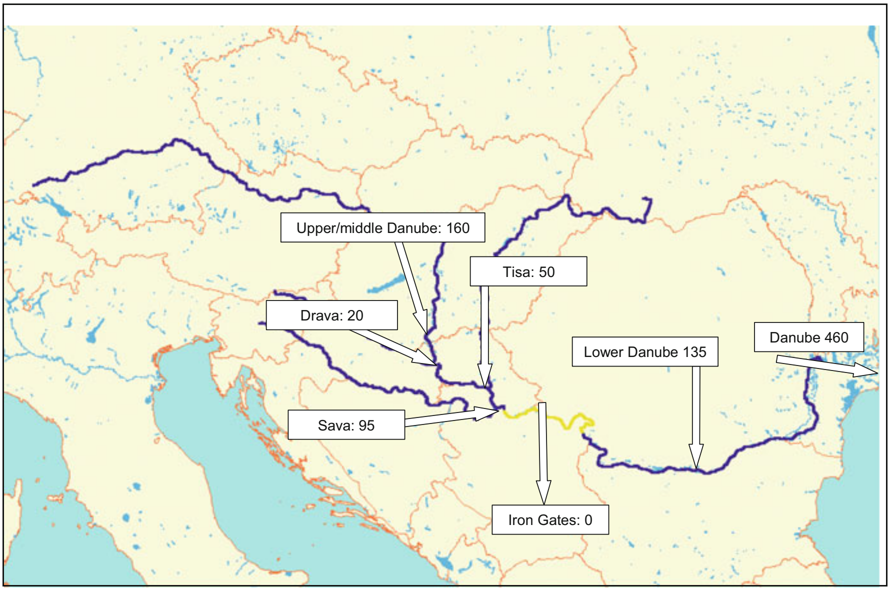


Fig. 6 DIN in-stream loads (kt/year) of the Danube River (based on data from 1997 to 2001)

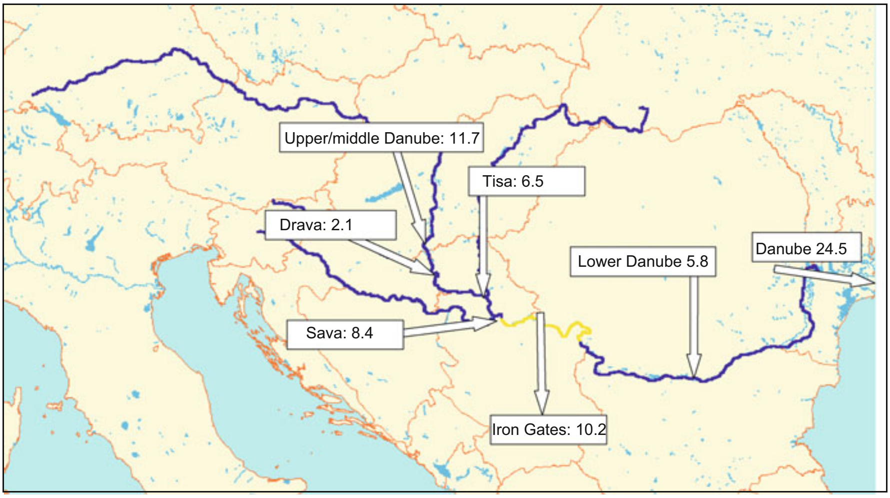
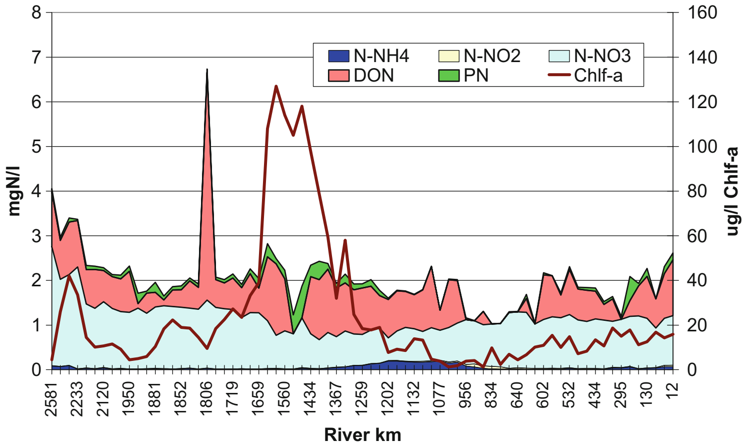


Fig. 7 Estimated total phosphorus in-stream loads (kt/year) of the Danube River (based on data from 1997 to 2001)

were inconsistent, both in the middle and lower river sections. Furthermore, the loads at stations upstream of the Iron Gates section were significantly lower than expected based on our understanding of the Danube River system. Finally, in 2000–2001 a strong decrease of the calculated river load downstream of Pristol was observed, which was not there in the preceding years 1996–1999. Therefore, the phosphorus load information had to be interpreted on the basis of expert judgement. Again, the load from the Sava had to be calculated from the change in the in-stream load along the relevant Danube section, while the retention of phosphorus in the Iron Gates area was estimated as 1/3 of the incoming load (between Smederevo and Kladovo [15]).

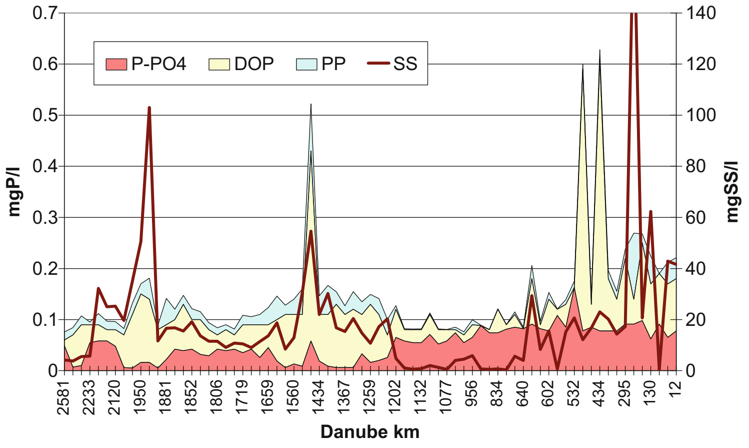
The data from the first Joint Danube Survey provided a valuable insight in the longitudinal concentration gradients of nitrogen and phosphorus and the speciation of these nutrients. Figures 8 and 9 show profiles along the Danube of the cumulative concentrations of N and P species, respectively. For interpretation purposes, Fig. 8 shows the concentration of chlorophyll a, which is an indicator for the concentration of phytoplankton. For N, nitrates are the dominant species. The total of nitrates, ammonium and nitrites represents a median fraction of 62%. Ammonium is only present in a noticeable amount downstream of the area of algae bloom (1,600–1,400 km), where it is formed as an intermediate product during the recycling of organic matter to nitrates. The median fraction of organic nitrogen is 38%. The share of particulate organic nitrogen is very small: the median fraction is 3%. This means that nitrogen is present almost completely in dissolved forms.

For interpretation purposes, Fig. 9 shows the concentration of suspended solid. For P, phosphates represent a relevant part of the total (median 37%), with other dissolved phosphorus (DOP) representing a similar fraction (median 46%).



N-NH4 = ammonium N-NO2 = nitrites N-NO3 = nitrates
DON = dissolved organic nitrogen PN = particular nitrogen Chlf-a = chlorophyll-a

Fig. 8 Profile of cumulative concentrations of N species along the Danube (Joint Danube Survey 1, August–September 2001)



P-PO4 = ortho-phosphates DOP = dissolved organic phosphates
PP = particular phosphates SS = suspended solids

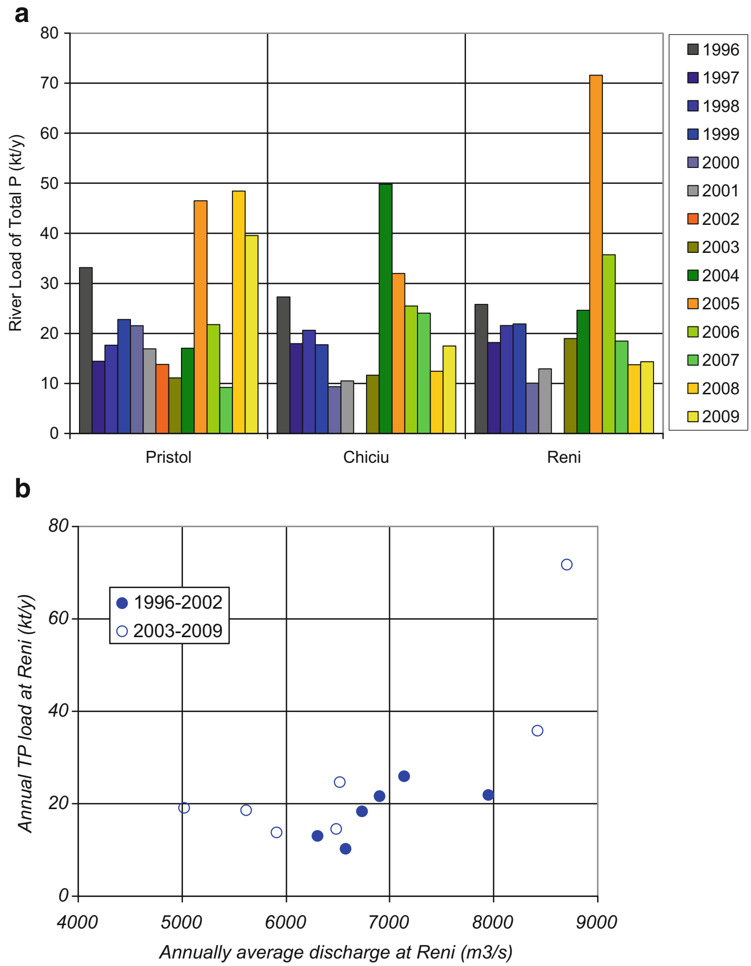
Fig. 9 Profile of cumulative concentrations of P species along the Danube (Joint Danube Survey 1, August–September 2001)

The share of particulate P is smaller (median 13%). It can be noted that the particulate fraction and SS almost disappear in the Iron Gates section downstream of 1,200 km.

We note that the JDS results are probably not representative for the whole year. The JDS represents a summer situation when algal activity is at its maximum and the concentrations of suspended solids are relatively low.

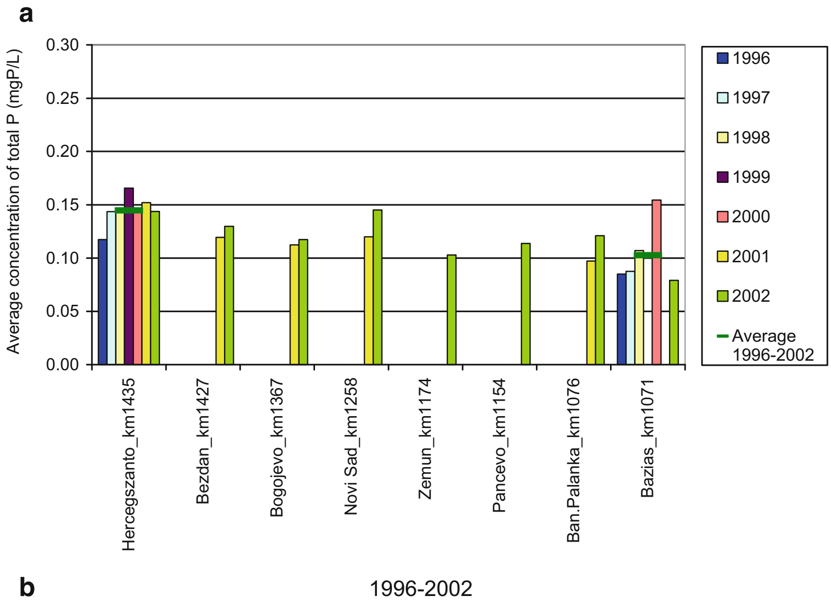
Since the finalisation of the *daNUbs* project, new data have become available to verify the expert judgements made at that time. In particular, the ongoing harmonised and basin-wide TNMN provides highly valuable additional data and information. Figure 10a shows the annual in-stream loads of phosphorus at three stations along the lower Danube (Pristol at 834 km, Chiciu at 375 km and Reni at 132 km), calculated from observed water quality data and discharge data. The loads for 1996–2001 have been copied from van Gils [9], while data for 2002–2009 have been derived from the TNMN Yearbooks [16]. Figure 10a shows the apparent strong decrease, both in space and in time, of the calculated river load downstream of Pristol in 2000–2001, as compared to the preceding years 1996–1999, which was observed during *daNUbs*. The new data for 2002–2009, however, illustrate that these spatial and temporal trends are not persistent, which was indeed the assumption made during *daNUbs*. Figure 10a shows an extremely strong interannual variability, which is partly correlated to the variable water flow, as illustrated in Fig. 10b.

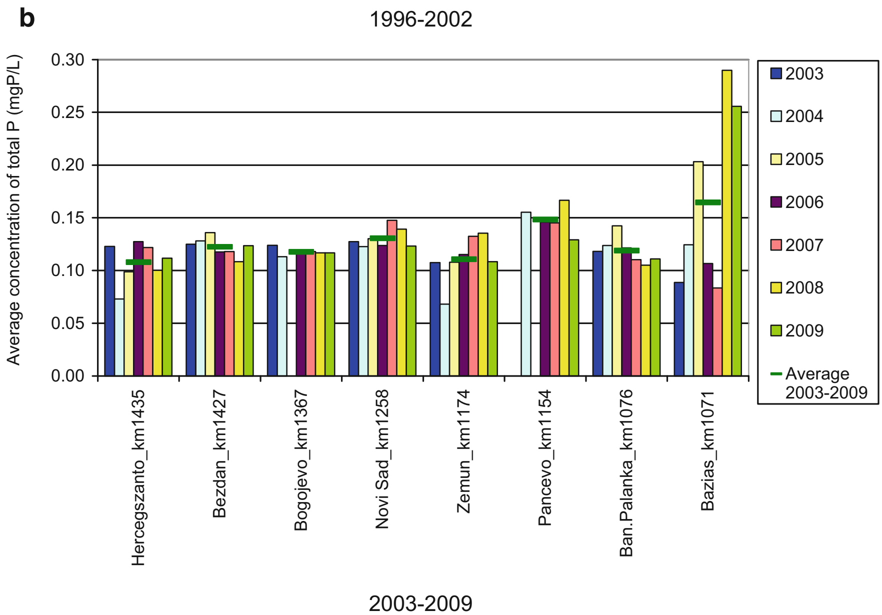
Another assumption made during *daNUbs* was that the observed concentrations at the station Bazias (Danube 1,071 km) were for some reason not representative for the Danube in-stream load of phosphorus upstream of the river section affected by the Iron Gates dams. In particular, to be representative these concentrations should have been higher. Figure 11 shows annually averaged observed concentrations of total phosphorus, calculated from TNMN data [16], along the river stretch between 1,435 and 1,071 km, for 1996–2002 (a) and for 2003–2009 (b). We note that the most upstream station Hercegszanto is situated in Hungary, that the most downstream station Bazias is situated in Romania and that the stations in between are all on the Serbian territory. We also note that the data for the Serbian stations as well as the data for 2003–2009 were not available at the time that the *daNUbs* project was carried out. The results illustrate the apparent decrease of the concentration in Bazias in 1996–2002 relative to the station Hercegszanto (Fig. 11a), which was assumed unrealistic in the *daNUbs* project. The results for 2003–2009 (Fig. 11b) suggest that this decrease is not there: the average value over 2003–2009 increases. The Serbian stations in between suggest that there is no consistent spatial gradient along the middle Danube. These observations confirm the assumptions made during *daNUbs*.



Loads for 1996-2001 have been copied from van Gils et al. (2004a). Data for 2002-2009 have been derived from the TNMN Yearbooks (e.g. ICPDR, 2009).

Fig. 10 Annual in-stream loads of phosphorus over the period 1996–2009, calculated on the basis of water quality monitoring data: (**a**) at three stations along the lower Danube (Pristol (834 km), Chiciu (375 km) and Reni (132 km)); (**b**) at Reni, plotted against the annually averaged water discharge





Station Hercegszanto is situated in Hungary; station Bazias is situated in Romania; the remaining stations are on Serbian territory.

Fig. 11 Annually averaged observed concentrations of total phosphorus at a sequence of stations along the middle Danube. (**a**) 1996–2002. (**b**) 2003–2009

3.2 Model Calibration and Validation

The DWQM needs to be able to represent the transport and retention of nutrients in the Danube river and its main tributaries with a sufficient accuracy. The developers validated this by comparing simulation results to field data. This validation was carried out on the basis of a list of concrete criteria, directly related to the ability of the DWQM to meet its objectives. Certain model formulations are of a (semi) empirical nature and contain parameters which may have to be tuned to the characteristics of the Danube River and its main tributaries. This process is called calibration. The number of parameters potentially subject to calibration is very large, and it is not possible to pay explicit attention to all of them [8]. The calibration effort was therefore concentrated on those parameters which affect the behaviour of the model the strongest, in view of the concrete criteria for validation mentioned above. Sensitivity analyses served as a supportive tool to find such parameters.

Among other things, the developers quantified the “goodness of fit” between the model results and the field data. This provides an objective and reproducible evaluation of the ability of the model to reproduce the field data. However, it was not possible to rely on this information only, for different reasons. In the first place, our ability to define a representative criterion for goodness of fit is limited, taking into account the complexity of the study area and the model formulations. Furthermore, the quality of the field data was sometimes limited. Therefore, qualitative and necessarily subjective judgements on the quality of the model have also been used, on the basis of simultaneous graphical presentation forms of field data and simulation results.

The first validation criterion reads: *the model should be able to adequately reproduce the (temporal and spatial variability of the) river hydraulics, insofar as it determines the water quality and the pollution loads to the Black Sea*. This criterion deals with the river discharge since it influences the diffuse emissions and the dilution of pollutants. The river velocity is important because it determines the residence time of the water in the river system. Together with the river depth and the bottom roughness, the velocity controls the shear stress, which determines the sedimentation and resuspension of particles. Finally, the river depth determines the relative importance of surface- and sediment-related processes, as well as the available light for phytoplankton growth. The model validation revealed that the dynamics of the river discharge is adequately reproduced, and the model generates a realistic behaviour with respect to the water depth and the water velocity.

The second validation criterion reads: *the model should be able to adequately reproduce the (temporal and spatial variability of the) concentrations of total nutrients*. This criterion deals primarily with the emissions and their disaggregation over time. Also the losses and storage of nutrients play a role. This aspect of the model validation could only partly be completed, due to data gaps: for nitrogen, we have to rely on data of dissolved inorganic nitrogen (DIN) only, while for phosphorus, the data show ambiguities (see Sect. 3.1 above). Assuming that our expert judgements to overcome these gaps are correct, the DWQM is reproducing the

Table 1 Simulated overall nutrient balances for the Danube Basin (multi-annual average, around the year 2000)

	N (kt/year)	N (%)	P (kt/year)	P (%)
Emissions to surface waters	687	100	67.8	100
Retention "small waters"	236	34	36.1	53
Inflow to DWQM	451	66	31.7	47
Retention in DWQM	16	2	7.6 (Iron Gates)	11
Outflow to delta	435	63	24.1	36

concentrations of total N and total P well. This conclusion was based among other things on thorough sensitivity analyses and subsequent parameter calibration, the evaluation of the formal goodness of fit and visual inspection of different types of graphical presentations comparing model results and measured concentrations.

Table 1 shows the overall nutrient balances derived from the validated model simulations. The table shows the total emissions to the surface waters and the retention of small surface waters not included in the DWQM, as calculated by MONERIS. The table also shows the remaining inflows to the Danube and its main tributaries, the retention along the larger rivers and the resulting outflow towards the Danube Delta. For nitrogen, about 63% of the emissions reach the Delta. The nitrogen retention is taking place almost exclusively in the small water courses not included in the DWQM. For phosphorus, about 36% of the emissions reach the Delta. In this case, there is significant retention along the Danube itself. The Iron Gates section of the Danube (yellow colour in Fig. 7) traps roughly 50% of the incoming inorganic particles (the model has been calibrated to reproduce the literature dedicated to this subject). Since phosphates sorb to these particles, also P is trapped in the Iron Gates section.

For P, another form of retention is taking place in floodplains. A clear example is the Gabcikovo section along the Slovak–Hungarian border. A *daNUbs* survey in the area during the major August 2002 flood demonstrated a substantial retention of suspended solids and phosphorus, due to the sedimentation of particles in the floodplains and old Danube branches (van Gils [9]). Because of the limited availability of detailed cross-sectional data, the model does not explicitly represent the floodplains along the Danube and the main tributaries and therefore cannot resolve this retention mechanism. We note that the frequency of the 1996–2001 TNMN water quality monitoring also does not resolve flood events responsible for this retention mechanism. Therefore, the retention may not show in the field data either.

This process is not only relevant along the main river but also in the smaller order tributaries. With every high-water period, sediment is deposited with P attached to it. This deposition process is probably partly counteracted by resuspension during the next flood and deposition further downstream. The literature provides evidence however that the river floodplains experience a net sedimentation.

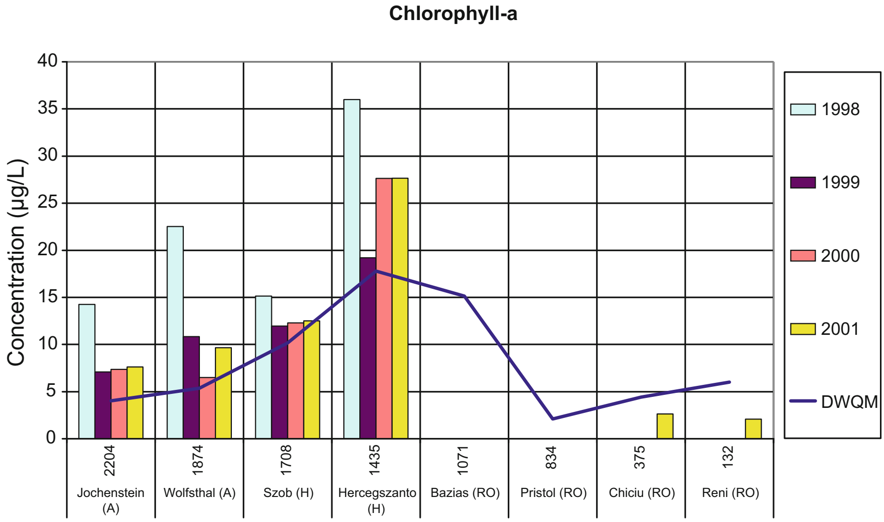


Fig. 12 Comparison of annually averaged simulated concentrations of chlorophyll a (µg/L) with annual averages of observed concentrations from the TNMN at selected stations

The third validation criterion reads: *the model should be able to adequately reproduce the (temporal and spatial variability of the) concentrations of the different nutrient species*. This criterion deals with the cycling of the nutrients induced by phytoplankton growth, the mineralisation of organic matter and the different processes related to inorganic nutrients. Since the phytoplankton dynamics of a river system are controlled by the water clarity which determines the light availability in the water column, also the suspended sediment dynamics and the particle light extinction characteristics are relevant. The model was calibrated to reproduce the available field data for chlorophyll a (see Figs. 12 and 13a).

Figure 13b, c shows that the model is able to reproduce the observed concentrations of *ortho*phosphates and dissolved inorganic nitrogen during JDS1 quite well. The DWQM does not reproduce the TNMN data for ammonium very well. These data show a sudden increase of the concentration of ammonium from Bazias (Danube 1,071 rkm) onwards, while the model shows only minor variations in the downstream direction. Note that the JDS results do not show such a gradient (Fig. 8). If the spatial gradient in the field data is realistic, we do not know what causes it and therefore cannot make the model reproduce it.

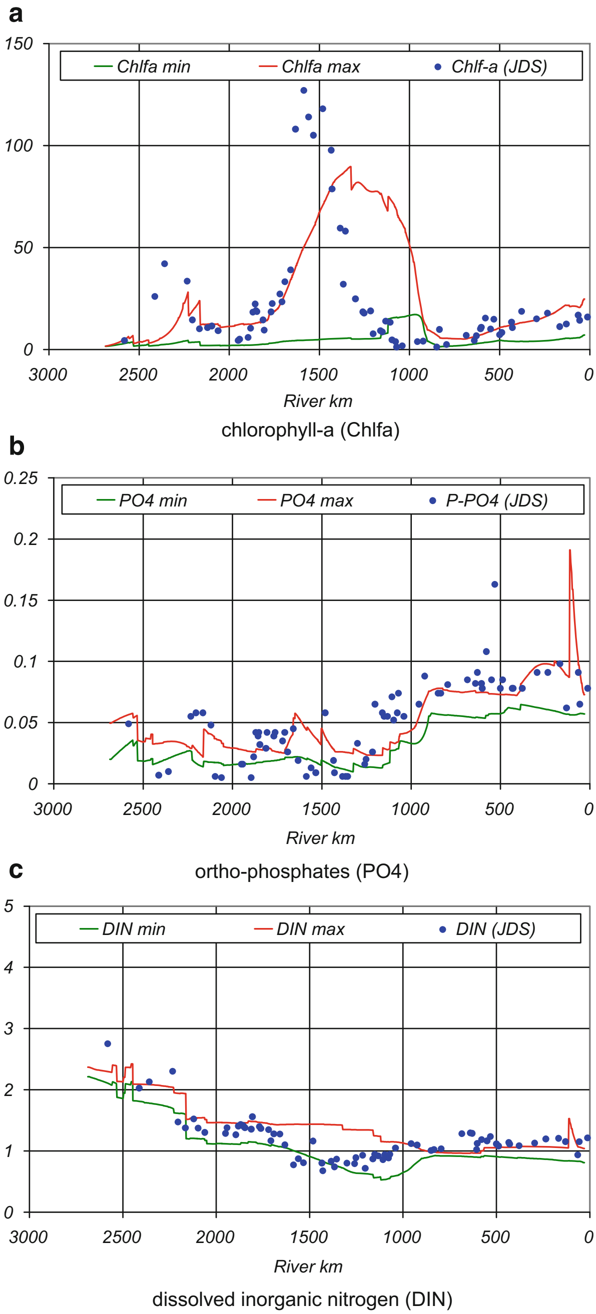


Fig. 13 Observed concentrations along the Danube during JDS1 (*blue dots*) and simulated concentrations during the survey period (*green*, minimum value; *red*, maximum value) for various parameters: chlorophyll a as μg/L (**a**), *ortho*phosphates as mgP/L (**b**) and dissolved inorganic nitrogen as mgN/L (**c**)

4 Prognosis of Future Situation

During the *daNUbs* project, the MONERIS and DWQM models have been used to make a prognosis of the expected water quality in the Danube outflow (for further assessment by Black Sea researchers). This has been done for five different scenarios varying from a "business as usual" to a "deep green" scenario. A detailed description of this exercise is provided by van Gils et al. [17].

Figure 14 provides the calculated Danube River loads towards the Danube Delta, for the present situation (year 2000, Sc0) and the scenarios Sc1 to Sc5. The results show that the loads may increase or decrease as compared to the present situations, dependent on the assumed socio-economic development of the Danube countries in each of the scenarios. It is also clear that the phosphorus loads show a stronger response to socio-economic changes than the nitrogen loads. The error bars in Fig. 14 show the variability of the loads, induced by differences in the river hydrology. This variability is significant. For phosphorus, the variability induced by socio-economic factors dominates the hydrological variability, but for nitrogen both are of the same order. This means that the effect of pollution reduction measures on the Danube nitrogen loads can be hidden by natural hydrology-induced variability.

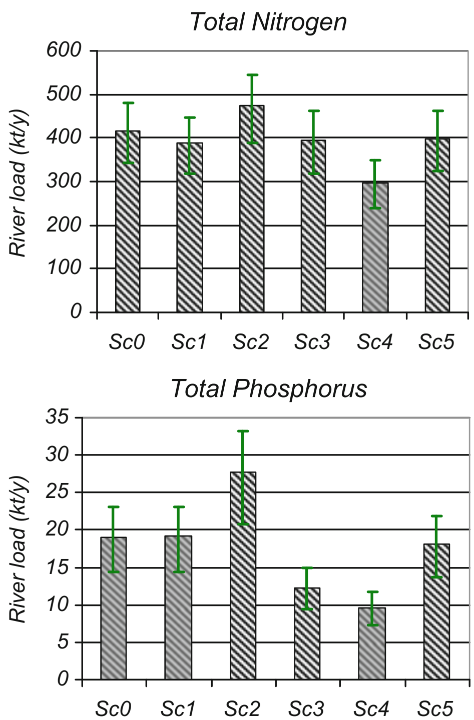


Fig. 14 Calculated Danube River loads towards the Danube Delta, for the present situation (Sc0) and the scenarios Sc1 to Sc5. The *error bars* show the variability of the loads, induced by differences in the river hydrology

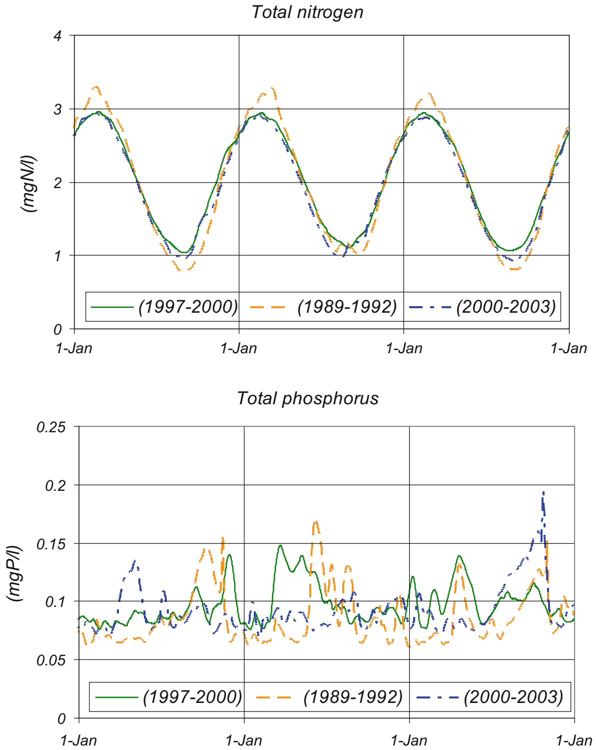


Fig. 15 Disaggregation over time of the concentration of total N and total P for scenario 5, for a period with low river discharges (1989–1992), a period with intermediate discharges (2000–2003) and a period with high river discharges (1997–2000)

Figure 15 shows simulated time series at the Danube outflow point for a period of 3 years assuming scenario 5 (a "policy" scenario representing the implementation of the Water Framework Directive and other EU water-related legislation throughout the Danube Basin). Again, the impact of the river hydrology is shown, by using three different historical periods as hydrological forcing for the model simulations.

5 Closing Remarks

The work that formed the basis for the present chapter has been carried out in the period 2002–2006. Meanwhile, new data have become available, and scientists and water managers have continued their efforts to improve the quality of the available data. Thus, a renewed effort to evaluate and if possible improve the work presented here done would be possible. Based on the *daNUbs* experience, the modelling could be further improved with respect to (a) the production, transport and retention of sediment; (b) the explicit modelling of floodplains as a sink of sediment and phosphorus (on all spatial scales); and (c) the consistent treatment of the temporal and spatial scales (which implies integrating the MONERIS and DWQM models and their underlying concepts).

Acknowledgements The author acknowledges that the results presented here come from the project "Nutrient Management in the Danube Basin and its Impact on the Black Sea" (*daNUbs*) supported under contract EVK1-CT-2000-00051 by the Energy, Environment and Sustainable Development (EESD) Programme of the 5th EU Framework Programme. The author furthermore acknowledges the cooperation with the *daNUbs* researchers during the project, especially with Dr. Helmut Kroiss, Dr. Matthias Zessner, the late Dr. Horst Behrendt and Dr. Adrian Constantinescu, as well as the valuable support of the International Commission for the Protection of the Danube River and its expert groups, especially Dr. Igor Liska, Dr. Mihaela Popovici and Dr. Liviu Popescu.

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Pollution by Heavy Metals in the Danube River Basin

Ferenc László

Abstract Heavy metals were identified as relevant pollutants of the Danube River some decades ago. This chapter reviews and evaluates the concentrations of heavy metals measured in the Danube and its tributaries by the monitoring activities of the International Commission for the Protection of the Danube River (ICPDR) – the TransNational Monitoring Network and Joint Danube Surveys 1 and 2.

Keywords Cadmium, Danube, Heavy metals, Lead, Mercury, Nickel

Contents

1 Introduction

The term "heavy metals" is used in broad sense in this chapter. The nonmetallic trace element arsenic and the non-heavy metal aluminium are included in the expression.

Heavy metals can be present in industrial, municipal and urban runoff, causing adverse effects in the aquatic ecosystem when the concentration in the water as well as in the sediment exceeds the tolerance limits. Furthermore, heavy metals in water

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can limit drinking water supplies and affect aquatic organisms, livestock and wildlife, and after bioaccumulation it may enter to the food chain causing environmental and public health risks.

The water solubility of most of the elements concerned 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. Depending on turbulence, flow velocity in the surface waters, the sedimentation and resuspension is in a dynamic equilibrium. Changing redox conditions, particularly in the case of reductive (anaerobic) media, mobilization and increasing bioavailability, may increase the adverse effects [1].

2 ICPDR Transnational Monitoring Network

Heavy metals were identified as relevant pollutants in the Danube already in the 1980s when several heavy metals were included in the "Bucharest Declaration" water quality monitoring of the Danube. This monitoring later turned into the operation of the ICPDR TransNational Monitoring Network which provides the data on heavy metals in the Danube and its main tributaries on a regular basis.

Table 1 shows the statistical values of dissolved heavy metal concentrations in the Danube and tributaries for the period 1996–2009 based on ICPDR TNMN database [2].

The ranges of the individual measured values and also the ranges of the annual average values are rather wide both in the river Danube and in its tributaries.

The maximum of the annual average values of the priority heavy metals cadmium and mercury in the Danube and tributaries exceeded the Annual Average Environmental Quality Standards (AA-EQS) set in the Directive 2008/105/EC [3] as well as the EQS for cadmium in the Directive 2013/39/EC [4]. The maximum individual measured values of these elements exceeded the Maximum Allowable Concentration Environmental Quality Standards (MAC-EQS) which are the same in both Directives.

Regarding the other priority heavy metals lead and nickel, violation of AA-EQS from the Directive 2008/105/EC did not occur in the Danube and tributaries in 1996–2009, while the stronger AA-EQS for these metals in the Directive 2013/39/EC were exceeded by the maximum average concentrations of lead and nickel in the Danube and its tributaries. In this case however the Directive 2013/39/EC refers to bioavailable concentration of the substances which was not assessed. Similarly the newly introduced MAC-EQS for lead and nickel in the Directive 2013/39/EC were exceeded by the maximum individual measured values in the Danube and its tributaries.

Loads of selected heavy metals in the Danube Delta are being regularly monitored by the ICPDR to assess the effect on the Black Sea (Table 2). The annual dissolved cadmium load decreased in the period 2008–2011 and the annual dissolved lead, mercury and copper loads increased in the period 2008–2010 and

Table 1 Statistical values of dissolved heavy metal concentrations in the Danube and tributaries in the years 1996–2009

	Number of monitoring sites	Range of values		Average		AA-EQS		MAC-EQS	
		Min	Max	Min_{avg}	Max_{avg}	2008/105/EC	2013/39/EC	2008/105/EC	2013/39/EC
Danube									
Cd (μg/l)	34	<0.01	20	0.019	1.5	0.08–0.25	0.08–0.25	0.45–1.5	0.45–1.5
Pb (μg/l)	34	0.02	41	0.036	2.0	7.2	1.2	–	14
Hg (μg/l)	34	<0.01	19	<0.01	0.13	0.05	–	0.07	0.07
Ni (μg/l)	34	0.05	64	0.15	7.2	20	4	–	34
As (μg/l)	34	0.09	15.3	0.19	2.1				
Al (μg/l)	17	<1.0	1,100	8.8	70				
Cr (μg/l)	34	0.04	197	0.05	2.1				
Cu (μg/l)	34	0.05	303	0.64	20.1				
Zn (μg/l)	34	0.32	1,190	0.44	40.0				
Tributaries									
Cd (μg/l)	43	0.004	10	<0.01	1.0	0.08–0.25	0.08–0.25	0.45–1.5	0.45–1.5
Pb (μg/l)	43	0.01	34.1	0.014	6.0	7.2	1.2	–	14
Hg (μg/l)	36	<0.01	3.1	<0.01	0.37	0.05	–	0.07	0.07
Ni (μg/l)	43	0.05	87	0.53	5.6	20	4	–	34
As (μg/l)	42	0.02	42	0.2	4.1				
Al (μg/l)	17	0.8	1,660	10.7	88.2				
Cr (μg/l)	50	<0.07	48	0.098	5.6				
Cu (μg/l)	53	<0.01	185	0.11	22.7				
Zn (μg/l)	55	0.01	886	0.22	144				

Min_{avg} Minimum of the annual average values, Max_{avg} Maximum of the annual average values, *AA-EQS* Annual Average Environmental Quality Standard, *MAC-EQS* Maximum Allowable Concentration Environmental Quality Standard

Table 2 Annual data on loads in the Danube at Reni (132 km) in the period 2008–2011

Year	Discharge (m^3/s)	Load (t/year)			
		Dissolved cadmium	Dissolved lead	Dissolved mercury	Dissolved copper
2008	5,909	26.3	186	7.7	425
2009	6,492	21.3	269	10.8	430
2010	9,598	16.5	501	11.5	779
2011	5,303	14.8	330	1.5	464

decreased in 2011 (ICPDR 2010, 2011, 2012). The load values are however influenced by the annual discharges, and moreover, for any thorough statistical assessment of the Danube loads, a larger dataset would be needed.

3 Research Surveys

Several survey type research investigations contributed to information on heavy metals in the Danube water, sediment and biota, e.g. the field study of Equipe Cousteau [5], the Environmental Programme for the Danube River Basin [6], the survey trip of the MS "Burgund" [7], the Danube Regional Project [8] and the Joint Danube Survey 1 [9] and Joint Danube Survey 2 [1].

A study of the Equipe Cousteau Danube Programme [5] focussed on chemical analysis of sediment samples from the Danube River. The sampling sites were selected from monitoring stations of the "Bucharest Declaration", hot spot areas and confluences of main tributaries of the Danube. The levels of mercury in the lower reaches of the river were generally two or threefold higher than those of the region above river km 2,000. Mercury concentrations above 0.8 mg/kg occurred in the Lower Danube reaches. Similarly, in the lower reach of the river, the concentrations of cadmium were significantly higher than those in the upper reaches.

A Slovakian-Hungarian bottom sediment survey programme was carried out in the frame of the "Quality of sediment and biomonitoring" project in the Environmental Programme for the Danube River Basin (1998). Two sampling campaigns were performed along the Danube and its tributaries between Greifenstein (1,949 river km) and Budapest (1,632 river km) in the years 1995 and 1996.

The measured heavy metal concentrations of the bottom sediment were compared with Dutch and Canadian guideline values (Table 3).

Mercury showed uniform distribution in the Danube River. The concentration values were around the Dutch target value and the Canadian lowest effect limit.

Cadmium showed a distribution pattern very similar to mercury. Several samples from both the Danube and tributaries contained cadmium above the Canadian lowest effect limit, but the Dutch limit value was exceeded at a few sites in the tributaries only.

Table 3 Limit values for evaluation of heavy metals in sediment (Environmental Programme for the Danube River Basin 1998)

Heavy metal	Value in mg/kg dry weight sediment			
	Dutch guidelines		Canadian guidelines	
	Target value	Limit value	Lowest effect	Severe effect
Hg	0.3	0.5	0.2	2
Cd	0.8	2	0.6	10
Pb	85	530	31	250
Cu	36	36	16	110
Cr	100	380	26	110
Zn	140	480	120	820
As	29	55	6	33
Ni	35	–	16	75

Lead concentrations were below the Dutch target value in all the Danube sediment samples.

Copper showed higher values than the Dutch limit values in most of the samples from both the Danube and the tributaries.

Zinc concentrations were found between the Dutch target and limit values in all the Danube and most of the tributary samples.

Nickel concentration showed the highest variation in both the Danube and the tributary samples. About 25% of the Danube and tributary sediment samples exceeded slightly the Dutch target value, and about 75% of the samples exceeded the Canadian lowest effect limit.

Heavy metal concentrations were measured in suspended sediments along the Main, Main-Danube canal and Upper Danube section down to 1,433 Danube River km (Hungarian-Croatian border) during the survey trip of the MS "Burgund" [7].

The measured concentrations were compared with the following LAWA target values: $Pb < 100$ mg/kg, $Cr < 100$ mg/kg, $Cu < 60$ mg/kg, $Zn < 200$ mg/kg, $Cd < 1.2$ mg/kg, $Hg < 0.8$ mg/kg and $Ni < 50$ mg/kg. Along the studied Danube section, the lead, chromium, cadmium and mercury concentrations were below the target values in the suspended sediment samples, while copper, zinc and nickel concentrations exceeded the target values in some samples.

Heavy metals in sediments from the Iron Gate Reservoir were assessed using historical data and sediment survey results in the frame of the Danube Regional Project [8]. The project findings were the following: (i) compliance checking with different guideline values indicated the anthropogenic pollution of sediment in the Iron Gate region in the surface layer of sediment and in core samples as well, (ii) the sediment survey indicated that the longitudinal concentration distributions of contaminants did not show a typical pattern along the Danube section in the Iron Gate Reservoir, (iii) the vertical profiles of core samples revealed sediment pollution in the complete profile of the 50–80 cm thick core samples.

Table 4 Concentration ranges of dissolved heavy metals in water in JDS 1 and 2

Determinand (μg/l)	Danube		Tributaries	
	JDS 1	JDS 2	JDS 1	JDS 2
Cadmium	<0.2–0.5	<0.2	<0.2	<0.2
Lead	<1–1.38	<2.0	<1–1.2	<2.0–5.07
Mercury	<0.2	<0.05–0.071	<0.2	<0.05
Nickel	<1–3	<2.0–12.2	<1–6	<2.0–33.3
Arsenic	<1–4.55	<0.8–4.31	1.05–44.8	<0.8–13.2
Chromium	<1–1	<0.5–1.26	<1–1	<0.5–1.73
Copper	2–6	<2.0–4.59	2–16	<2.0–34.5
Zinc	<1–291	<5.0–16.1	3.27–66.3	<5.0–67.9

Table 5 Concentration ranges of heavy metals in suspended sediment in JDS 1 and 2

Determinand (mg/kg)	Danube		Tributaries	
	JDS 1	JDS 2	JDS 1	JDS 2
Cadmium	<1.1–7.6	0.294–2.23	<1.1–25.6	0.394–4.85
Lead	18.2–85.0	25.3–64.6	17.3–215	18.5–79.1
Mercury	<0.10–0.55	0.102–0.388	<0.10–0.79	0.060–1.21
Nickel	23.2–89.8	30.9–85.0	32.6–171	41.4–161
Arsenic	9.4–32.1	8.62–19.0	10.4–29	8.83–23.4
Chromium	32.9–107	40.8–94.3	55.0–149	38.0–127
Copper	28.3–194	37.7–111	26.9–95.5	34.4–230
Zinc	99–398	117–335	87–2,220	111–553
Aluminium	17,900–52,800	19,000–57,000	15,300–54,100	31,200–49,800
Iron	14,300–38,300	7,180–35,400	21,300–37,200	9,700–34,300
Manganese	565–4,028	770–3,150	963–3,340	1,060–4,120

The Joint Danube Surveys had wide spatial coverage in the Danube River Basin and were carried out in 2001 and 2007. Tables 4, 5 and 6 summarize the concentration ranges measured in water, suspended sediment and mussel samples during JDS 1 and 2.

The ranges of concentration of dissolved heavy metals in water detected during JDS1 and JDS2 are relatively low when compared to a wide concentration interval observed by the TNMN monitoring between 1996 and 2009. This can be explained by a single shot character of JDS data and also by the fact that JDS data on heavy metals in a particular matrix are homogeneous because they were produced by a single laboratory while TNMN data have been produced by a large number of national laboratories.

Reviewing the results from JDS 1 and 2, exceeding of MAC-EQS was not observed with the exception of one JDS2 Danube sample in which the MAC-EQS for mercury was exceeded. The situation is different when AA-EQS from the Directive 2013/39/EC are applied, as in this case higher concentrations were observed at some sites for nickel and lead. In this case however the Directive

Table 6 Concentration ranges of heavy metals in mussel samples in JDS 1 and highest concentrations during JDS 2

Determinand	Concentration ranges during JDS 1 (mg/kg dry weight)		Highest concentrations during JDS 2 (mg/kg dry weight)	Quality targets during JDS 1 (mg/kg dry weight)
	Danube	Tributaries		
Cadmium	0.1–35.9	0.2–16.4	29.6	4
Lead	0.5–49.9	0.7–31.7	9.8	10
Mercury	0.055–0.41	0.037–0.74	0.3	0.4
Nickel	0.44–4.69	0.49–9.43	5.16	10
Arsenic	0.08–1.23	0.06–0.81	2.7	20
Chromium	0.5–11.7	<MQL-24.1	4.9	6
Copper	4.5–178	4.3–54.0	37.5	20
Zinc	120–2,680	160–1,360	1,880	400

2013/39/EC refers to bioavailable concentration of the substances which was not assessed. A thorough assessment of cadmium and mercury was affected by relatively high limit of quantification when compared to AA-EQS.

Comparing the concentration of heavy metals in suspended sediment between JDS 1 and JDS 2, the spatial distribution of Cu, Zn, Cd and Ni was very similar to the distribution of Al and Fe during JDS 1 and JDS 2. This comparable trend was interpreted in both surveys as a reflection of geochemical background [10].

Mussels have been used for monitoring of heavy metals during JDS1 and JDS2. 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 one considers 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.

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 ranges of concentrations of the different elements during JDS1 are shown in Table 6 and compared with the maximum measured values during JDS2.

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 for As. Particular attention however has to be paid to the concentration of mercury because of the strict EQS for mercury in fish according to the Directive 2013/39/EC (20 μg/kg wet weight). Even though a direct comparison between concentrations in fish and mussel tissue is not possible, the concentration levels observed in mussels during Joint Danube Surveys and the relation between wet and dry weight mentioned above indicate that pollution of biota by mercury can be an issue for the Danube.

The first Danube River Basin Management Plan published in 2009 included the assessment of the chemical status which provided the first ever comprehensive overview of contamination of surface waters in the Danube River Basin by WFD priority substances. From this assessment, which is presented in detail in the first chapter of this book, it is apparent that mercury, cadmium, lead and nickel are on the top of the list of the priority substances causing bad chemical status in the Danube River Basin.

4 Conclusions

As shown by the results of the international monitoring activities, heavy metals are relevant pollutants of the Danube River and its tributaries. Heavy metal pollution has been monitored in different compartments/matrices of the aquatic environment of the Danube River Basin. The available monitoring data and survey results show wide concentration ranges both in the Danube River and its tributaries. The review of the results of the monitoring activities organized by the ICPDR revealed that the four metals listed in the Directive 2013/39/EC, mercury, lead, nickel and cadmium, are to be of concern with the view of achieving the good chemical status of water bodies in the Danube River Basin. For nickel and lead, the newly introduced strict AA-EQS are changing the overall picture as much more noncompliance is observed when evaluating the past results with the new EQS. To obtain a complete picture, the question of bioavailability has still to be considered. The key issue for mercury is the very low EQS in fish for which the sufficient results are not available, but the data for mussels indicate a potential problem. The cadmium classification is depending on water hardness classes.

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Identification of the Danube River Basin Specific Pollutants and Their Retrospective Risk Assessment

Jaroslav Slobodnik and Peter Carsten von der Ohe

Abstract Following the requirements of the European Water Framework Directive (WFD), a process of selecting pollutants relevant at the river basin scale started in 2001. In the Danube river basin, the process was aided by two Joint Danube Surveys (JDS1 and JDS2) organised by the International Commission for the Protection of the Danube River (ICPDR) in 2001 and 2007, respectively. This study was retrospectively analysing all data on organic substances identified in the water samples collected within the two surveys and comparing them to the latest Environmental Quality Standards (EQSs) as well as ecotoxicological threshold values (Predicted No Effect Concentrations; PNECs) that were not available at the time of writing the JDS1/2 Final Scientific Reports. The results showed that 26 out of 89 substances detected in the samples exceeded the EQS/PNEC values in at least one sampling site and 53 substances were found above their limit of quantification (LOQ) at more than five sampling sites within the basin. The above-mentioned 26 substances deserve closer attention as candidates for the list of Danube River Basin Specific Pollutants (DRBSPs).

A novel approach of ranking gas chromatography–mass spectrometry (GC–MS) nontarget screening data, based on the assessment of (1) available literature PNEC values (19 substances), (2) derived provisional PNEC (P-PNEC) values (160 substances) and (3) estimated concentrations of tentatively identified substances, has been applied too. Sixty-five out of a total of 179 compounds identified in the JDS samples exceeded the ecotoxicological threshold value in at least one sampling site, which makes them potential candidates for inclusion into future investigative monitoring schemes.

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Contents

1 Introduction

Article 16 of the WFD sets out the strategy to reduce the chemical pollution of European waters [1]. Thereby, the chemical status assessment is used alongside the ecological status assessment to determine the overall quality of a water body. The EQS Directive [2] and its recent update [3] establish EQSs, expressed as both annual average (AA) concentrations and maximum allowable concentrations (MACs) for 45 priority substances. Compliance with AA-EQSs and MAC-EQSs sets the chemical status of the water body as "good". However, under the WFD, Member States must also set quality standards (according to Annex V, 1.2.6) for "river basin specific pollutants" (listed in Annex VIII, 1–9) that are "discharged in significant quantities" and take action to meet those quality standards by 2015 as part of the ecological status (Article 4, 11, and Annex V, 1.3, WFD) [1]. EQSs are therefore key tools in assessing and classifying both chemical and ecological status. Whether a compound is "discharged in significant quantities" is commonly decided based on the substance's exposure level, referred to as Predicted Environmental Concentration (PEC). This in turn is compared to ecological safety threshold expressed as PNEC. PEC/PNEC risk ratios above 1 would trigger the substance's inclusion in the routine monitoring and the derivation of a legally binding EQS.

Despite the majority of the Danube countries have already defined their national RBSPs and related EQSs, there is no recent update of the Danube river basin-wide list of specific pollutants. The currently valid list includes only arsenic, chromium, copper and zinc without specifying their EQSs. A prioritisation methodology to select RBSPs in a wider European context, including the data from the Danube river basin, was introduced by von der Ohe et al. [4]. It was based on the methodology developed by the prioritisation working group of the NORMAN network [5] and has more recently been applied for the prioritisation of the monitoring data from the Slovak Republic [6]. All of the prioritisation efforts run so far either at the EU, river basin or national level concluded that there is a need for more occurrence and ecotoxicity data of high quality. This has been understood also at the design of the Danube surveys.

The aim of this study was to prioritise among the large number of substances detected in the surface water samples during the JDS1 and JDS2 in order to identify substances of basin-wide relevance. At the time of writing this paper, there was no discussion on prioritisation criteria acceptable by all ICPDR countries (e.g. minimum no. of countries/sites in which substance is present/exceeding ecotoxicological threshold value, commonly agreed PNECs, additional hazard criteria to be taken into account, etc.). Such concept is still under development at the research level, e.g. in the recently funded EU Framework Programme 7 project SOLUTIONS (www.solutions-project.eu). The discussion was focused therefore on simply highlighting substances which can have adverse effects on water fauna and flora by exceeding their respective "best available" PNECs and EQSs. Here, one should be aware that the EQS Directive came into force only a year after the end of the JDS2 (in 2008) and it has been significantly updated in 2013 [2, 3]. Also, when evaluating results of the JDS2, the concept of identifying RBSPs and setting up their EQSs at the river basin/national scale was largely new to the Member States. This paper presents the first attempt to revisit the results of the JDS1 and JDS2 and apply the latest ecotoxicological know-how to assess the risk by chemical pollutants identified in the Danube river basin before the JDS3 [7, 8].

2 Methods

The JDS1 sampling programme was carried out on 74 sampling locations on the main river and 24 locations on the main tributaries. During the JDS2, 96 sites were sampled by the JDS2 Core Team along a 2,600 km stretch of the Danube, 24 of which were located in the mouths of tributaries or side arms. Additional 28 sites were sampled by National Teams during longitudinal surveys on selected Danube tributaries.

3 Prioritisation Based on Occurrence and (Predicted) Toxicity Data

A prioritisation process was carried out using target analysis data from the surveys. With a few exceptions, each substance was determined in a single laboratory to allow for pollution trend analysis without taking into account differences induced by varying analytical methodologies. Provisional threshold values (P-PNECs) based on a read-across modelling approach were used to estimate the toxicity of compounds for which no literature data existed [9, 10]. A stepwise procedure to derive respective P-PNEC thresholds was described by von der Ohe et al. [4]. The prioritisation consisted of simple comparison of the measured maximum concentration value per pollutant to the respective EQS/PNEC/P-PNEC value. P-PNECs were used only in the absence of EQS or literature-based PNEC. Since only a single dataset (one value per compound/site/time) was evaluated, it was considered not to use neither the full-scale NORMAN prioritisation approach [5] nor the more simplified risk-assessment approach using only two indicators (the frequency of exceedance and the extent of exceedance of the lowest PNEC) that was previously applied to 500 target compounds in four river basins (including Danube) across Europe [4].

4 Prioritisation of GC–MS Data

The main objective of the gas chromatography–mass spectrometry (GC–MS) screening of the JDS samples was to identify and trace pollution trends of "unknown" substances, which are not included (1) in the routine monitoring schemes of the Danube countries and (2) among the JDS target parameters. Such pollutants are often termed as "emerging substances" and may be candidates for future regulation, depending on the research of their (eco)toxicity, their 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 the electron impact (EI) mode are widely accepted as unique fingerprints of individual organic compounds and can be compared against existing databases. 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 these unknown substances, e.g. based on the overall ecotoxicity of the sample, the frequency of occurrence of these substances and their concentrations or evidence of biological impact in the vicinity of the sampling site(s).

The JDS1 water samples were prepared using liquid–liquid extraction with dichloromethane, whereas in the JDS2, a more advanced technique (i.e. the Stir Bar Sorptive Extraction (SBSE) [11]) was applied. Both techniques concentrate hydrophobic compounds from water to an extraction solvent, and the extraction efficiency depends on the compounds partition coefficients. 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 suspended particulate matter (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. For more details on analytical procedures, see [8]. Mass spectra obtained in the EI ionisation scan mode were used for the identification of unknowns. A compound has been considered as tentatively identified only in cases when both conditions below were fulfilled:

- Match of the mass spectrum of the observed substance with that in a commercially available mass spectral library (NIST, Wiley) is $>70\%$.
- Proposed structure is confirmed by manual interpretation.

Nevertheless, one should be aware that the risk of false identification can be avoided only by analysis of a chemical standard, which is in some cases not available and must be synthesised first. The mass spectra and raw mass chromatograms obtained with all relevant metadata were therefore stored in the ICPDR's Water Quality Database, so that they were made available for any future independent confirmation/re-evaluation, whereas mass spectra were transferred later on to the online NORMAN MassBank [12].

Concentration values of the detected compounds were estimated based on comparison of their signal to the signal generated by the known concentration of an internal standard [13, 14]. In the procedure, a signal of the quantification ion of the internal standard (e.g. m/z 214 for propazine) was compared with the signal of its overall mass spectrum, which resulted in estimation of its relative intensity (ca. 12% of the total response for propazine, RSD 0.93%, $n = 6$). The same procedure was applied to the unknown compound (selection of the most abundant ion, determination of its intensity relative to the overall intensity of the whole mass spectrum). The ratio between signals of quantification ions of the unknown substance to the known internal standard was then corrected for their percentage representativeness of the whole spectrum. It should be made clear that the method provides only rough estimations of actual concentrations. However, additional comparisons obtained with numerous standard compounds showed that the error is usually contained within one order of magnitude, which is well within the range of uncertainty associated with the respective ecotoxicological effect thresholds. In the next step, P-PNEC values were derived for the 179 identified substances using available ecotoxicity data and predictions [9, 10].

5 Results

5.1 Target Analyses

The results of the prioritisation of target substances are presented in Table 1. Altogether, 25 substances exceeded lowest PNEC value in at least one of the investigated sites in the JDS1 and JDS2. The list is dominated by the two WFD priority substances PFOS and tributyltin cation (TBT), whose maximum concentrations were exceeding the EQS values 155 and 60 times, respectively. PFOS was detected at 85 sites, compared to TBT that was found only at eight sites. This indicates that PFOS might be of more basin-wide significance. Nevertheless, the extremely low EQS of 0.0002 μg/L for TBT is hardly reachable by any analytical methodology in routine water laboratories, and hence, the results obtained at the sub-ng/L level in the JDS2 could be considered unique in the European context. Other WFD priority substances like fluoranthene, nonylphenols, DEHP and atrazine were found in more than 30 sites. With exception of atrazine, some of their concentrations exceeded the EQS values, and therefore, their presence in the river basin scale should be carefully monitored. The EQS values of polyaromatic hydrocarbons benzo(g,h,i)-perylene (0.002 μg/L), indeno(1,2,3-cd)-pyrene (0.002 μg/L) and benzo(b)fluoranthene (0.03 μg/L) as set in the older version of the EQS Directive [2] were exceeded; however, they were found less frequently, and according to the latest upgrade of the EQS Directive [3], "their corresponding AA-EQS in water refer to the concentration of benzo(a)pyrene, on the toxicity of which they are based. Benzo(a)pyrene can be considered as a marker for the other PAHs, hence only benzo(a)pyrene needs to be monitored for comparison with the biota EQS or the corresponding AA-EQS in water". No excessive concentrations of benzo(a)pyrene were found. Trichlorobenzenes were detected only at three and one site in the JDS1 and JDS2, respectively.

Next to the above WFD priority substances, which must be monitored anyway by all Member States (and thus by majority of the Danube countries including those with the Associated Member State status), the list contains additional 15 pollutants of concern. The pesticide terbuthylazine and its degradation product desethylterbutylazine were detected at 78 and 75 sites in the JDS2, and desethylterbutylazine was exceeding frequently the lowest PNEC. A widespread use of the highly polar pesticides bentazone and 2,4-D was detected across the basin with concentrations exceeding the lowest PNECs. Similar to other pesticides, one should be aware of their seasonal application and possible "missing of the pollution peak". These substances certainly belong to those that should be followed in a more systematic manner. The nonregulated member of the nonylphenols family – nonylphenol-1-carboxylate (determined at 86 sites in the JDS2) – seems to be of even higher relevance than the nonylphenol itself. Xylenes and toluene were not on the list of target parameters in the JDS2; however, their frequent occurrence and exceedance of the lowest PNECs in the JDS1 indicate that even these volatile compounds could be followed more closely in future investigative screenings. The endocrine

Table 1 Results of prioritisation based on the occurrence of target analysis substances and (predicted) toxicity data

No.	CAS No.	Name	2001 Min[b]	2001 Max[c]	2001 n[d]	2007 Min[b]	2007 Max[c]	2007 n[d]	EQS[e]	Old Lowest PNEC[f]	S[g]	New lowest PNEC[h]	Excess[i]
1	1763231	Perfluorooctane sulfonic acid and its derivatives (PFOS)[a]				0.00068	**0.101**	85	0.00065	1.3	B		**155**
2	36643284	Tributyltin compounds (Tri-butyltin cation)[a]				0.0053	**0.012**	8	0.0002	0.0026	L		**60**
3	30125634	Terbuthylazine-desethyl				0.001	**0.120**	75		0.0084	P	0.0024	**50**
4	3115499	Nonylphenol-1-carboxylate				0.018	**3.30**	86		0.07	P		**47**
5	206440	Fluoranthene[a]				0.00014	**0.160**	34	0.0063	0.031	L		**25**
6	95476	*o*-Xylene	0.1	**94**	17					4.4	L	4.4	**21**
7	53167	Estrone				0.001	**0.071**	8		1	B	0.0036	**20**
8	191242	Benzo(g,h,i)-perylene[a]				0.004	**0.031**	2	0.002	0.002	B		**16**
9	84852153	Nonylphenols (4-Nonylphenol)[a]				0.021	**3.30**	102	0.3	0.08	B		**11**
10	193395	Indeno(1,2,3-cd)-pyrene[a]				0.021	**0.021**	1	0.002	0.002	B		**11**
11	108883	Toluene	0.1	**75**	22					10.8	L		**6.9**
12	117817	Di(2-ethylhexyl)-phthalate (DEHP)[a]	0.037	**2.8**	43	0.23	**8**	101	1.3	0.0004	B		**5.8**
13	63387280	BDE-206				0.0000004	**0.000013**	12		0.000003	B		**5.2**
14	80057	Bisphenol A				0.005	**0.490**	32		2.1	B	0.1	**4.9**
15	25057890	Bentazone				0.001	**0.240**	80		4.5	L	0.06	**4.0**
16	1330207	m(p)-Xylene	0.1	**12**	20					3.7	L	4.4	**2.7**
17	15307865	Diclofenac	0.0022	**0.130**	18	0.001	**0.052**	60		1.2	B	0.05	**2.6**

(continued)

Table 1 (continued)

No.	CAS No.	Name	2001 Min[b]	2001 Max[c]	2001 n[d]	2007 Min[b]	2007 Max[c]	2007 n[d]	EQS[e]	Old Lowest PNEC[f]	S[g]	New lowest PNEC[h]	Excess[i]
18	298464	Carbamazepine	0.27	**0.27**	1	0.003	**1**	86		113	B	0.5	**1.9**
19	205992	Benzo(b)fluoranthene[a]				0.000017	**0.049**	23	0.03	0.013	B		**1.6**
20	120821	1,2,4-Trichlorobenzene[a]	0.1	**0.5**	3	0.6	**0.6**	1	0.4	0.9	P		**1.5**
21	12002481	Trichlorobenzenes[a]	0.1	**0.6**	3				0.4	0.9	P		**1.5**
22	83158	*N*-acetyl-4-aminoantipyrine	0.04	**2.8**	16					7,102	B	2.1	**1.3**
23	1912249	Atrazine[a]	0.02	**0.8**	83	0.001	**0.140**	97	0.6	0.14	L		**1.3**
24	5915413	Terbuthylazine				0.001	**0.250**	78		0.003	L	0.22	**1.1**
25	94757	2,4-Dichlorophenoxyacetic acid (2,4-D)				0.002	**0.190**	76		41	L	0.2	**1.0**

All concentrations are in μg/L

[a]WFD priority substance regulated at the EU level

[b,c]Minimum and maximum concentration of a substance determined in JDS1 (2001) and JDS2 (2007)

[d]Number of sites with concentration of a substance above the limit of quantification (LOQ) of the analytical method used

[e]Environmental Quality Standard as in the EQS Directive (2008/105/EC and 2013/39/EU)

[f]Predicted No Effect Concentration value available in 2008

[g]Predicted No Effect Concentration value available in 2014

[h]Key reference: B – Baseline prediction from the QSAR model, L – literature data

[i]Maximum concentration of a substance (all sites) divided by the Lowest PNEC

disrupting compounds estrone and bisphenol A were present at eight and 32 sites, respectively, frequently exceeding the newly proposed PNECs. The pharmaceuticals diclofenac and carbamazepine were detected at 60 and 86 sites during the JDS2 versus 18 and 1 sites during the JDS1, respectively. This might indicate that (1) the use of substances is increasing and (2) that they are not sufficiently retained by wastewater treatment technologies used within the basin. Diclofenac was already included in the proposal for the update of the EQS Directive [15] but finally not considered for inclusion among the WFD priority substances with the justification that more evidence on its occurrence in Europe is needed. The substance is now on the EU watch list of substances to potentially be included in the national monitoring programmes [3]. *N*-Acetyl-4-aminoantipyrine was screened only during the JDS1; however, it showed to be present at 16 sites. Hence, a follow-up monitoring to assess the effects of this pharmaceutical would be recommended. BDE-206 is not among the isomers to be followed according to EQS Directive, and according to its latest update (2013/39/EU), it is not even foreseen to monitor BDEs in the inland surface waters. Still, the extremely low P-PNEC is a matter of concern and might require more background ecotoxicological studies.

Perfluorononanoic acid (PFNA) and perfluorooctanoic acid (PFOA) occurring at 58 and 85 sites, respectively, were originally scoring high in the above list using the P-PNEC values of 0.0004 and 0.003 μg/L at the time. This has been changed recently on the basis of an assessment report carried out by the German Federal Environmental Agency indicating that the ecotoxicological effects of these two substances were grossly overestimated by the available QSAR models [9].

In general, one can see two trends in developing lowest PNECs and EQSs in Table 1, where (1) the QSAR model-based predictions are usually contained within one order of magnitude of the finally agreed EQS and where (2) more recent PNECs (or EQS) are usually lower considering new experimental evidence. Two striking exceptions are PFOS and DEHP, where the differences are related to the outcomes of very sensitive chronic toxicity tests not well accounted for by the acute-based computer models. In contrast, a higher PNEC 0.22 μg/L was assigned to terbuthylazine due to the latest chronic toxicity studies in Ökotoxzentrum EAWAG in Switzerland. The "old" lowest PNECs in Tables 1 and 2 were based on the knowledge and model predictions available in 2008. Due to a systematic work of the NORMAN network (www.norman-network.net) dealing with emerging substances, many PNECs were updated and presented in this study as "new" lowest PNECs. One should be aware that the PNECs and EQSs are a part of dynamically developing system fed by frequent new knowledge in ecotoxicology. Therefore, "old" data on the river basin or European scale should be reassessed from time to time. An information on the latest lowest PNECs can be sought at http://www.norman-network.net/empodat/.

Table 2 Results of prioritisation based on the GC–MS screening data and (predicted) toxicity data

No.	CAS No.	Name	2001 Min[a]	2001 Max[b]	2001 n[c]	2007 Min[a]	2007 Max[b]	2007 n[c]	Old Lowest PNEC[d]	S[e]	New Lowest PNEC[f]	Excess[g]
1	629787	Heptadecane	0.07	**2.0**	7	0.054	**0.11**	3	0.0002	B		**9,091**
2	112801	9-Octadecenoic acid	0.13	**6.8**	46	0.25	**0.32**	2	0.002	B		**3,396**
3	143282	9-Octadecen-1-ol (Z)	1.59	**1.6**	1				0.0011	B		**1,455**
4	2091294	9-Hexadecenoic acid	0.1	**32**	51	1.5	**1.48**	1	0.032	B		**1,000**
5	28623463	Nonadecanenitrile				0.52	**0.52**	1	0.0006	B		**869**
6	111068	Hexadecanoic acid, butyl ester				0.021	**0.18**	17	0.0002	B		**844**
7	57103	Hexadecanoic acid	0.3	**15**	55	0.32	**1.52**	3	0.021	B		**705**
8	6765395	1-Heptadecene	0.2	**0.200**	1				0.0004	B		**540**
9	34347289	2,2-Dehydrocholesterol	0.045	**0.53**	44				0.0013	B		**408**
10	142916	1-Methylethyl ester hexadecanoic acid	0.047	**0.250**	6				0.0008	B		**312**
11	108383	1,3-Dimethylbenzene	0.038	**736**	10				4.4	L		**167**
12	124265	Octadecanamide	0.71	**0.71**	1				0.0046	B		**154**
13	301020	9-Octadecenamide	0.21	**0.74**	2	0.18	**0.18**	2	0.0074	B		**100**
14	629629	Pentadecane				0.072	**0.14**	3	0.0015	B		**92**
15	629798	Hexadecanenitrile				0.1	**1.00**	1	0.0120	B		**83**
16	115866	Triphenyl phosphate	0.095	**2.5**	2	0.021	**0.05**	4	0.87	L	0.03	**83**
17	506127	Heptadecanoic acid	0.27	**0.27**	1				0.0034	B		**79**
18	544763	Hexadecane				0.094	**0.11**	2	0.0015	B		**71**
19	112538	1-Dodecanol				2.7	**4.81**	3	0.075	P		**64**
20	96764	2,4-bis(1,1-Dimethylethyl)-phenol	0.056	**25**	31	0.0096	**0.04**	2	0.44	P		**57**
21	1330865	Hexanedioic acid, diisooctyl ester				0.029	**0.06**	3	0.001	B		**55**
22	2719622	(1-Pentylheptyl)-benzene				0.017	**0.09**	9	0.0016	B		**53**
23	4534536	(1-Methyldodecyl)-benzene				0.011	**0.03**	6	0.0006	B		**50**
24	2400002	(1-Ethyldecyl)-benzene				0.017	**0.08**	7	0.0016	B		**49**

25	1120361	1-Tetradecene				0.3	**0.32**	2	0.0068	B		**47**
26	544638	Tetradecanoic acid	0.045	**1.9**	55	0.015	**1.03**	10	0.05	P		**38**
27	2719633	(1-Butyloctyl)-benzene				0.011	**0.06**	11	0.0016	B		**39**
28	2719611	(1-Methylundecyl)-benzene				0.027	**0.06**	5	0.0016	B		**38**
29	4534503	(1-Butylnonyl)-benzene				0.013	**0.02**	5	0.0006	B		**35**
30	25154523	Nonylphenol	0.17	**1.7**	3	0.042	**0.04**	1	0.05	B		**34**
31	2719644	(1-Propylnonyl)-benzene				0.014	**0.05**	9	0.0016	B		**33**
32	3055956	Pentaethylene glycol monododecyl ether				0.78	**0.78**	1	0.029	B		**27**
33	4536883	(1-Methyldecyl)-benzene				0.038	**0.09**	12	0.0043	B		**22**
34	1002842	Pentadecanoic acid	0.05	**0.8**	24	0.028	**0.41**	3	0.039	P		**20**
35	4537159	(1-Butylheptyl)-benzene				0.02	**0.08**	12	0.0043	B		**19**
36	2437561	1-Tridecene				0.26	**0.26**	1	0.018	B		**14**
37	629505	Tridecane				0.014	**0.33**	24	0.023	B		**14**
38	128370	2,6-Bis(1,1-Dimethylethyl)-4-methyl-phenol				0.017	**1.14**	24	0.08	B		**14**
39	4536861	(1-Propyloctyl)-benzene				0.023	**0.06**	11	0.0043	B		**14**
40	142165	Bis(2-Ethylhexyl) maleate				0.008	**0.04**	3	0.0035	B		**11**
41	4537148	(1-Pentylhexyl)-benzene				0.01	**0.04**	11	0.0043	B		**8.8**
42	4536305	2-(Dodecyloxy)-ethanol				0.29	**0.63**	4	0.078	P		**8.1**
43	629594	Tetradecane				0.031	**0.08**	2	0.0095	B		**8**
44	84742	Di-*n*-butyl phthalate (DBP)	0.069	**3.1**	44	0.028	**5.82**	27	0.74	L	0.74	**7.9**
45	120729	1H-indole	2.8	**6.4**	2				1	L		**6.4**
46	84695	Diisobutyl phthalate	0.054	**1.5**	89	0.059	**5.40**	91	0.9	B		**6**
47	18300919	Pentadecanenitrile				0.17	**0.17**	1	0.031	B		**5.6**
48	108689	3,5-Dimethylphenol	33	**33**	1				6	P		**5.5**
49	1120214	Undecane				0.017	**0.63**	18	0.12	P		**5.2**
50	66408557	5,9,13-Trimethyl-4,8,12-tetradecatrienal				0.096	**0.10**	1	0.02	B		**4.8**
51	1222055	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylin-deno[5,6-c]pyran (Galaxolide)	0.37	**0.37**	1	0.012	**0.38**	9	0.028	B	0.083	**4.6**

(continued)

Table 2 (continued)

No.	CAS No.	Name	2001 Min[a]	2001 Max[b]	2001 n[c]	2007 Min[a]	2007 Max[b]	2007 n[c]	Old Lowest PNEC[d]	S[e]	New Lowest PNEC[f]	Excess[g]
52	112403	Dodecane				0.017	**0.30**	24	0.065	P		**4.6**
53	5746587	12-Methyl-tetradecanoic acid				0.31	**0.31**	1	0.071	P		**4.4**
54	124185	Decane				0.088	**0.80**	10	0.24	P		**3.3**
55	110270	Tetradecanoic acid, 1-methylethyl ester				0.024	**0.03**	2	0.013	B		**2.4**
56	3055934	Diethylene glycol monododecyl ether				0.25	**0.36**	2	0.18	B		**2**
57	6382076	*p-tert*-Amyl phenoxy ethanol	3.1	**3.1**	1				1.88	P		**1.6**
58	629630	Tetradecanenitrile				0.12	**0.12**	1	0.081	B		**1.5**
59	143077	Dodecanoic acid	0.08	**0.08**	1	0.025	**0.12**	5	0.08	P		**1.5**
60	98828	Isopropylbenzene	3.6	**3.6**	1				2.6	L		**1.4**
61	933982	1-Ethyl-2,3-dimethylbenzene	0.089	**0.68**	2	0.016	**0.02**	1	0.51	P		**1.3**
62	5466773	2-Propenoic acid, 3-(4-Methoxyphenyl)-, 2-ethylhexyl ester				0.012	**0.10**	6	0.076	B		**1.3**
63	4537115	(1-Butylhexyl)-benzene				0.013	**0.01**	1	0.011	B		**1.2**
64	2531842	2-Methyl-phenanthrene				0.12	**0.24**	2	0.21	P		**1.1**
65	638539	Tridecanoic acid				0.06	**0.06**	1	0.057	P		**1.1**

All concentrations are in μg/L
[a,b]Minimum and maximum concentration of a substance determined in JDS1 (2001) and JDS2 (2007)
[c]Number of sites with concentration of a substance above the limit of quantification (LOQ) of the analytical method used
[d]Predicted No Effect Concentration value available in 2008
[e]Predicted No Effect Concentration value available in 2014
[f]Key reference: *B* Baseline prediction from the QSAR model, L literature data
[g]Maximum concentration of a substance (all sites) divided by the Lowest PNEC

5.2 Nontarget Screening

A novel prioritisation approach has been tested on substances tentatively identified by nontarget screening of surface water samples using GC–MS. Based on the obtained spectral information, chemical structures of 158 analytes could be proposed in the JDS2 samples [8]. An additional 43 compounds remained unidentified. Screening of 98 water samples in the JDS1 revealed the presence of 96 provisionally identified analytes. Similarly, estimated concentrations of identified substances were compared with their predicted ecotoxicological threshold values. The results of prioritisation are shown in Table 2.

In agreement with the results of JDS1 [7], phthalates and fatty acids belonged to the most ubiquitous compounds detected. Phthalates are commonly used as plasticisers, industrial and lubricating oils, defoaming agents, cosmetics and insect repellents. The most widespread representative of this group was isobutyl phthalate, present in 89 and 91 samples in the JDS1 and JDS2, respectively. Dibutyl phthalate, which is already on the list of RBSPs for Slovakia and Finland, was detected in 44 and 27 samples in the JDS1 and JDS2, respectively.

Fatty acids enter the environment mainly from degradation of petroleum hydrocarbons and animal and vegetable fats, which can be used as indicators for 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 phenanthrene were characteristic for the Arges tributary, site downstream of Bucharest after confluence of the Danube with the Arges tributary and the Rusenski Lom tributary at Beli Lom, Pisanetz.

Alkyl-substituted benzenes represent typical degradation products of petroleum hydrocarbons coming mainly from oil pollution from navigation and combustion of fuels. They were found in larger numbers and quantities in the JDS2 samples: especially in the river stretch from river kilometres 1,040 to 840 (Iron Gate reservoir (Golubac/Koronin) – Pristol/Novo Selo Harbour) as well as at Paks (rkm 1,533) and at sites Deggendorf and Niederalteich and the 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 *sunscreen 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)) and *other cosmetic ingredients* (ethylene-, diethylene-, triethylene- and pentaethylene glycol monododecyl ethers, 2-hydroxybenzoic acid pentyl ester, dipropylene glycol 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).

However, comparing to the lowest PNEC values, not all of these substances present a threat to the environment (cf. Table 2). During the JDS 2, galaxolide was found at the highest level at the Arges tributary, indicating pollution by urban wastewater from Bucharest. It was also recorded in wastewaters from Budapest at about tenfold lower concentration levels, reflecting potentially lower pollution. The compound was also present in the tributaries Morava, Olt, Iskar and Rusenski Lom. Galaxolide was also detected at the Arges tributary 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 organophosphate flame retardants (OPFR) and nitrogen-containing compounds (alkylnitrobenzenes, nitriles, amines). Despite not exceeding the lowest PNEC values, tributyl phosphate, belonging to the first group, was present in most JDS2 samples in the stretch from river kilometre 795 (JDS68 – Calafat) to the Black Sea, with the highest concentration at the Arges tributary. In the upper part of the Danube, this compound was only present in two samples. The majority of the OPFRs, including triphenyl phosphate (exceeding the lowest PNEC at 4 sites), 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 OPFRs 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 OPFRs in the DRB certainly deserves serious attention and further investigations.

The highest number of organic compounds, including a wide variety of aromatic hydrocarbon derivatives and personal care products, was identified mostly in samples from the Arges tributary. For example, the WFD PS nonylphenol was detected in the Arges tributary, which is in line with the findings of target analyses, showing its highest concentration (3.28 μg/L) at the same site.

Despite using advanced identification methodologies, about 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 the retrospective identification of these yet unknown compounds in the future.

6 Conclusions

A retrospective assessment of the results from JDS1 (2001) and JDS2 (2007) was made using the latest know-how provided by the NORMAN network on the ecotoxicological threshold values of individual pollutants detected in the surface water samples. A list of 25 compounds exceeding the ecotoxicological threshold value in at least one sampling site within the Danube river basin was drafted. A ratio obtained by comparing the maximum concentration of a substance from all available measurements to the lowest PNEC or EQS value was used for the ranking/ prioritisation of these substances. Whenever no ecotoxicity study was available, a provisional PNEC (P-PNEC) value was proposed using a QSAR-based or read-across modelling approach [9, 10].

A novel approach of ranking substances from nontarget screening was applied in which concentration of each detected compound was semi-quantitatively estimated and compared to the latest available PNEC or P-PNEC value. The overall goal was not to exactly quantify the risk caused by pollutants that occurred at the basin level, but to create a list of candidate substances which may become future RBSPs.

The widespread occurrence and the potential effects of emerging substances in Europe are under the scrutiny of the NORMAN network, which is supporting the WFD CIS Working Group Chemicals working under EC DG ENV and their prioritisation efforts towards the upgrade of the list of WFD priority substances and the related watch list. Close cooperation with the NORMAN network is recommended in order to harmonise strategies for deriving common DRBSP EQSs, developing methodologies for their analysis, setting up schemes for investigative monitoring and developing adequate measures for their removal. The remaining challenge is to identify and prioritise toxic compounds that stay "unknown" due to their missing mass spectra in the currently available libraries.

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EU WFD Organic Priority Substances in Water, Suspended Particulate Matter, Sediments, and Biota in the Danube

Alfred Rauchbüchl

Abstract Since its publication in the year 2000, the EU Water Framework Directive (WFD) became the most important legal act for water protection not only within the European Union but also in the Danube River Basin. In its strategy against water pollution, the WFD identifies priority substances (PS). PS are hazardous chemical compounds forming a special threat to the quality of surface waters. The goal is to reduce concentrations of all PS at least below substance-specific environmental quality standards (EQS). EQS are concentration limit values derived on the basis of ecotoxicological substance data and additional information. In the Danube River Basin, the level of contamination of the Danube and its tributaries by PS was investigated within the monitoring activities of the International Commission for the Protection of the Danube River Basin (ICPDR). Especially the results of ICPDR's research expeditions in 2001 and 2007, the Joint Danube Surveys, revealed the exposure situation for PS in different aquatic matrices. For the subgroup of organic PS, widespread pollution problems with partial exceedance of the respective water EQS were found for nonylphenol, a decomposition product of surfactants, the plasticizer di(2-ethylhexyl) phthalate, and tributyltin compounds, formerly used in antifouling paints for ships. The mostly banned pesticide atrazine could also be found in many water samples. For all other PS, only local problems were identified or they have not been detected at all. The results for suspended particulate matter, sediment, and biota support the findings above.

Keywords Danube, Joint Danube Survey, Pollution, Priority substance, Water framework directive

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Contents

1 The Water Framework Directive

In the 1970s, the first legal instrument of the European Union to protect surface waters against pollution by hazardous substances was introduced with the enforcement of the Dangerous Substances Directive (DSD) [1] and its daughter directives. In the following years, these legal acts were supplemented by a number of use-oriented directives and decisions which covered different other aspects of water protection (e.g., Nitrates Directive [2], Industrial Emissions Directive [3]). With increasing pressures on surface waters and groundwater, it became clear that existing legislation was not capable to guarantee the preservation and improvement of European waters in the long term. In the 1990s, therefore, work started on reshaping water legislation, and in December 2000, eventually, the Water Framework Directive (WFD) [4] of the European Union was enforced (more details can be found in [5]). This legal act forms the basis for a new and comprehensive water policy within the EU.

The outstanding goal of the WFD is to achieve a good status for all surface waters and groundwater until 2015. For the status assessment, surface waters and groundwater are formally divided into “water bodies,” coherent subunits of the river basin district [6]. For each water body, a set of quality elements has to be evaluated and compared to the environmental objectives given for all types of waters in Annex V of the WFD. The quality elements are grouped to define the ecological status (biological, hydromorphological, and physicochemical quality elements including hazardous substances of relevance in a specific river basin) and the chemical status (hazardous substances regulated on community level). The combination of these two assessments leads to the overall result revealing whether a water body has achieved good status.

The operational tool to pursue the WFD goals is the River Basin Management Plans (RBMPs). To set up this plan for a catchment area, several consecutive steps have to be carried out: identification of pressures, analysis of impacts, identification of risks to fail good status, monitoring and assessment of status, and development and implementation of measures to improve water bodies in bad status. The results of these analyses and the necessary measures for improvement are compiled in the RBPMs. According to the WFD, the first edition of the RBPMs had to be put into force in 2009. Currently, the second cycle of analysis and assessment for update of the RBPMs in 2015 is ongoing. The results of river basin characterization and the coordinated measures for the international catchment area of the Danube River were summarized in the Danube RBMP 2009, prepared by the International Commission for the Protection of the Danube River (ICPDR) [7].

2 Priority Substances

The WFD defines hazardous substances as "substances or group of substances that are toxic, persistent and liable to bio-accumulate and other substances or group of substances which give rise to an equivalent level of concern." Two groups of hazardous substances are defined: According to the subsidiary principle, on community level, only substances shall be regulated posing a threat to a majority of European waters, therefore named priority substances (PS). Pollutants with only local or regional impacts have to be handled on member state level (belonging to the quality elements of the ecological surface water status). According to WFD Article 16, the European Commission is obliged to submit a proposal for a PS list ranking substances according to their risk to the aquatic environment due to their intrinsic properties and exposure.

The selection and prioritization for PS are challenging because of the large number of potential candidates and the huge amount of high-quality data needed to assess risk and exposure. The basic measure for the ranking of candidate substances is the ratio of the predicted environmental concentration (PEC) to the predicted no-effect concentration (PNEC). PEC values are calculated with the help of exposure models taking into account data on production, use, and release potential for a certain substance. Ideally, instead of PEC values, data of monitored concentrations of a pollutant can be used. PNECs are derived, inter alia, based on ecotoxicological endpoints for water organism, determined to the greater part in standardized laboratory tests (see Sect. 3). Substances with a PEC/PNEC ratio greater than 1 pose a risk to the aquatic environment.

In 2001, the EC submitted a first proposal [8] identifying 33 substances and substance groups as PS of which 11 were designated as priority hazardous substances (PHS) and 14 as PHS candidates (in the meantime, this decision process has been finalized resulting in 13 PHS). For PHS, due to their extremely dangerous properties, the phase-out and cessation of discharges, emissions, and losses is the midterm goal of the WFD. For PS, the WFD demands a continuous reduction of emissions into the aquatic environment.

3 Derivation of Environmental Quality Standards

The environmental quality standards (EQS) provide legally binding concentration limits for hazardous substances in surface waters ensuring protection of the environment and humans, mainly derived on the basis of ecotoxicological effect data.

For hazardous substances, the basic principles for derivation of EQS are laid down in Annex V, point 1.2.6 of the WFD. The development of a detailed method for the first PS list was carried out by a consultant [9, 10]. Based on this work and after a tedious legislative procedure, the EQS for PS were put into force in December 2008 ("EQS Directive" [11]). The directive lays down EQS for inland surface waters and other surface waters (transitional, coastal, and marine waters). Both sets of EQS comprise Annual Average-EQS (AA-EQS) protecting against long-term/chronic exposure to PS and Maximum Allowable Concentration-EQS (MAC-EQS) protecting against short-term/acute effects due to pollutant concentration peaks. In addition, the directive includes EQS for 8 remaining substances of the 17 dangerous substances of the DSD, which have not been identified as PS. The existing standards for these substances have proved to be useful, so their regulation on community level was maintained.

The AA-EQS is compared to the annual average concentration of monthly measurements of 1 year and the MAC-EQS to the single measurement of the same period. Only if in both assessments the monitoring results do not exceed the respective EQS values for all 41 hazardous substances the water body is assigned "good chemical status." Table 1 summarizes the 41 substances regulated on community level for the time being, the substance status, and the EQS for inland surface waters.

While MAC-EQS are based on acute ecotoxicological effects, AA-EQS take into account both chronic and acute effects. Figure 1 gives an overview of the derivation process for freshwater AA-EQS.

In the first step, on the basis of substance properties and agreed trigger criteria, it is decided which additional risk scenarios besides the water phase (pelagic community) are relevant (sediment/benthic community, top predators via prey/biota, and humans via food intake/biota and drinking water). For example, if the substance has no potential to bioaccumulate, the risk for top predators via prey and humans via food intake need not to be considered.

In the next step, the necessary data are compiled and checked for their usability (relevance and reliability). On the basis of this filtered data set, specific quality standards (SQS, synonymous to PNEC) for the relevant risk scenarios are derived: The lowest no-effect concentration (NOEC) is identified and an appropriate

Table 1 Hazardous substances regulated on community level, substance status, and environmental quality standards for inland surface waters [11]

No.	Name of substance	Status[a]	CAS number[b]	AA-EQS[c] Inland surface waters[d]	MAC-EQS[e] Inland surface waters[d]
(1)	Alachlor	PS	15972-60-8	0.3	0.7
(2)	Anthracene	PHS	120-12-7	0.1	0.4
(3)	Atrazine	PS	1912-24-9	0.6	2.0
(4)	Benzene	PS	71-43-2	10	50
(5)	Brominated diphenylether[f]	PHS	32534-81-9	0.0005	Not applicable
(6)	Cadmium and its compounds (depending on water hardness classes)[g]	PHS	7440-43-9	≤0.08 (Class 1)	≤0.45 (Class 1)
				0.08 (Class 2)	0.45 (Class 2)
				0.09 (Class 3)	0.6 (Class 3)
				0.15 (Class 4)	0.9 (Class 4)
				0.25 (Class 5)	1.5 (Class 5)
(6a)	Carbon tetrachloride	OP	56-23-5	12	Not applicable
(7)	C10–C13 chloroalkanes	PHS	85535-84-8	0.4	1.4
(8)	Chlorfenvinphos	PS	470-90-6	0.1	0.3
(9)	Chlorpyrifos (chlorpyrifos-ethyl)	PS	2921-88-2	0.03	0.1
(9a)	Cyclodiene pesticides	OP		$\Sigma = 0.01$	Not applicable
	Aldrin		309-00-2		
	Dieldrin		60-57-1		
	Endrin		72-20-8		
	Isodrin		465-73-6		
(9b)	DDT total[h]	OP	Not applicable	0.025	Not applicable
	para–para'-DDT	OP	50-29-3	0.01	Not applicable
(10)	1,2-Dichloroethane	PS	107-06-2	10	Not applicable
(11)	Dichloromethane	PS	75-09-2	20	Not applicable
(12)	Di(2-ethylhexyl)-phthalate (DEHP)	PS	117-81-7	1.3	Not applicable

(continued)

Table 1 (continued)

No.	Name of substance	Status[a]	CAS number[b]	AA-EQS[c] Inland surface waters[d]	MAC-EQS[e] Inland surface waters[d]
(13)	Diuron	PS	330-54-1	0.2	1.8
(14)	Endosulfan	PHS	115-29-7	0.005	0.01
(15)	Fluoranthene	PS	206-44-0	0.1	1
(16)	Hexachlorobenzene	PHS	118-74-1	0.01[i]	0.05
(17)	Hexachlorobutadiene	PHS	87-68-3	0.1[j]	0.6
(18)	Hexachlorocyclohexane	PHS	608-73-1	0.02	0.04
(19)	Isoproturon	PS	34123-59-6	0.3	1.0
(20)	Lead and its compounds	PS	7439-92-1	7.2	Not applicable
(21)	Mercury and its compounds	PHS	7439-97-6	0.05[k]	0.07
(22)	Naphthalene	PS	91-20-3	2.4	Not applicable
(23)	Nickel and its compounds	PS	7440-02-0	20	Not applicable
(24)	Nonylphenol (4-nonylphenol)	PHS	104-40-5	0.3	2.0
(25)	Octylphenol ((4-(1,1′,3,3′-tetramethylbutyl)-phenol))	PS	140-66-9	0.1	Not applicable
(26)	Pentachlorobenzene	PHS	608-93-5	0.007	Not applicable
(27)	Pentachlorophenol	PS	87-86-5	0.4	1
(28)	Polyaromatic hydrocarbons (PAH)[l]	PHS	Not applicable	Not applicable	Not applicable
	Benzo(a)pyrene	PHS	50-32-8	0.05	0.1
	Benzo(b)fluoranthene	PHS	205-99-2	$\Sigma = 0.03$	Not applicable
	Benzo(k)fluoranthene	PHS	207-08-9		
	Benzo(g,h,i)perylene	PHS	191-24-2	$\Sigma = 0.002$	Not applicable
	Indeno(1,2,3-cd)pyrene	PHS	193-39-5		
(29)	Simazine	PS	122-34-9	1	4
(29a)	Tetrachloroethylene	OP	127-18-4	10	Not applicable
(29b)	Trichloroethylene	OP	79-01-6	10	Not applicable
(30)	Tributyltin compounds (tributyltin cation)	PHS	36643-28-4	0.0002	0.0015

(31)	Trichlorobenzenes	PS	12002-48-1	0.4	Not applicable
(32)	Trichloromethane	PS	67-66-3	2.5	Not applicable
(33)	Trifluralin	PS	1582-09-8	0.03	Not applicable

[a] *OP* other pollutant: substance formerly regulated by DSD and not identified as PS, *PS* priority substance, *PHS* priority hazardous substance
[b] *CAS* Chemical Abstracts Service
[c] This parameter is the EQS expressed as an annual average value (AA-EQS). Unless otherwise specified, it applies to the total concentration of all isomers
[d] Inland surface waters encompass rivers and lakes and related artificial or heavily modified water bodies
[e] This parameter is the EQS expressed as a maximum allowable concentration (MAC-EQS). Where the MAC-EQS are marked as "not applicable," the AA-EQS 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
[f] For the group of priority substances covered by brominated diphenylethers (No. 5) listed in Decision No. 2455/2001/EC, an EQS is established only for the sum of congener numbers BDE 28, 47, 99, 100, 153, and 154
[g] For cadmium and its compounds (No. 6), the EQS values vary depending on the hardness of the water as specified in five class categories (Class 1, <40 mg CaCO3/L; Class 2, 40 to <50 mg CaCO3/L; Class 3, 50 to <100 mg CaCO3/L; Class 4, 100 to <200 mg CaCO3/L; and Class 5, ≥200 mg CaCO3/L)
[h] DDT total comprises the sum of the isomers 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (CAS number 50-29-3; EU number 200-024-3); 1,1,1-trichloro-2-(o-chlorophenyl)-2-(*p*-chlorophenyl) ethane (CAS number 789-02-6; EU number 212-332-5); 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (CAS number 72-55-9; EU number 200-784-6); and 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane (CAS number 72-54-8; EU number 200-783-0)
[i] Water EQS may only be applied in combination with biota EQS for hexachlorobenzene: 10 μg/kg (wet weight)
[j] Water EQS may only be applied in combination with biota EQS for hexachlorobutadiene: 55 μg/kg (wet weight)
[k] Water EQS may only be applied in combination with biota EQS for mercury and its compounds: 20 μg/kg (wet weight)
[l] For the group of priority substances of polyaromatic hydrocarbons (PAH) (No. 28), each individual EQS is applicable, i.e., the EQS for benzo(a)pyrene, the EQS for the sum of benzo(b)fluoranthene and benzo(k)fluoranthene, and the EQS for the sum of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene must be met

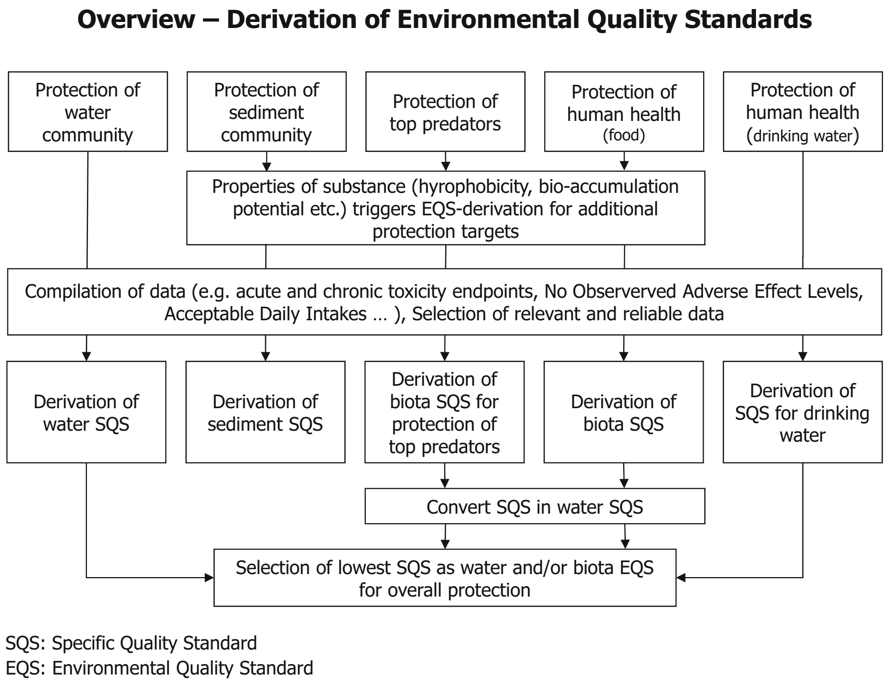

Fig. 1 Overview of derivation steps for environmental quality standards according to [12]

assessment factor (AF) in the range 2–1,000 applied (i.e., division of the lowest concentration by AF) to obtain the SQS/PNEC. The AFs account for:

- Uncertainties in transfer of ecotoxicological endpoints from laboratory tests to the environment
- Completeness of data set (data gaps)
- Effects on endocrine system of aquatic organisms
- Synergistic toxic effects of pollutant mixtures (no consolidated approach for assessment of pollution mixtures is available presently)

The "assessment factor method" was developed to deal with limited data sets for pollutants of interest. In the meantime, many substances are well characterized regarding their adverse environmental effects, and large data sets are available for risk assessment and QS derivation. In this case, a statistical method can be applied, the so-called species sensitivity distribution, where the SQS/PNEC is derived as percentile of ecotoxicity data distribution.

The SQS for other matrices than water are back-calculated to the water phase with the help of bioaccumulation factors, etc. The SQS with the lowest (corresponding) water value is selected as water and/or biota EQS for the substance ensuring overall protection.

More details on EQS derivation (MAC-EQS, metals, etc.) can be found in the EU CIS Guidance Document No. 27 "Technical Guidance for Deriving

Environmental Quality Standards" [12]. This document lays down the advanced methodology of EQS setting for the revision of the PS list based on the original method [9, 10].

4 Monitoring of Priority Substances in the Danube River Basin

4.1 Routine Monitoring Programs

The first coordinated monitoring program within the Danube River Basin was already initiated under the Bucharest Declaration, which was signed by the Danube riparian countries in 1985. The focus of this monitoring network was to evaluate water quality in the cross section of the river at the borders of the riparian states. Monitoring activities were heavily intensified after the signing of the Danube Convention in 1994 eventually leading the setup of the "Trans-National Monitoring Network" (TNMN) in 1996. In 2007, the monitoring network was reshaped to adapt it to the requirements of the WFD. Within the joint monitoring under TNMN, the water quality is determined at over 100 sampling sites at the Danube River and its tributaries 12 times per year, at selected monitoring stations 26 times per year for reliable load calculations. The list of determinants comprises basic physicochemical parameters, nutrients, metals and selected pollutants (all measurements in the water phase), and biological parameters. Up to now, only a few WFD priority substances (namely, cadmium, lead, nickel, mercury, atrazine, lindane (γ-hexachlorocyclohexane), PAH, and trichloromethane) and some EU-regulated "other pollutants" (carbon tetrachloride, *p*,*p*′-DDT, tetrachloroethylene, trichloroethylene) are monitored within the framework of TNMN. Analyses are carried out by national reference laboratories in the riparian countries. With regard to the mentioned organic priority substances, it has to be noted that these substances were only partly analyzed at TNMN stations and the assessment of available data is additionally complicated by varying limits of quantification (LOQ). Nevertheless, the available data show that lindane and trichloromethane can hardly be detected. Atrazine can be quantified in some cases, but only single values exceed the AA-EQS in some tributaries. The mean values are well below the EQS. Result details of the TNMN program can be found in the annual TNMN reports starting from 1996 [13].

Another part of TNMN functions as surveillance and operational monitoring according to WFD providing data for the Danube River Basin Management Plan. The priority substances which were identified in the first Danube River Basin Management Plan as causing poor chemical status in the surface water bodies in catchments larger than 4,000 km^2 are described in the chapter by Liska [14].

4.2 Joint Danube Surveys

At the end of the 1990s, the idea came up to supplement the results of the existing monitoring programs with a research expedition to give a longitudinal multidisciplinary overview of the water quality of the Danube River. The need to close the data gaps regarding priority substances, geographically and with respect to the substances not monitored within TNMN, was one of the important motivations for the organization of the first Joint Danube Survey of the ICPDR in 2001(JDS 1) and remained as one of the most important goals for the second survey in 2007 (JDS 2).

The lessons learned in the first years of routine monitoring led to a different approach for analysis during the Joint Danube Surveys: Within these measurement campaigns, groups of substances are measured by one laboratory for all samples of the same type of the survey. This avoids problems with bias and differing LOQs and guarantees comparability of data along the whole stretch of the Danube River. The results of both surveys can be found in the respective scientific reports [15, 16].

4.3 Results and Assessment of Organic Priority Substances According to WFD

During JDS1 (2001), lots of experience were gained regarding sampling and analysis of PS. This knowledge, the even broader scope of investigation with respect to matrices analyzed combined with the comparability of data due to the "one substance-one laboratory" principle, makes the results for priority substances of JDS2 (2007) the most valuable data set for the basin-wide assessment of this substance group. The following summary assessments are therefore based on JDS2 results, with a comparison of JDS1 outcome, where possible.

It is the character of the survey to provide only a snapshot of the exposure situation (one result for a single sample per sampling site and matrix). For full chemical status assessment, the WFD demands 12 monthly measurements per year. It therefore has to be stressed that the JDS results can only give an indication of the chemical status at each sampling site and must not be mixed up with chemical status assessment on a water body basis which lies within the responsibility of the riparian states.

In this chapter, most of the organic PS are addressed. The findings for metals are discussed in the chapter by László [17] and the results for polycyclic aromatic hydrocarbons (PAH) in the chapter by Literathy [18].

Table 2 summarizes the analytical results of organic PS in the water phase. For these PS, the whole water sample (including suspended particulate matter) was analyzed because due to their hydrophobic properties for most of these substances, the partition equilibrium is shifted from the dissolved to the adsorbed state. For each substance, the range of concentrations found and the percentage of results above LOQ are given. Many results were below the respective LOQ. In these cases, for the

Table 2 Summary of results of JDS2 for priority substances and certain other pollutants in the water phase of the Danube River and its tributaries

No.	Name of substance	JDS2 results	% >LOQ	AA-EQS	MAC-EQS
(1)	Alachlor	(0.05)	0	0.3	0.7
(3)	Atrazine	(0.005)–0.56	>50	0.6	2.0
(4)	Benzene	(0.3)	0	10	50
(5)	Brominated diphenylether	(0.002)[a]	0	0.0005	–
(6a)	Carbon tetrachloride	(1.2)	0	12	–
(7)	C10–C13 chloroalkanes	n.a	–	0.4	1.4
(8)	Chlorfenvinphos	(0.005)	0	0.1	0.3
(9)	Chlorpyrifos (chlorpyrifos-ethyl)	(0.005)	0	0.03	0.1
(9a)	Cyclodiene pesticides			$\Sigma = 0.01$	–
	Aldrin	(0.01)	0		
	Dieldrin	(0.021)	0		
	Endrin	(0.023)	0		
	Isodrin	(0.005)	0		
(9b)	DDT total	(0.007)	0	0.025	–
	para–para-DDT	(0.007)	0	0.01	–
(10)	1,2-Dichloroethane	(0.7)	0	10	–
(11)	Dichloromethane	(0.5)	0	20	–
(12)	Di(2-ethylhexyl)-phthalate (DEHP)	(0.2)–**4.53**	~100	1.3	–
(13)	Diuron	(0.001)	0	0.2	1.8
(14)	Endosulfan	(0.005)	0	0.005	0.01
(16)	Hexachlorobenzene	(0.02)	0	001	0.05
(17)	Hexachlorobutadiene	(0.1)	0	0.1	0.6
(18)	Hexachlorocyclohexane	(0.02)	0	0.02	0.04
(19)	Isoproturon	(0.001)–0.016	<1	0.3	1.0
(24)	Nonylphenol (4-nonylphenol)	0.02–**3.28**	100	0.3	2.0
(25)	Octylphenol ((4-(1,1′,3,3-′-tetramethylbutyl)-phenol))	(0.005)–0.022	20	0.1	–
(26)	Pentachlorobenzene	(0.018)	0	0.007	–
(27)	Pentachlorophenol	(0.1)	0	0.4	1
(29)	Simazine	(001)–0.055	3	1	4
(29a)	Tetrachloroethylene	(0.5)–0.8	2	10	–
(29b)	Trichloroethylene	(1.7)	0	10	–
(30)	Tributyltin compounds (tributyltin-cation)	(0.0002)–**0.014**	34	0.0002	0.0015
(31)	Trichlorobenzenes	(0.5)–**0.6**	<1	0.4	–
(32)	Trichloromethane	< (1.8)	0	2.5	–
(33)	Trifluralin	(0.005)–0.01	<1	0.03	–

[a]Detected in some water samples in concentrations between limit of detection and LOQ

lower end of the range, the LOQ in parenthesis is filled in. If all values were <LOQ, only the LOQ in parenthesis is given. If the maximum of results exceeds the respective EQS, the figure is displayed in bold.

The results of Table 2 show that for the major part of PS no or only local pollution problems could be identified in the water phase (no or only a few percent of results above LOQ). In contrast, for atrazine, alkylphenols, DEHP, and tributyltin compounds the results indicate a basin-wide pollution. Another group of substances where a widespread environmental contamination can be anticipated due to production and use is brominated diphenylethers. The real extent of pollution is concealed by the lack of analytical routine methods with sufficient analytical performance. Relevant concentrations can only be quantified with sophisticated analytical techniques. For some other compounds with high adsorption and bioaccumulation potential, water data alone are not sufficient to assess the real extent of pollution. For these compounds, supplementary data in sediment, suspended particulate matter, and/or biota were collected. These results are assessed in combination with the water data in the following sections.

4.4 Alkylphenols: Nonylphenol, Octylphenol

Nonylphenol, predominantly 4-iso-nonylphenol (NP), is a decomposition product of alkylphenol ethoxylates (APEO), surface active substances which were in widespread use in the last decades. More than 50% of produced NP went to manufacture of APEO. Other uses of NP were modified phenolic resins, plastics, stabilizers, and phenolic oximes. In 1997, 73,500 t of NP was produced within the EU; 3,500 t of exports and 8,500 t of imports give 78,500 t of NP used [19]. Of all possible octylphenol (OP) isomers, only 4-(1,1,3,3-tetramethylbutyl)-phenol (4-*tert*-octylphenol) seems to be of relevance due to the manufacturing process. Production in the EU is reported to be 6,800 t in 1998; thereof, 5,000 t is estimated to be used for the production of octylphenoxyethoxylates [19]. The use pattern seems to differ to some extent from NP. The ratio of NP and OP production is reflected in analytical results of environmental samples for these compounds. In the meantime, alkylphenols were banned in the EU [20] due to their endocrine-damaging potential.

JDS2 results of NP and OP revealed that at least NP was ubiquitous in the water phase in the whole catchment area at the time of investigation. NP was found in nearly all water samples at concentrations up to a maximum value of 3.28 μg/L. The highest concentrations, exceeding the AA-EQS and MAC-EQS for NP, were found in tributaries in the lower Danube region. The highest NP concentration in the Danube was measured at a sampling station downstream Novi Sad in Serbia (0.14 μg/L). OP could be only found in quantifiable concentrations at three sampling sites: the same sites where NP EQS were exceeded.

The main source for NP and OP are untreated urban and industrial waste waters. But even effluents of waste water treatment plants (WWTP) contribute remarkably to NP pollution of the aquatic environment. High concentrations in the intake of the WWTP result in relevant concentrations in the effluent, despite of high removal rates for NP.

The findings in suspended particulate matter (SPM) and sediment support the water results for NP. Quantifiable amounts in SPM can be found at all sampling sites along the Danube. The maximum value (0.280 mg/kg dry matter) was found downstream Budapest where the main sewage plant was under construction at the time of the survey. The impact of the Budapest sewage could be seen for more than 200 km. Also the tributaries Tisza (89 mg/kg dry matter) and Velika Morava (74 mg/kg dry matter) were obviously influenced by untreated or insufficiently treated waste water. The level of NP in SPM samples of the upper part and the lower part of the Danube was always lower than 0.05 mg/kg dry matter with small variations. OP was only found in some 30% of SPM samples, with a maximum value of 0.043 mg/kg also downstream of Budapest.

Sediment results give a similar ratio of detectable concentrations for NP and OP as for SPM. NP could be quantified in nearly all sediment samples, OP only in 20% of the samples. Concentration ranges from LOQ (0.01 mg/kg dry weight) up to 1.8 mg/kg for NP and from LOQ (0.005 mg/kg) to 0.026 mg/kg. Hot spots are sampling sites in tributaries and in the lower stretch of the Danube.

4.5 Di(2-Ethylhexyl)Phthalate (DEHP)

The main use of di(2-ethylhexyl)phthalate (DEHP) was as plasticizer, mainly in polyvinylchloride (PVC) polymers. The content in flexible polymer materials was up to 30–40% (w/w). In the 1990s, the production in Western Europe was in the order of magnitude of several 100,000 t/year [21]. The global release into the environment via air was estimated between 10,000 and 150,000 t/year [22]. Therefore, it is not surprising that DEHP can still be found in high concentrations in different environmental samples (soil, sewage sludge, water, biota).

As a consequence of the widespread use of DEHP-containing plastics and the relatively high volatility of phthalate, it is ubiquitously present. This also creates a serious problem for analytical laboratories. Due to high blank values, additional uncertainty is introduced in the analytical process, which is reflected in elevated quantification limits. For this reason, LOQs of 0.2 μg/L for whole water samples and of 0.30 mg/kg dry matter were achieved for suspended particulate matter for analysis of JDS2 samples.

In all water samples of JDS2 – except four samples from the upper reach of the Danube – DEHP was detected. The highest concentration was found at the Austrian–Slovakian border (Wildungsmauer, 4.5 μg/L) and downstream Budapest (Dunavoldfar, 4.4 μg/L). Elevated concentrations of DEHP were detected in the middle stretch of the Danube, whereas the concentrations in the upper part and the lower part of the river were below 1 μg/L. Quite a number of single measurements exceed the AA-EQS for DEHP of 1.3 μg/L which is a strong indication that the good chemical status could be failed in some water bodies.

DEHP could be quantified in all suspended matter samples of JDS2 concentrations above 0.3 mg/kg dry matter. Samples of the tributaries Tisza (10 mg/kg dry matter) and Sava (5.0 mg/kg dry matter) showed the highest values of all samples. Elevated concentrations were also found in the German stretch and in the middle section of the Danube. The sharp rise of DEHP concentrations downstream Budapest again indicated the influence of insufficiently treated household and industrial sewage.

During JDS2, DEHP was also found in all sediment samples analyzed. The ubiquitous occurrence of DEHP in all water, suspended matter, and sediment samples underlines the relevance of DEHP as a priority substance for contaminating the Danube River. For most of the sediments, concentrations ranged between 0.1 and 1.0 mg/kg dry mass, and only few samples exhibited significantly higher amounts of DEHP. However, no clear trend in DEHP contamination along the course of the Danube River could be identified. Maximum DEHP levels of more than 16 mg/kg dry matter were found in a sample collected downstream Arges in Romania, i.e., the same sediment that already exhibited elevated amounts of NP. During JDS1, DEHP was also found in almost all sediments under investigation with a maximum concentration of 170 mg/kg dry weight which also was found in a sediment sample near Arges. Comparing JDS1 and JDS2 results for DEHP, suspended matter show higher concentrations especially in the middle part of the Danube, whereas sediment samples indicate an improvement of sediment quality with regard to phthalates.

4.6 Tributyltin Compounds

Tributyltin compounds (TBT) were used as antifouling paints (80%), fungicides, and various biocides used in preparations and products. In 2002, the use of tri-substituted organotin compounds was about 1,600 t in the EU. In the meantime, the use of TBT as antifouling agent was forbidden by EU chemicals law (REACH [20]). Therefore, the application of tri-substituted organotins decreased to about 350 t/a and of TBT to about 250 t/a. According to its use as antifouling agent, the pollution by TBT is mainly caused by diffuse emissions from ship hulls and emissions of TBT during activities in shipyards and dockyards. Despite the ban as a biocide in antifouling paints, diffuse emissions of TBT from ship hulls and contaminated harbor and river sediments still go on although they will gradually diminish [23].

During JDS2, TBT was analyzed in 23 selected water samples together with 4 other organotin compounds and was found only in 8 of the 23 samples in concentrations above the LOQ of 0.2 ng/L with a maximum concentration of 14 ng/L. All other organotin compounds analyzed could not be detected or were below LOQ in the water samples. For TBT the LOQ of the method applied during

JDS2 and the AA-EQS were equal, which means that all positive results were an indication of good chemical status failure. The EQS for acute effects of 15 ng/L was not exceeded by the single measurements.

Suspended matter samples were collected at the same sample sites which were selected for water analysis of organotin compounds. Tributyltin compounds could only be found in 3 of the 23 samples but with a maximum concentration of 230 μg/kg dry matter. This high level was determined for the suspended matter collected in Serbia downstream Belgrade.

The fraction of samples with concentrations of TBT above LOQ was even lower for sediments. Only 9 of 124 samples showed positive evidence for TBT with a maximum of 12 μg/L dry matter.

TBT was additionally analyzed in mussel samples. In contrast to the other matrices in mussel tissue, TBT was the organotin compound with the highest abundance of all organotin compounds investigated. Out of 25 mussel samples, 24 showed positive results with a maximum value of 1,200 μg/kg dry weight and with mean and maximum value a factor 6 higher than concentrations of other organotins. The maximum for TBT in mussel samples was detected at a site downstream Novi Sad in Serbia.

4.7 *Polybrominated Diphenylethers*

Polybrominated diphenylethers (PBDE, in the context of the WFD also named brominated diphenylethers – BDE) were broadly used as flame retardants in polyurethane foams for furniture and upholstery as well as in plastic housings of electronic equipment in recent decades. Combined figures for production and import of PBDE in the EU were some 10,000 t/year at the end of the 1980s [24]. In the meantime, due to the identified risks, the amount used went down to several hundred t/year; eventually, production and use were banned. Huge amounts of PBDE are still physically bound in products and enter the environment by diffusion.

PBDEs are persistent. They show low water solubility but a high binding affinity to particles and a distinct tendency to accumulate in sediments and biota. The decisive-specific quality standard was the one for protection of human health via food consumption. Due to the high accumulation potential in fish, mussels, etc., the back-calculation from biota SQS led to very low AA-EQS for the water phase of 0.0005 μg/L.

Three technical mixtures of PBDEs were used as flame retardants referred to as pentabromo diphenylether, octabromo diphenylether, and decabromo diphenylether. At the time of preparation of the first PS list only for pentabromo diphenylether, a risk to the aquatic environment was identified and the substance group therefore included in the PS list. The technical products contain a mixture of several congeners of brominated diphenylethers (compounds based on the same chemical structure, a diphenylether, but with differing number and position of bromine atoms; the 209 possible different congeners are identified, besides their correct nomenclature names, via number codes). For the commercial product pentabromo diphenylether, tetra- and pentabromo compounds were identified as the main components and tri-, hexa-, and heptabromo congeners as impurities. For monitoring purposes, the six most important congeners of pentabromo diphenylether have been selected (BDE 28, 47, 99, 100, 153, 154; see also Table 1, footnote f). The sum of measured concentrations has to be compared with the AA-EQS.

PBDE water concentrations at EQS level are hardly accessible with analytical routine techniques; therefore, water data for PBDE are scarce. Also during JDS2, the achievable LOQ for lower and medium brominated diphenylethers was 0.002 μg/L (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183; BDE 28 was not analyzed) and for highly brominated diphenylethers 0.005 μg/L (BDE 203, BDE 205), a factor 4–10 above the AA-EQS. PBDEs were not found in amounts above the respective LOQs in any water sample. Only in a few samples, BDE 47 and BDE 99 were measured in concentrations between LOD and LOQ (Romanian reach of Danube). Comparison with former data is not possible because PBDE was not analyzed in water samples in JDS1.

Also in sediments, just two compounds of the regulated PBDE group (BDE 99, BDE 100) could be detected in only one sample. Conversely, decabromo diphenylether (BDE 209) was quantified in all sediment samples and turned out to be relevant for contamination of the Danube River sediment. The 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 in a sediment sample from the Serbian tributary Velika Morava. Detailed analysis of the results of polybrominated diphenylethers received from JDS2 is provided in the chapter by Umlauf et al. [25].

4.8 Organochlorine Compounds

Chlorinated compounds form the biggest group of the PS list (including the "other pollutants") and comprise of substances used mainly as solvents (carbon tetrachloride, 1,2-dichloroethane, dichloromethane, tetrachloroethylene, trichlorobenzene, trichloroethylene, trichloromethane), insecticides (chlorfenvinphos, chlorpyrifos, aldrin, dieldrin, endrin, isodrin, DDT, endosulfan), bactericides/fungicides (hexachlorobenzene, pentachlorophenol), and intermediates in chemical processes (pentachlorobenzene, trichlorobenzenes). The main use of C10–C13 chloroalkanes was as cooling lubricant in metal works. The commercial product is a mixture of several thousand isomers with different chain length and chlorination degree. As an agreed method has been made available only recently, this substance group was not analyzed during JDS2.

With exception of chlorpyrifos, the production and use of the listed organochlorine compound are banned or restricted, for some of them since decades (e.g., DDT). The use of chlorinated solvents is allowed in part but only in closed-loop circuits to minimize emissions to air and water. The bans and restrictions for organochlorine compounds are laid down in international treaties (Stockholm Convention [26], Convention on Long-range Transboundary Air Pollution [27]) and EU regulations (Regulation on persistent organic pollutants [28], REACH [20]).

For all organochlorine compounds, the results of JDS2 target analyses (Table 2) revealed that these substances were hardly detectable in the water phase and all quantifiable concentrations are well below the respective EQS. But for some of the substances, the LOQ of the applied method was higher than the EQS (dieldrin, endrin, pentachlorobenzene, hexachlorobenzene, trichlorobenzene). Furthermore, organochlorine compounds with higher molecular weight and chlorination degree tend to adsorb on sediment and suspended matter and have a high bioaccumulation potential (for hexachlorobenzene and hexachlorobutadiene biota, EQS of 10 and 55 μg/kg wet weight are stipulated, respectively). Accordingly, data on organochlorine compounds in sediment, SPM, and biota are an important supplement to water monitoring results. Target analysis of sediment and SPM showed only a low content of organochlorine compounds in a few samples, mainly in the middle and lower stretch of the Danube River. Fish samples, in the contrary, show quantifiable concentrations of hexachlorobenzene > hexachlorobutadiene > 1,2,4-trichlorobenzene and pentachlorobenzene in muscle tissue and liver. The concentrations for hexachlorobenzene come close but did not exceed the biota EQS. The higher concentrations of hexachlorobenzene in the upper reach of the Danube River were assigned to historic pollution stemming from chemical industry facilities already under remediation. These data for organochlorine compounds are supplemented by an in-depth analysis which is given in the chapter by Umlauf et al. [25].

4.9 Polar Pesticides

The herbicides alachlor, atrazine, diuron, isoproturon, simazine, and trifluralin were broadly used in agriculture and other applications in recent decades. Mainly due to their persistence in soil and the resulting groundwater contamination in combination with their toxicity to aquatic organisms, the authorizations on the basis of the EU Plant Protection Products Regulation [29] for alachlor, atrazine, simazine, and trifluralin were withdrawn between 2004 and 2007. Atrazine and simazine were already banned in some member states since the 1990s and 2000, respectively. Diuron and isoproturon are still authorized. While isoproturon-containing products are approved in most EU countries, the number of diuron-containing formulations on the markets has been successively reduced in the last years.

According to their polarity, the water solubility of these compounds is moderate to high with a low tendency to adsorb to SPM, showing only moderate bioaccumulation potential. Analysis is therefore focused on the water phase. Despite its ban, atrazine and its most important degradation product desethylatrazine were found in many water samples during JDS2. Most concentrations were in the range 0.01–0.02 μg/L with a maximum for atrazine of 0.056 μg/L in a Romanian tributary, more than a factor of 10 below the AA-EQS. For all the herbicides, the overview in Table 2 reveals that they hardly could be detected. The concentrations of the few positive results were far below the respective EQS. It has to be noticed that monitoring during JDS2 took place in August and September. At least for the two authorized pesticides, it can be anticipated that the concentrations in surface waters are probably higher during the application periods, mainly in spring.

4.10 Benzene

In the meantime, the use of benzene is largely restricted according to Annex XVII of the REACH regulation with two exceptions: motor fuels and industrial uses (when legal emission limit values are not exceeded). In both application fields, rather huge amounts of benzene are used. Nevertheless, during JDS2, benzene was not detected in any of the analyzed water samples.

5 Conclusions and Outlook

5.1 Trends in Environmental Concentrations for PS

As already mentioned in the discussion of substances and substance groups, PS production and use are limited or even banned. Especially for the frequently detected alkylphenols, DEHP, tributyltin compounds, and atrazine, it can be expected that environmental concentrations will further go down. Due to the low AA-EQS in water for brominated diphenylethers, the actual exposure situation could only be partly evaluated, but also PBDE use is limited and an improvement of the environmental status with regard to this substance group is likely. Despite these trends toward a meaningful long-term monitoring, it is important to further shift the focus from the water phase to suspended matter, sediment, and/or biota depending on physical properties and behavior of the respective pollutants. This is also reflected on the European level in the increasing number of EQS laid down for biota (see below).

The development in concentrations of identified local pressures for some of the other PS depends on the source of the respective pollution. It's up to the riparian countries to identify these sources and develop measures for their sanitation. For EU member states, this is already obligatory, and the first River Basin Management Plans (RBMPs) addressing these problems are in place since 2009. The 2015 update of the RBMPs is currently in preparation. During this exercise, the efficiency of the actual reduction measures have to be assessed and the measures modified if necessary.

Pollution problems affecting more than one riparian state are addressed in the Danube RBMP prepared by the ICPDR in 2009 [30]. Although this document is based on the obligations of the Water Framework Directive, also information on the water quality status and measures for non-EU member states within the Danube River Basin are included.

For some of the PS, however, even basin-wide measures might not be sufficient. Due to the physical properties, certain PS are subject to long-range air transport and therefore could be found even in remote areas far away from the location of their production and use. From the list of organic PS relevant for the Danube River Basin, tributyltin compounds and PBDE have been marked as such "ubiquitous persistent, bioaccumulative, and toxic" substances (uPBT) by the European Commission [31]. Thus, the goal of reduction and phase-out of emissions for these substances can only be reached if the measures already implemented on an international level are intensified and effectuated [26, 27].

5.2 New PS and Revision of Existing PS

Identification and regulation of PS is a dynamic process. WFD Article 16 provides for a regular revision of the PS list. Although the first revision was delayed in August 2013, the new PS Directive was published eventually [31]. The new directive will extend the PS list with 12 substances, 6 of them were identified as PHS (underlined below):

- Pesticides and biocides: aclonifen, bifenox, cybutryne, cypermethrin, dichlorvos, <u>dicofol</u>, <u>heptachlor and heptachlor epoxide</u>, <u>quinoxyfen</u>, terbutryn
- Industrial chemicals: <u>hexabromcyclododecane (HBCDD)</u>, <u>perfluorooctane sulfonic acid, and its derivatives (PFOS)</u>
- Byproducts of combustion processes: <u>dioxins and dioxin-like compounds</u>

For dicofol, PFOS, dioxins, and dioxin-like compounds, HBCDD and heptachlor and heptachlor epoxide biota EQS were derived.

In the revision proposal, also pharmaceutical substances (α-ethinyl estradiol, β-estradiol, diclofenac) were included for the first time, but their regulation in the PS list was postponed due to uncertainties regarding the exposure situation.

In parallel, also the existing PS have been revised. On the basis of new data, EQS have been adapted and lowered in most cases. For brominated diphenylethers, fluoranthene, and PAH, biota EQS were defined. Water AA-EQS for brominated diphenylethers, hexachlorobenzene, hexachlorobutadiene, mercury and its compound, and some compounds belonging to PAH were withdrawn. New substance information led to a change of status of 2 PS (DEHP, Trifluralin) to PHS. The new directive has to be transposed into national law of the member states until September 2015.

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Semivolatile Organic Compounds in Water, Suspended Particulate Matter, Sediments and Biota in the Danube

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Abstract During the second joint Danube survey (JDS 2) in autumn 2007, water, sediment, suspended particulate matter and mussel samples were collected from 23 sites covering the River Danube and important tributaries from Germany until the Black Sea. The compound classes investigated were polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochlorine pesticides (OCPs) and polyaromatic hydrocarbons (PAHs).

The results revealed no exceeding of the environmental quality standards (EQS) according to the Directive 2008/105/EC for all investigated compounds except the $\sum$benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, where the concentrations at most sites were close to the EQS of 2 ng/L. In five sites the EQS were slightly exceeded, with a maximum concentration 3.1 ng/L close to Bratislava.

OCP concentrations in water were orders of magnitude below the EQS except for HCH that reached levels up to 25% of the EQS in the lower Danube. Maximum PBDE concentration in water was at 20% of the EQS.

The longitudinal concentration profiles in water and sediment suggest DDT, HCH and to a lower extent chlordane and heptachlor releases into the lower Danube originating from left bank sources and tributaries especially Arges, Siret and Prut. PBDEs showed a maximum in the middle Danube stretch impacted from releases from the right bank tributaries such as Drava, Sava and Velika Morava.

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Concentrations in the investigated compartments were generally at the lower end of the concentration ranges typically found in European freshwaters.

Keywords Dissolved phase, Joint Danube survey 2, Mussels, Organochlorine pesticides (OCPs), Polyaromatic hydrocarbons (PAHs), Polybrominated diphenyl ethers (PBDEs), Polychlorinated biphenyls (PCBs), Polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), Sediment, Suspended particulate matter

Contents

Abbreviations

AA-EQS	EQS based on the average concentration of the substance concerned calculated over a 1-year period
ASE	Accelerated solvent extraction
B(ghi)P	Benzo(g,h,i)perylene
BDE	Brominated diphenyl ether
cDeca-BDE	Commercial decabromodiphenyl ether
cOcta-BDE	Commercial octabromodiphenyl ether

cPenta-BDE	Commercial pentachlorodiphenylether
CTRTAP	Convention on long-range transboundary air pollution
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DL-PCBs	Dioxin-like PCBs
EC6-PCBs	Sum of PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180
EI mode	Electron ionisation, electron impact
EPA	Environmental protection agency
EQS	Environmental quality standard
GC	Gas chromatography
GFF	Glass fibre filter
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HRMS	High-resolution mass spectrometry
ICPDR	International commission for the protection of the Danube River
I-TEQ	International toxicity equivalent
JDS	Joint Danube survey
JRC	Joint Research Centre of the European Commission
Ko/w	Octanol/water partition coefficient
LRMS	Low-resolution mass spectrometry
MAC-EQS	EQS based on the maximum allowable concentration
Milli-Q	Trademark by Millipore Corporation to describe 'ultrapure' water of 'Type 1', as defined by various authorities (e.g. ISO 3696)
NOEC	No observed effect concentration
OCPs	Organochlorine pesticides
PAHs	Polyaromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-*p*-dioxins and -dibenzofurans
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PTV	Programmed temperature vaporisation
SIM	Single ion monitoring
SOCs	Semivolatile organic compounds
SPM	Suspended particulate matter
UBA	Umweltbundesamt/federal environment agency
WFD	Water Framework Directive
WHO	World health organisation
WHO-TEQ	Toxicity equivalent acc. to WHO
XAD2	Nonpolar resin generally used for adsorption of organic substances from aqueous systems

1 Introduction

The target compounds of the cross-matrix screening programme were polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), organochlorine pesticides (OCPs) and polyaromatic hydrocarbons (PAHs), all of them semivolatile organic compounds (SOCs) with high octanol/water partition coefficients (Ko/w) and low vapour pressures. As a result of their lipophilicity, persistence and low-volatility SOCs tend to accumulate in the sediments and biota of aquatic environments.

In the aqueous phase, SOCs distribute between dissolved phase and suspended particulate matter (SPM), depending on their Ko/w and the amount and adsorptive properties of the SPM. The transport of the nonpolar SOCs in the river is mainly associated with the hydraulic remobilisation of sediments into the water column and the subsequent transport and re-sedimentation of the SPM.

An important objective of the second joint Danube survey (JDS 2) was to check the compliance with the environmental quality standards (EQS) according to the Directive 2008/105/EC [31].

Beyond the scope of the compliance checking spatially overlapping data from sediment, SPM, water and biota were generated, which would allow an insight into the interactions between the aquatic compartments relevant for storage, remobilisation, transport and bioaccumulation of SOCs.

2 Experimental

2.1 Overview on the Sampling Sites

Samples were collected from 23 sites on the Danube and its key tributaries over a distance of 2,600 km from Germany until the Black Sea. The selection of sites was based on the Transnational Monitoring Network of the International Commission for the Protection of the Danube River (ICPDR) and took into account transboundary aspects and major pollution sources. A geographical overview on the ‘23 super sites’ is given in Fig. 1.

At the end of the upper stretch (km 1,800), the river Danube reaches approximately one third of its final discharge into the Black Sea, with the tributary Inn (km 2,225) contributing about 50% of the discharge volume at km 1,800.

At the end of the middle stretch (Iron Gate at km 933), approx. 90% of the final discharge into the Black Sea appears. The most important tributaries are the Rivers Drava (km 1,379), Tisa (km 1,215), Sava (km 1,170) and to a smaller extent Velika Morava (1,103); they all contribute around 60% to the discharge of the Danube at the Iron Gate.

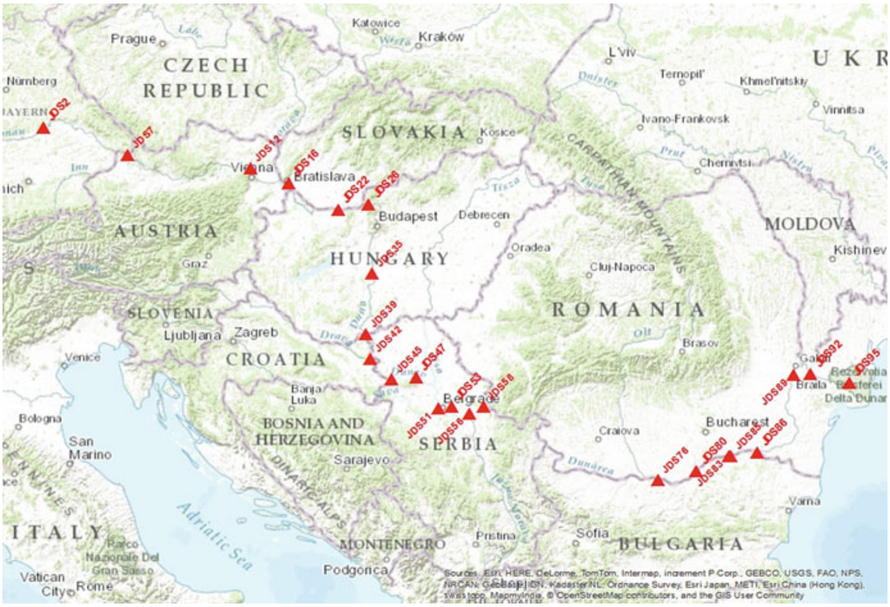

Fig. 1 Location of the 23 sampling stations for cross-matrix screening of SOCs

In the lower Danube, between the Iron Gate and the Black Sea, only a small increase of the discharge appears, mainly caused by the Rivers Siret (km 154) and Prut (km 135), contributing with about 5% to the discharge into the Black Sea.

More detailed information about the sampling sites can be found in the JDS 2 logbook under http://www.icpdr.org/jds/diary_sites.

2.2 *Investigated Compound Classes*

2.2.1 Polycyclic Aromatic Hydrocarbons

In aquatic systems PAHs tend to associate with SPM and accumulate in sediments but – compared to other SOC compound classes – only to some extent in biota, since they can be more easily metabolised than the halogenated aromatic SOC classes discussed below. Their transport within rivers is mainly driven by the hydraulic dynamics between with sediments and SPM. 16 EPA priority PAH plus benzo(e)pyrene and benzo(j)fluoranthene were analysed in water, SPM and sediments. The individual PAHs analysed were acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene, benzo(j)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-c,d)pyrene, phenanthrene and pyrene.

2.2.2 Organochlorine Pesticides

In aquatic systems OCPs tend to associate more (DDT) or less (HCH) with SPM and to accumulate in sediments and biota. Their transport within rivers is mainly driven by the hydraulic dynamics between with sediments and SPM. OCPs are toxic (including endocrine disruption) to aquatic organisms and mammals.

The individual OCPs and related metabolites analysed were α-HCH, aldrin, β-HCH, *cis*-chlordane, *cis*-nonachlor, dieldrin, endosulfan-α, endosulfan-β, endosulfan sulphate, endrin, γ-HCH (Lindane), hexachlorobenzene (HCB), heptachlor, heptachlor-*endo*-epoxide, heptachlor-*exo*-epoxide, Mirex, *o*,*p*-DDD, *o*,*p*-DDE, *o*, *p*-DDT, oxychlordane, *p*,*p*′-DDD, *p*,*p*-DDE, *p*,*p*-DDT, *trans*-chlordane, *trans*-nonachlor, δ-HCH, ε-HCH, isodrin and methoxychlor.

2.2.3 Indicator Polychlorinated Biphenyls

In aquatic systems PCBs tend to associate with SPM and accumulate in sediments and biota. Their transport within rivers is mainly driven by the hydraulic dynamics between with sediments and SPM.

Among the 209 isomers present in technical PCB mixtures, 6 Indicator PCBs (EC6-PCBs) have been selected for the characterisation of the presence of PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, PCB-180). The sum of their concentration is commonly reported as ‘Sum of Indicator PCBs’.

2.2.4 Polychlorinated Dibenzo-*p*-Dioxins and Dibenzofurans and Dioxin-Like Polychlorinated Biphenyls (DL-PCBs)

In aquatic systems PCDD/Fs and DL-PCBs tend to associate with SPM and accumulate in sediments and biota. Their transport within rivers is mainly driven by the hydraulic dynamics between with sediments and SPM.

Due to the risk for wildlife and humans arising from PCDD/Fs in sediments, quality objectives for PCDDs and PCDFs have been set. Out of eight approaches available [1], the tissue residue-based (TRB) method is the most commonly used. This method defines a safe chemical concentration in sediment, which results in an acceptable tissue concentration in biota. A no observed effect concentration (NOEC) of 200 pg of international toxicity equivalent (I-TEQ)/g dry weight (d.w.) in sediment was derived, but since only few chronic toxicity data were available, a safety factor of 10 was applied, which resulted in the proposal of a ‘safe sediment value’ of 20 pg I-TEQ/g d.w. [2].

The PCDD/F and DL-PCBs analysed were the 29 2,3,7,8 chlorine-substituted congeners included in the WHO-TEQ scheme.

2.2.5 Polybrominated Diphenyl Ethers

In aquatic systems PBDEs tend to associate with SPM and accumulate in sediments and biota. Their transport within rives occurs to a large extent associated with SPM and is driven by the hydrodynamics between water and sediments.

PBDEs were produced mainly in three commercial formulations, the so-called Deca-, Octa- and Penta-mixtures.

Commercial decabromodiphenyl ether (cDeca-BDE) consists mainly of BDE 207, BDE-208 and BDE-209.

Commercial octabromodiphenyl ether (cOcta-BDE) consists mainly of BDE 183, 196, 197 and 203. cOcta-BDE has recently been proposed to be added to the list of POPs under the UNECE convention on long-range transboundary air pollution (CTRTAP).

The commercial pentachlorodiphenylether (cPenta-BDE) mixture is included in the priority substance list of the WFD. The related AA-EQS for inland waters is 0.5 ng/L for the $\sum$ of BDE 28, 47, 99, 100, 153 and 154. In Europe the use of cPenta-BDE and cOcta-BDE is prohibited since 2003 [3].

The PBDEs analysed in this study were BDE-17 (Tri), BDE-28 (Tri), BDE-47 (Tetra), BDE-49 (Tetra), BDE-66 (Tetra), BDE-85 (Penta), BDE-99 (Penta), BDE-100 (Penta), BDE-153 (Hexa), BDE-154 (Hexa), BDE-183 (Hepta), BDE-196 (Octa), BDE-197 (Octa), BDE-203 (Nona), BDE-206 (Nona), BDE-207 (Nona), BDE-208 (Nona) and BDE-209 (Deca).

2.3 Materials and Methods

The Danube and its tributaries show low contamination levels with SOCs when compared to other European Rivers. During JDS 1 it had appeared that classic standard methods for water analyses based on liquid/liquid extraction of sample volumes of around one litre fail in the quantification of a series of compounds and often do not fit even the requirements for the compliance checking of existing EQS. Moreover the intention of the JDSs is not only compliance checking but also the creation of an overview of the baseline contamination, which, supplemented later on trough subsequent surveys, shall allow to look into time trends also for compounds that do not yet pose a risk. Also for the estimation of the pollutant loads into the Black Sea, sound data are needed, since flux estimates cannot be based on 'less than' concentration values.

In order to increase the sensitivity of quantification and with regard to the EQS set in the WFD, we used large volume sampling techniques both for SPM and the dissolved phase and quantified where necessary with HRMS, thus increasing the sensitivity by approximately an order of magnitude when compared to LRMS.

2.3.1 Sampling

Sediment

Sediments were obtained from 23 sites, among them 14 sites where both sides of the river were sampled. Sediments were sampled by sampling net, taking upper layer (ca. 5–10 cm) of the sediment at the places of the Kick & Sweep sampling for macro-zoobenthos and phyto-benthos. Ca. 10 kg sample was transported to the ship in PP buckets. This was followed by on-board grain size fractioning with wet sieving in order to separate the <63 μm fraction for analyses. The samples were stored in dark at 4°C and sent to the laboratory of Umweltbundesamt GmbH Vienna for freeze-drying.

Water: Dissolved Phase

Dissolved phase water samples were collected in situ on 50 g XAD-2 contained in modified extraction cartridges of the ASE extraction system. The methodology allowed to sample between 10 and 49.5 L of water, depending on the residence time at the sampling sites.

Water was pumped at a rate of 200 mL/min with a LIQUIPORT KNF NF 1.100 FT.18S PTFE-coated diaphragm pump (KNF FLODOS AG, Switzerland) through 8 mm i.d. Teflon tubing directly from the Danube River over a 293 mm (diameter) glass fibre filter (GFF) and the filtrate was extracted online by a modified ASE cartridge containing 50 g XAD 2 [4]. In some cases two cartridges were connected in series to check for eventual breakthrough. The GFF was transferred for transport and storage in a 500 mL Schott Duran borosilicate bottle and frozen until further processing, whereas the XAD containing cartridges were put in a fridge and transported back to the laboratory (arrived in blocks approximately one week after sampling at the lab), stored again at 4°C and processed in February 2008 by pressurised liquid extraction using a Dionex accelerated solvent extractor (ASE 300, Dionex Corporation, USA).

Two breakthrough experiments were executed (JDS 22 and JDS 92). For most PAHs breakthrough on the 2nd cartridge was <4% except for fluorene and phenanthrene which ranged up to 11% and 13%, respectively, in site JDS 22. The breakthrough for OCPs varied from a minimum of 2% for HCHs to the maximum of 15% for oxychlordane and from a minimum of 7% for PCB-28 to the maximum of 34% for PCB-189.

Suspended Particulate Matter

Twenty-three SPM samples were collected with a continuous-flow centrifuge mostly during cruising, while contemporarily the dissolved-phase water samples were collected through the Filter/XAD system described above. Centrifugation, preservation and storage were performed on board of Argus. The centrifuge was a Z61H from Carl Padberg Zentrifugenbau GmbH (Germany), operating at a cylinder speed of 17,000 rpm. Sampling typically took from 30 min to several hours, depending on the concentration of suspended solids in water. Preservation was attained through keeping the samples in the dark and refrigerated (or on ice during transportation) at between −20 and −50°C (ISO 5667-15). After shipping to UBA Vienna, the SPM samples were lyophilised and shipped to the JRC.

Mussel

Mussel samples were *Anodonta anatina*, *Sinanodonta woodiana*, *Unio pictorum* and *Unio tumidus* taken on 24 sites that were only partially identical with the 23 sites selected for the inter-matrix comparison. The samples were kept in the dark and refrigerated (or on ice during transportation) at between −20 and −50°C (ISO 5667-15). After shipping to UBA Vienna, the mussel samples were lyophilised and shipped to the JRC.

2.3.2 Analytical Methodology

A sample preparation method for determination of PCDD/Fs, EC-6 PCBs and DL-PCBs was adopted to include PBDEs in the analysis [5–7]. The analysis of all compounds was done using isotope dilution and GC/MS techniques, starting from one extract, where isotope-labelled standards were added for each analyte prior to extraction.

Ten percent of the extract was separated to analyse PAHs and OCPs (except for the dissolved phase where PCBs, PBDEs and PAH were analysed in the raw extract before splitting the sample). In the remaining 90% of the extract, PCDD/F, PCBs and PBDEs were analysed.

Materials

68-CVS and 68-LCS were native and 13C-labelled internal standards for 12 congeners' DL-PCBs (Wellington Laboratories Guelph, Ontario, Canada). EC-4058 was native for Indicator PCBs (CIL, Andover, Massachusetts, USA). 13C-labelled PCB-111 and PCB-170 were used as recovery standards (Wellington Laboratories Guelph, Ontario, Canada). EPA-1613CVS, EPA1613LCS and EPA-1613ISS were native, 13C-labelled internal and recovery standards, respectively, for 17 PCDD/Fs. The standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada). Ten 13C-labelled PBDE congeners were used as internal standards (in accordance with IUPAC nomenclature: BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-197, BDE-207 and BDE-209), nine were present in MBDE-MXE-STK solution (in accordance with IUPAC nomenclature: BDE-28, BDE-47, BDE-99, BDE-153, BDE-154, BDE-183, BDE-197, BDE-207 and BDE-209) and one BDE-100 was added from the solution MBDE-100. 13C-labelled BDE-126 and BDE-206 were used as recovery standards. BDE-MXE was native solution. All PBDE standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

Ten deuterated PAH isomers, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(g,h,i)perylene, benzo(b)fluoranthene, dibenz(a,h)anthracene, fluoranthene, fluorene and indeno(1,2,3-c,d)pyrene, were used as internal standards; deuterated acenaphthene, benzo(e)pyrene, benzo(k)fluoranthene and pyrene were used as recovery standards. All PAH standards were obtained from Dr. Ehrenstorfer GmbH, Augsburg, GER.

OCP internal standards were 13C labelled except for d8 *p,p*-DDD. Isotope-labelled aldrin, α-HCH, γ-HCH, *cis*-nonachlor, dieldrin, α-endosulfan, β-endosulfan, endrin, heptachlor, heptachlor-*endo*-epoxide (*trans*, isomer A), HCB, Mirex, *o,p*-DDD, *o,p*-DDT, Oxy-chlordane (gamma), *p,p′*-DDE, *p,p′*-DDT, *trans*-chlordane (gamma) and *trans*-nonachlor were used as internal standards.

13C-labelled β-HCH, *o,p*-DDE and *p,p′*-DDD were used as recovery standards. All OCP standards were obtained from Cambridge Isotope Laboratories.

All organic solvents used were Dioxin analysis grade (Sigma-Aldrich, Buchs SG, Switzerland). Sulphuric acid was 98% extra pure (VWR International s.r.l., Milan, Italy). Clean-up of PCDD/Fs, PCBs and PBDEs was conducted on ready to use multilayer (acidic silica, basic alumina and carbon) columns (Fluid Management Systems (FMS) Inc., Watertown, MA, USA).

Treatment of Solid Samples

The freeze-dried solid samples were extracted with a mixture of *n*-hexane/acetone (220/30) by Soxhlet for 48 h after spiking with isotope-labelled surrogate standards. For bottom sediments and SPM, copper powder was added to the solvent during the extraction to remove sulphur. For the further analysis of SPM, sediments and biota, 10% of the Soxhlet extract was separated to execute the combined clean-up of PAHs and OCPs. The remaining 90% of the extract was subjected to an automated clean-up for the purification and separation of the fractions containing PCDD/Fs, PCBs and PBDEs.

PCDD/Fs, PCBs and PBDEs

After treatment of the raw extract with conc. H_2SO_4 extract purification was executed with an automated clean-up system (Power-Prep P6, Fluid Management Systems (FMS) Inc., Watertown, MA, USA). This system was previously described [8] and uses a multilayer silica column (acid/neutral), basic alumina and carbon column combination. Two fractions were collected: one containing mono-*ortho* PCBs, Indicator PCBs and PBDEs and one for non-*ortho* PCBs and PCDD/Fs.

OCPs and PAHs

The n-hexane extracts from solid samples were submitted to a clean-up using 2 g of deactivated (10% H_2O) Alumina-B (Supelco) over a SPE cartridge containing 5 g of Florisil (Waters, WAT043370). The samples were eluted with 40 mL of CH_2Cl_2/*n*-hexane (1:2) vol/vol. After evaporation of the extract to 100 μL, the syringe standards for PAHs and OCPs were added. The sample was analysed in separate runs for OCPs and PAHs.

Treatment of Dissolved-Phase Water Samples

Dissolved-phase water samples were collected on 50 g XAD-2 contained in modified extraction cartridges of the ASE extraction system [4]. The cartridges were extracted using the Dionex ASE 300 applying in a first extraction methanol (3 cycles each with a static time of 5 min at 75°C, heat-up time of 5 min, a flush volume of 100%, a purging time of 60 s and a pressure of 1,500 psi) and in a second extraction *n*-hexane (same parameters as for methanol), respectively.

Surrogate standards were added to the hexane phase of the ASE after extraction. The methanol and hexane phases were combined in a separator funnel, and ca. 60–80 mL (1/3 of the volume of the methanol phase) Milli-Q water was added for improved phase separation.

After phase separation the methanol phase was collected in the ASE bottles and the hexane phase transferred into vials for concentration.

The methanol phase was extracted three times with 20 mL *n*-hexane and the hexane phases combined with the first extract from the ASE.

The combined extract was evaporated to 0.5 mL under purified N_2 using a TURBOVAP workstation (Zymak) and transferred into a 2 mL conic vial.

Labelled syringe standard (internal standard recovery check) was added before the final evaporation to 50 μL under a gentle stream of purified N_2.

PCBs, PBDEs and PAHs were analysed in the raw extract before splitting the sample. Subsequently 10% of the raw extract was separated for clean-up for OCPs (as described above for solid matrices). Extract purification of the remaining 90% was executed with an automated clean-up system (Power-Prep P6, Fluid Management Systems (FMS) Inc., Watertown, MA, USA) described above to obtain the fraction containing PCDD/Fs and coplanar PCBs.

Instrumental Analyses

All instrumental analyses of PCDD/Fs, PCBs and PBDEs were based on isotope dilution using HRGC-HRMS (high-resolution gas chromatography-high-resolution mass spectrometry) for quantification on the basis of EPA 1613 [32], EPA 1668 [33] and EPA 1614 [34] methods. OCPs were analysed using isotope dilution with HRGC-HRMS for quantification on the basis of an in-house method applying the QA/QC criteria laid down in the methods above for PCDD/Fs, PCBs and PBDEs.

Non-*ortho* PCBs, PCDD/Fs, PBDEs and OCPs were analysed on double HRGC (Thermo Trace GC Ultra, Thermo Electron, Bremen, Germany) and were coupled with a DFS high-resolution mass spectrometer (HRMS) (Thermo Electron, Bremen, Germany) operating in the EI mode at 45 eV with a resolution of >10,000. For non-*ortho* PCBs, PCDD/Fs, the two most abundant ions of the isotopic molecular cluster were recorded for both native and labelled congeners.

For tri- to octa-brominated congeners, two ions of the isotopic molecular cluster were recorded; for nona- and deca-brominated congeners, two isotopic ions of the cluster M ± 2Br were recorded for both native and labelled congeners. The quantified isomers were identified through comparison of retention times of the corresponding standard and the isotopic ratio of the two ions recorded.

Non-*ortho* PCBs, PCDD/Fs and OCPs were separated on a BP-DXN 60 m long with 0.25 mm i.d. (inner diameter) and 0.25 μm films (SGE, Victoria, Australia). The following gas-chromatographic conditions were applied for non-*ortho* PCBs, PCDD/Fs: split/splitless injector at 280°C, constant flow at 1.0 mL min^{-1} of He, GC-MS interface at 300°C and a GC programme rate starting at 160°C with a 1 min hold, then 2.5°C min^{-1} to 300°C and a final hold at 300°C for 8 min.

Gas chromatographic conditions for OCPs were split/splitless injector at 250°C, constant flow at 1.0 mL min^{-1} of He, GC-MS interface at 270°C and a GC programme rate: 100°C with a 1 min hold, then 10°C min^{-1} to 300°C and a final hold at 300°C for 9 min.

PBDEs were analysed on a Sol-gel-1 ms, 15 m with 0.25 mm i.d. and 0.1 μm film GC column (SGE, Victoria, Australia). The following gas-chromatographic conditions were applied: PTV injector with temperature programme from 110 to 300°C at 14.5°C s^{-1}, constant flow at 1.0 mL min^{-1} of He, GC-MS interface at 300°C and a GC programme rate (110°C with a 1 min hold, then 20°C min^{-1} to 300°C and a final hold at 300°C for 6 min). The selection of the chromatographic conditions was optimised following the literature indications [5,9–11].

Mono-*ortho* PCBs and Indicator PCBs were analysed on a GC (HP-6890, Hewlett Packard, Waldbronn, Germany) coupled with a VG Autospec Ultima high-resolution mass spectrometer (Micromass, Manchester, UK) operating in EI mode at 34 eV with a resolution of >10,000.

Mono-*ortho* PCBs were separated on a HT-8 capillary column, 60 m long with 0.25 mm i.d. and 0.25 μm film (SGE, Victoria, Australia).

Gas chromatographic conditions for mono-*ortho* PCBs were split/splitless injector at 280°C, constant flow at 1.5 mL min^{-1} of He, GC-MS interface at 280°C and a

GC programme rate starting from 120°C with 20°C min^{-1} to 180°C, 2°C min^{-1} to 260°C and 5°C min^{-1} to 300°C isotherm for 4 min.

PAHs were analysed by GC/LRMS consisting of a GC (6,890 N Agilent Technologies) coupled to a low-resolution mass selective detector (5,973 Agilent Technologies), an autosampler and a PTV injector (CIS 4 Gerstel). The GC-MS was operated in single ion mode (SIM), and quantification was performed by using ten deuterated internal standards and four syringe standards. The GC separation was performed on a J&W DB-5MS capillary column (60 m × 0.25 mm × 0.25 μm).

Gas chromatographic conditions for PAHs were split/splitless PTV injection (temperature ramp 80–300°C at 12°C s^{-1}, constant flow at 1 mL min^{-1} of He, GC-MS interface at 300°C and a GC programme rate: starting from 100°C for 1 min isotherm with 7°C min^{-1} to 280°C for 12 min isotherm, with 12°C min^{-1} to 310°C for 28 min isotherm.

QA/QC

The quantified isomers were identified through retention time comparison of the corresponding standard, and the isotopic ratios between two ions were recorded for all halogenated compounds analysed.

Reference materials were analysed in parallel with sediments and SPM samples for PCDD/Fs, DL-PCBs and PBDEs. The concentrations detected were in accordance with the reference values.

Levels of analytical blanks obtained during the clean-up process were at least 5–10 times lower of the reported concentrations for all compounds studied. The blank level was not subtracted. The reported detection limits were calculated on the basis of a signal to noise ratio of 3/1.

Several duplicate samples were performed in order to keep under control the QA/QC and the method reproducibility for the compounds where reference materials were not available. During the analysis of OCPs, a *p,p′*-DDT standard was injected every tenth sample in order to check for DDT degradation inside the injector system. If degradation occurred the liner was replaced and the GC column cut or replaced.

3 Results and Discussion

In the following an overview on the average abundance of the pollutants in sediments, SPM, dissolved phase and mussels will be given and EQS values will be discussed as far as applicable. In addition Danube downstream concentration profiles will be discussed. PCDD/Fs and DL-PCBs are reported as 2,3,7,8 TCDD toxicity equivalents applying the WHO 1998-TEFs [12].

Sediment can be considered as long- to mid-term memory of pollutant discharges into the Danube River. Changes in pollutant loads in sediments occur in the range of decades. Therefore the concentrations in the sediments from different sampling stations can be compared even though not collected contemporarily.

By looking at the concentration changes in sediments downstream the Danube, it is possible to locate sources or the influence from incoming ‘clean tributaries’. The occurrence of a source is furthermore indicated through differences in concentrations between left- and right-hand sediment samples, since inlets from one side of the river need many kilometres to mix homogeneously along the medial profile of the river.

The downstream concentration profile in SPM and water is more a snapshot and depends very much on the momentary hydraulic conditions (sedimentation/ remobilisation) in the watershed, as a significant fraction of SOCs is transported associated with SPM. Due to the ‘short memory’ of the water column, the samples taken during JDS 2 cannot be regarded as taken contemporarily. Therefore, the water data are less suitable for the indication of spatial patterns of contamination and should not be over-interpreted with that respect. To localise current sources of contamination, annual concentration averages of the water column obtained with a considerably dense temporal resolution would be needed.

Mussels were analysed only for PCBs and PCDD/Fs and cPenta-BDE.

All concentration data reported for solids are given on a dry weight basis.

The results presented for all SPM-associated concentrations in the water column were calculated from the concentrations measured by the JRC in the SPM samples generated with a centrifuge along the transects and the suspended solid concentrations in water measured gravimetrically by the ‘Institute for Limnology’ in Mondsee, Austria, from filtration samples taken contemporaneously during JDS 2.

3.1 Compliance with EQS Set by the Directive 2008/105/EC

For all priority substances, EQS in inland surface waters were set as the annual average concentration (AA-EQS) and for some of them also as maximum allowable concentration (MAC-EQS). In Table 1 the results obtained during JDS 2 are compared with the corresponding EQS.

3.1.1 OCPs and cPenta-BDE

The concentrations of OCPs and cPenta-BDE in water were all below related annual average (AA)-EQS, most of them by more than one or two orders of magnitude. Only HCHs reached the order of magnitude of the AA-EQS along the lower stretch of the Danube downstream river km 1,000. Average cPenta-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 AA-EQS of 500 pg/L.

Table 1 Overview on concentrations in the water subject to WFD EQS

$n = 23$	Av (ng/L)	Med (ng/L)	Range (ng/L)	AA-EQS (ng/L)	MAC-EQS (ng/L)
Anthracene	0.47	0.39	0.13–1.5	100	400
Fluoranthene	3.1	2.9	1.8–6.8	100	1,000
Benzo(a)pyrene	0.72	0.73	0.4–1.2	50	100
∑Benzo(g,h,i)perylene, Indeno(1,2,3-cd)pyrene	1.5	1.3	0.43–3.2	2	–
∑Benzo(b)-, benzo(k)fluoranthene	1.4	1.3	0.42–3.8	30	–
Naphthalene			<250*	2,400	–
HCHs (∑α-, β-,γ-,δ-, ε-HCH)	2.7	0.79	0.17–11.4	20	40
HCB	0.059	0.050	0.02–0.11	10	50
p,p′-DDT	0.047	0.028	0.006–0.26	10	–
Total DDT (∑*p,p′*-DDT, *p,p′*-DDE, *p, p′*-DDD, *o,p*-DDT)	0.21	0.13	0.038–1.2	25	–
Cyclodiene pesticides (∑aldrin, dieldrin, endrin, isodrin)	0.023	0.025	0.002–0.046	10	–
Endosulfan (∑α-, β)	0.012	0.010	0.004–0.017	5	10
cPenta-BDE, ∑BDE-28, 47, 99, 100, 153,154)	0.057	0.051	0.025–0.12	0.5	–

AA-EQS is the EU quality standard for the annual average concentration in inland surface waters; MAC-EQS is the maximum allowable concentration

3.1.2 PAHs

The concentration of most of the PAHs in water was at least one order of magnitude below the AA-EQS except for the ∑benzo(g,h,i)perylene and indeno(1,2,3-cd) pyrene, where the limit was exceeded in 5 sites out of 23 (Fig. 2). The stations were 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). However, the maximal concentration was around 1.6 times the AA-EQS during one day in summer 2007. Thus, the annual average concentration might as well be below the EQS. Therefore, and since no MAC-EQS exists for ∑benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, it remains unclear whether or not the Danube is within the EQS for these compounds.

Naphthalene data reported in Table 1 have been analysed by Literathy et al. [13] during the JDS 2 survey. All samples were below the LOQ of 0.25 μg/L of the ISO 17993 method applied, thus clearly below the AA-EQS of 2 ng/L.

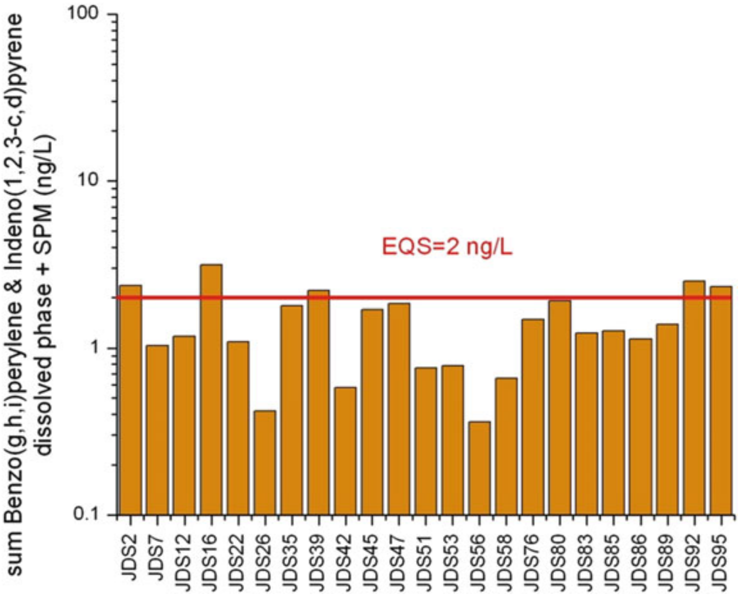

Fig. 2 Concentration of ∑benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene in water

3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

3.2.1 Overview on All Matrices

PAHs were determined in sediments, SPM and the dissolved phase.

The reported ∑PAH data refer to the ∑16 EPA priority PAH plus benzo(e) pyrene and benzo(j)fluoranthene in water, SPM and sediments (Fig. 3, Table 2).

Among the ∑16 EPA PAH, no explicit quantitative data could be obtained for naphthalene, acenaphthylene and acenaphthene, since the extraction conditions, optimised for PCDD/Fs and PBDEs, lead to low recoveries for the volatile PAHs. However, the semi-quantitative results obtained for the naphthalene, acenaphthylene and acenaphthene in SPM and sediments suggest a minor contribution to the ∑EPA PAH between 7% and 4% at average. We assume therefore that the ∑PAH data reported here can be compared with literature data referring to ∑EPA PAH.

Most sediment and SPM samples display moderate ∑PAH concentrations in a range of 250–750 μg/kg with extreme values of up to 2,600 μg/kg for SPM. 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 [14]. From the River Seine estuary, PAH data from SPM are available. The ∑11 PAH determined there overlaps with the ∑PAH from the JDS 2 except for fluorene, anthracene and dibenz(a,h)anthracene, which play only a minor role in the sediment pattern. For sediments a median of

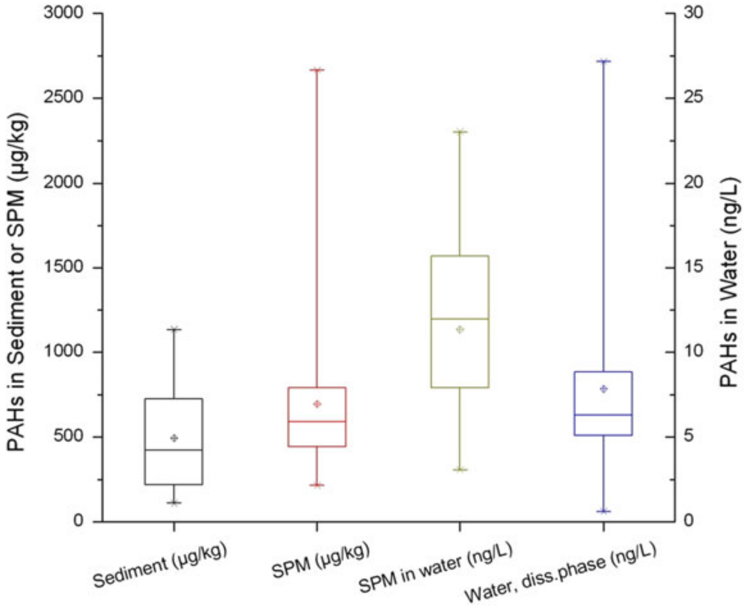

Fig. 3 ∑PAH concentrations in all abiotic compartments, box-whiskers diagram, *boxes* represent the 25/75 percentiles with median (——) and average (⌖), and the whiskers represent minimum and maximum values

Table 2 ∑PAH concentrations in all abiotic compartments

	Sediment (µg/kg)	SPM (µg/kg)	Water SPM (ng/L)	Water dissolved (ng/L)
Average	493	696	11	7.8
Median	407	590	12	6.3
Min	111	216	3.1	0.62
Max	1,135	2,665	23	27
25-Percentile	220	436	6.9	5.0
75-Percentile	712	787	15	8.8

2.65 mg/kg is reported [15], which corresponds to the extreme value in SPM measured during JDS 2. In ten sediment samples taken in 2002 along the German stretch of the Danube, ∑16 EPA PAH concentrations of 0.24–5.3 mg/kg were reported [16].

Among all sediment sites sampled during JDS 1, the ∑16 EPA PAH ranged between 2 and 16 mg/kg at 16 sites, which is considerably higher than the maximum level of 1.3 mg/kg detected during JDS 2. This suggests a decrease in PAH content in the Danube sediments since 2001. However, before concluding, the techniques applied for the sediment sampling during both campaigns should be carefully evaluated for their inter-comparability. Among the PAHs that were quantified in sediments and SPM, the most abundant were fluoranthene and pyrene.

In the water column, significant amounts of PAHs are associated with SPM, in particular the higher boiling compounds. Average (dissolved plus SPM) concentrations of ∑PAH around 17 ng/L and a maximum of 35 ng/L were detected in water, which is at the lower end of typical findings in the River Elbe [14]. The comparably low contamination level with PAHs in Danube water is further illustrated by comparing with data from the Seine estuary where an average/median concentration of 187/172 ng/L has been reported for the ∑11 PAH [15].

3.2.2 Downstream Concentration Profile

Sediments (Fig. 4)

The sediments at site JDS 02_L display comparably high PAH concentrations, which indicate an input from the tributary Altmuehl/Rhein-Main-Donau Channel, supported by the comparably high PAH content of the SPM (not reported here) at this site.

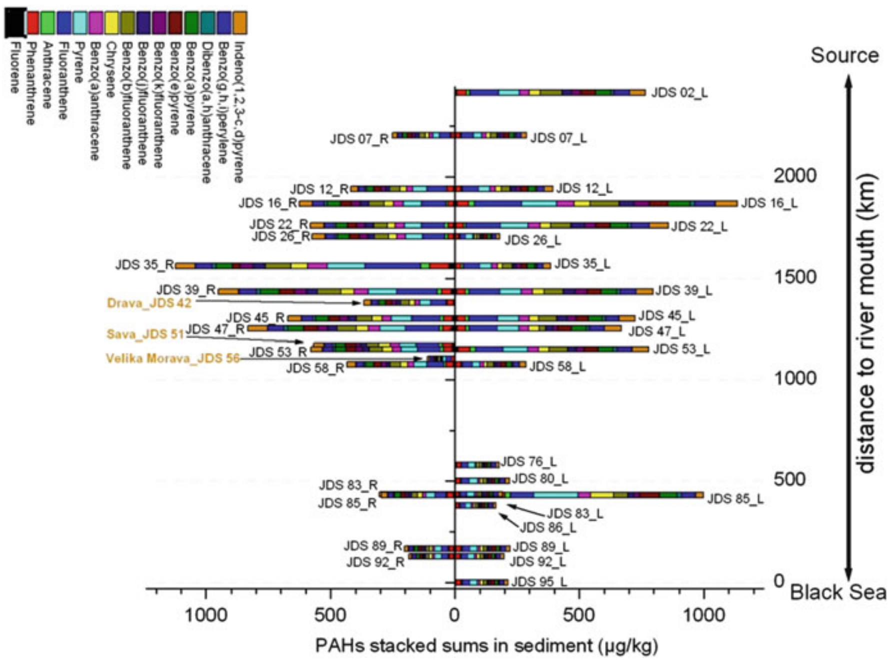

Fig. 4 Downstream concentration profile of PAHs in sediments

Site JDS 07 (AT) after the inlet of the tributary Inn shows lower concentrations similar on both sides of the Danube, which suggests a diluting effect of the River Inn for PAHs in the Danube.

Site JDS 16 shows an increase in concentration, in particular on the left-hand side downstream of the inlet of the tributary Morava, indicating an input from the tributary Morava.

Site JDS 22 shows a similar asymmetry in concentrations with a higher concentration on the left-hand side downstream of the inlet of the tributary Vah, which indicates a moderate input from the tributary Vah.

Site JDS 26 shows a concentration drop on the right bank downstream the mouth of the tributaries Hron and Ipoly, which indicates a dilution due to low PAH levels of the rivers Hron and Ipoly.

Site JDS 35 shows a strong asymmetry in the sediments with high concentrations on the left-hand side. This might be still due to the dilution influence of the rivers Hron and Ipoly entering left bank upstream. Another possibility is an unknown source (since no tributary enters in this section) on the right-hand side.

At site JDS 42 the sediment sample was taken inside the tributary Drava entering the Danube from the left bank. The sediments in the River Drava contain considerably less PAH than the Danube itself, and also the PAH content in the SPM is low, which indicates a dilution due to low PAH levels in the tributary Drava.

The sediments at site JDS 51 taken in the tributary Sava displayed about two times lower PAH levels in the sediments, when compared to the samples from the corresponding Danube stretch. Site JDS 56 inside the tributary Velika Morava displayed even five times lower PAH concentrations in sediments and SPM. This indicates a diluting effect of both tributaries as regards PAHs.

The sampling sites downstream the Iron Gate reservoir mostly display comparable low PAH concentrations in the sediments and SPM, indicating a sink for SPM-associated PAHs in the reservoir.

PAH inputs downstream the Iron Gate seem to be low, except at the inlet of the tributary Arges entering from the left-hand side between the sampling sites JDS 83 and JDS 85. A significant rise of PAHs in sediments after the inlet is visible in between sampling stations JDS 83 and JDS 85_L, indicating the tributary Arges being a source of PAHs into the lower stretch of the Danube. However, in this case, there was no confirmation through the SPM data, which points to historic inputs rather than recent ones.

Site JDS 89, which according to the cruise protocol is suspected to be impacted by an oil refinery, shows no abnormalities regarding PAHs in sediments, SPM and water.

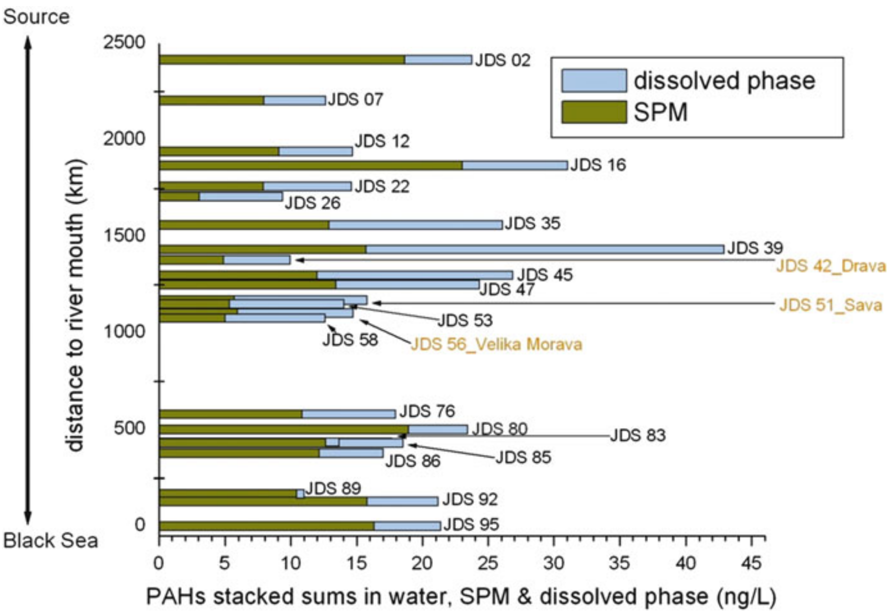

Fig. 5 Downstream concentration profile of PAHs in water

Water (Fig. 5)

Looking at the whole water column, the ∑PAHs show a more equilibrated situation with low concentrations in the tributaries Drava (JDS 42), Sava (JDS 51) and Velika Morava (JDS 56) as observed in the sediments above. The maximum concentration of ∑PAH in the water was 42 ng/L found at JDS 39 (border station HU/HR), with a comparably high contribution from the dissolved phase.

3.3 *Hexachlorocyclohexane (HCH, ∑α-, β-, γ-, δ-HCH)*

3.3.1 Overview on All Matrices

The group of HCHs includes eight isomers. The EQS for HCH refers to α-, β-, γ- and δ-HCH, the four 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 (Fig. 6, Table 3).

Sediments and SPM display similar concentrations with average values below 1 μg/kg. For comparison: In the River Elbe, average values in the sediments of the CZ stretch were around 15 μg/kg (0.69–104 μg/kg), followed by levels up to 224 μg/kg after the confluence of the contaminated tributary Mulde in Germany [17]. In the water column, HCHs were detected almost exclusively in the dissolved phase.

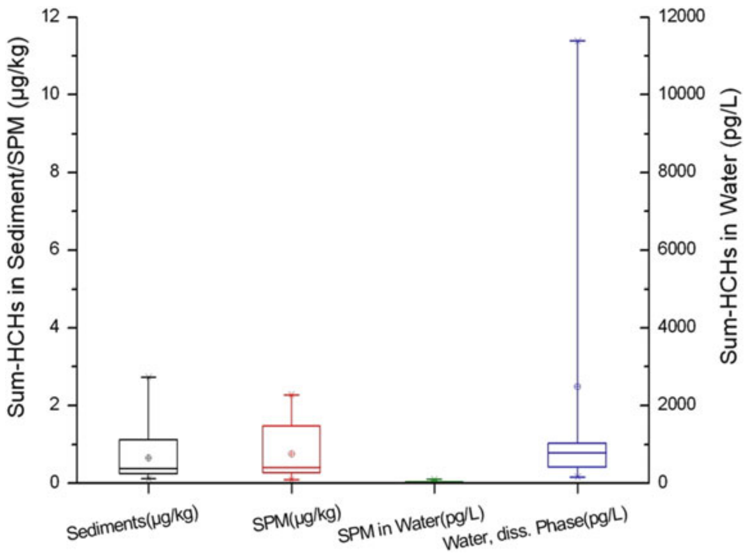

Fig. 6 ∑HCH concentrations in all abiotic compartments

Table 3 ∑HCH concentrations in all abiotic compartments

	Sediment (µg/kg)	SPM (µg/kg)	SPM in water (pg/L)	Water dissolved (pg/L)
Average	0.66	0.77	23	2,489
Median	0.35	0.42	5.1	752
Min	0.12	0.091	1.2	164
Max	2.7	2.3	105	11,386
25-Percentile	0.25	0.26	2.4	414
75-Percentile	1.1	1.5	42	2,431

For HCHs in water, the AA-EQS is 0.02 µg/L and the MAC-EQS is 0.04 µg/L; both of them were not exceeded. The maximum of ∑HCHs in the water column was 0.011 µg/L at site JDS 85 downstream of Arges (RO/BG).

3.3.2 Downstream Concentration Profile

Sediment (Fig. 7)

In the sediments HCH concentrations display a higher abundance in the samples taken on the left-hand side.

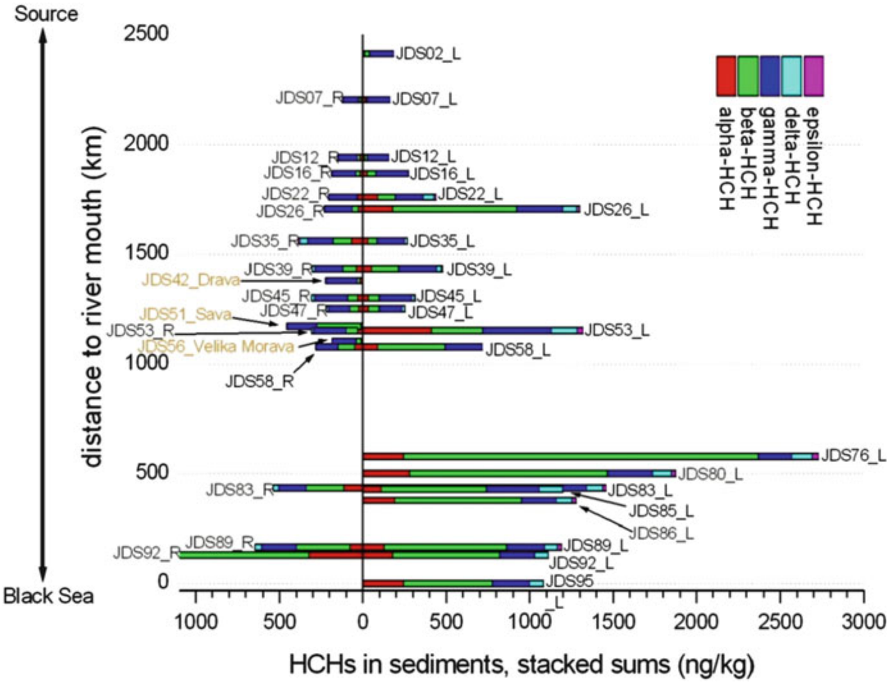

Fig. 7 Downstream concentration profile of HCHs in sediments

The sediments on the left-hand side of the middle stretch display the two distinct maxima: first at JDS 26 (HU), indicating a historic influence of the Hron (km 1,716) and Ipoly (km 1,708) tributaries entering only a few kilometres upstream on the left-hand side (in the tributary Hron high Lindane concentrations were detected during JDS 1), and second at JDS 53 (RS), downstream Pancevo situated on the left-hand side of the Danube, where high Lindane concentrations were detected also during JDS 1. Sediments taken in the tributaries Drava (JDS 42), Sava (JDS 51) and Velika Morava (JDS 56) display low concentration levels similar to those in the Danube sediments taken on the right-hand side.

In the lower Danube stretch from JDS 76 (RO/BG) downstream, a general tendency towards higher concentrations was observed. JDS 76 is located only 26 km downstream of the Olt Tributary entering from the left-hand side, where high Lindane concentrations were found also during JDS 1. The increase in HCH concentrations in the dissolved phase downstream the Olt Tributary goes along with a change of the HCH concentration pattern.

Water (Fig. 8)

Similar to the sediments, the downstream profile in the dissolved phase displays low HCH concentrations in the upper and middle stretch. A sharp increase was observed starting from site JDS 76 (RO/BG) downstream the Olt Tributary that had shown high Lindane concentrations during JDS 1 as well.

Most sites downstream the Olt Tributary remain at a high HCH level in the dissolved phase. The historic signals observed more upstream in the sediments at JDS 26 and JDS 53 are no longer visible in the dissolved phase.

The samples from the tributaries Drava (JDS 42) and Sava (JDS 51) display slightly lower concentrations than the Danube itself. The Velika Morava Tributary (JDS 56) shows, as for SPM, slightly higher concentrations in the dissolved phase as well.

The sharp increase in HCH concentrations in the dissolved phase of the lower stretch goes along with a significant change of the HCH concentration pattern: In the upper stretch of the Danube (JDS 02 to JDS 16), the sum of HCHs consists almost exclusively of γ-HCH. In the section between JDS 22 and JDS 58, the abundance of α-, β-HCH equals that of γ-HCH, and from site JDS 76 all sites showing high HCH concentrations in the dissolved phase are dominated by α- and β-HCH. A similar tendency can be seen in the sediment and to a lower extent in SPM (not reported here).

We got no explanation for the low HCH values observed at the sites JDS 80 and JDS 89. In the whole section of the lower Danube downstream the Iron Gate, no important tributaries come in, which might have caused a dilution effect explaining

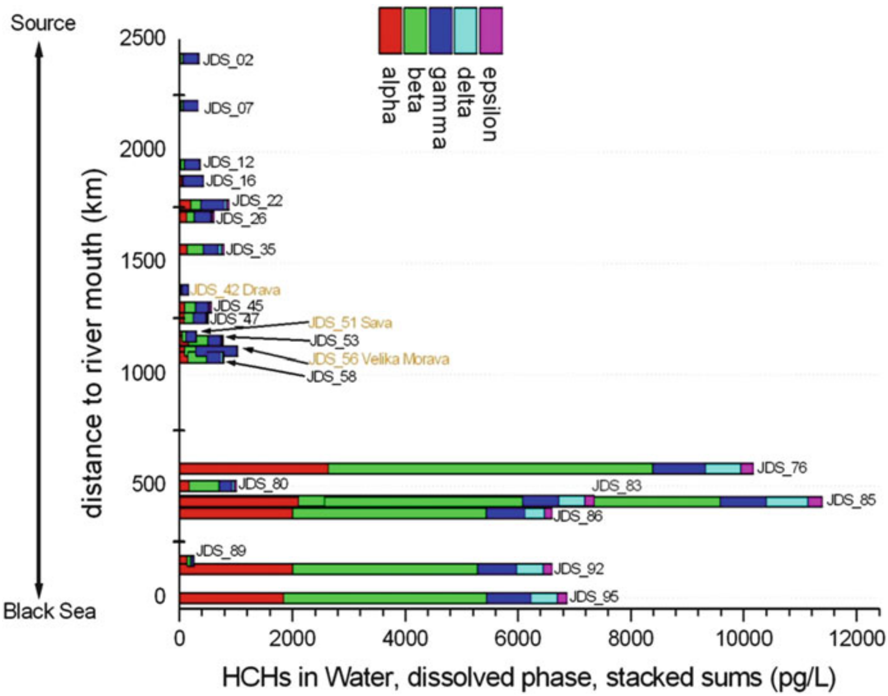

Fig. 8 Downstream concentration profile of HCHs in water, dissolved phase. HCHs associated with SPM are negligible

the locally low HCH findings on these two sites. A sampling error seems unlikely, since the concentration of other compounds as PCBs, PBDEs and OCPs in the dissolved phase do not show comparable spatial variations in that stretch.

3.4 Hexachlorobenzene

3.4.1 Overview on All Matrices

Average concentrations in sediments and SPM were around 1 and 0.65 μg/kg, respectively.

In the water column HCB was detected both in SPM and the dissolved phase, with a tendency towards the dissolved phase in the upper stretch and a stronger association with SPM in the lower stretch. The maximum value for HCB at site JDS 92 (RO) was 0.11 ng/L, which is around two orders of magnitude below the respective AA-EQS of 10 ng/l and the MAC-EQS of 50 ng/l (Fig. 9, Table 4).

3.4.2 Downstream Concentration Profile

Sediment (Fig. 10)

In the sediments a tendency of enhanced HCB concentrations in the samples taken on the left-hand side can be seen; however, it is less pronounced as above for the

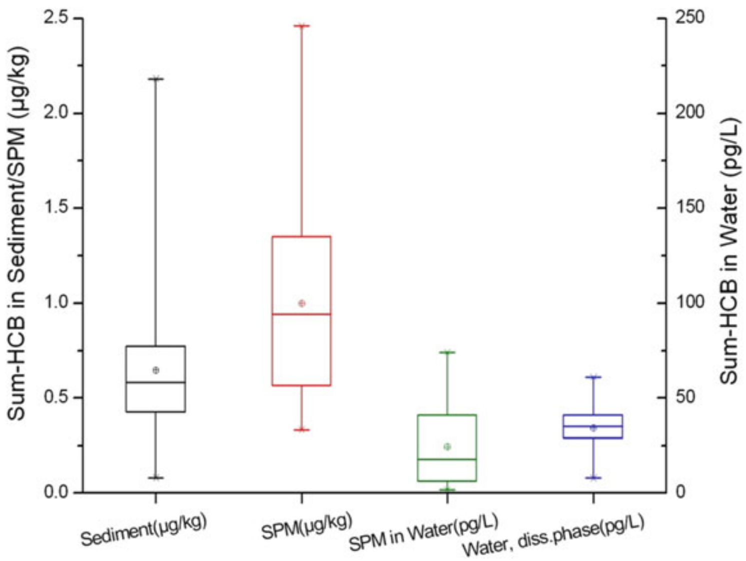

Fig. 9 HCB concentrations in all abiotic compartments

Table 4 HCB concentrations in all abiotic compartments

	Sediment (μg/kg)	SPM (μg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	0.65	1.0	25	34
Median	0.58	0.94	18	35
Min	0.081	0.33	1.8	7.9
Max	2.2	2.5	74	61
25-Percentile	0.42	0.51	6.1	28
75-Percentile	0.79	1.3	38	41

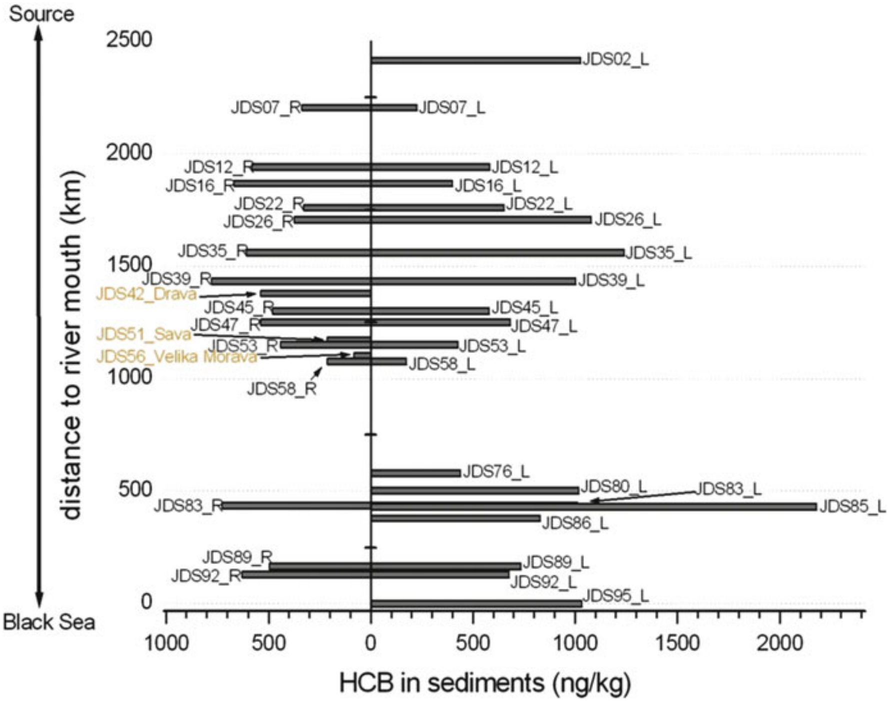

Fig. 10 Downstream concentration profile of HCB in sediments

HCHs. An influence of the tributary Altmuehl appears in the sediments at JDS 2 (DE), and comparably high levels at JDS 85 (RO) suggest a historic impact from the tributary Arges.

Water (Fig. 11)

In the water column, HCB does not show particular gradients in the downstream profile, except for slightly higher concentrations in the lower stretch, together with a higher abundance of SPM-associated HCB.

The SPM associate portion of HCB increases in the lower stretch.

The water samples from the tributaries Drava (JDS 42, HR/RS) and Velika Morava (JDS 56, RS) show comparable concentrations as in the Danube itself, whereas the sample from the tributary Sava (JDS 51, RS) displays lower concentrations.

3.5 DDT and Metabolites (p,p′-DDT, p,p′-DDE, p,p′-DDD, o,p-DDT)

3.5.1 Overview on All Matrices

Average concentrations of $\sum p,p'$-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT in sediments were 6.6 μg/kg and slightly lower in SPM with 4.4 μg/kg.

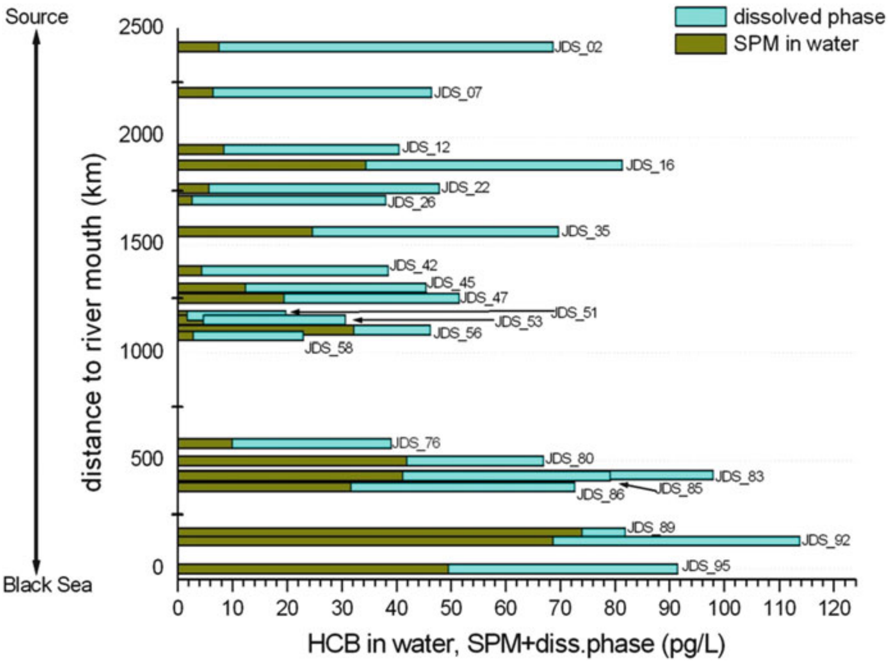

Fig. 11 Downstream concentration profile of HCB in water

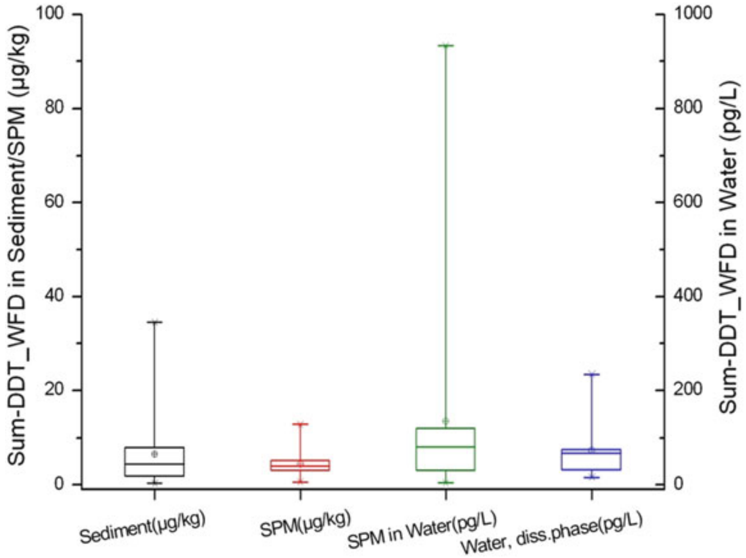

Fig. 12 ∑DDT and metabolite concentrations in all abiotic compartments

Table 5 ∑DDT and metabolite concentrations in all abiotic compartments

	Sediment (µg/kg)	SPM (µg/kg)	SPM in water (pg/L)	Water dissolved (pg/L)
Average	6.6	4.4	135	74
Median	4.5	4.0	81	66
Min	0.36	0.63	4.6	16
Max	35	13	933	234
25-Percentile	1.9	3.0	27	37
75-Percentile	7.8	5.0	111	75

In the water column, DDT and its metabolites were detected to a larger extent associated with SPM. The maximum concentration of $\sum p,p'$-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT in the water column was around 1.2 ng/L at sites JDS 92, 95 (RO), which is more than one order of magnitude below the AA-EQS of 25 ng/L.

This maximum corresponds to high DDT concentrations in SPM detected during JDS 1 (Fig. 12, Table 5).

3.5.2 Downstream Concentration Profile

Sediments (Fig. 13)

In sediments, DDT and metabolites show tendentially higher concentrations in the samples taken on the left-hand side, except at site JDS 92 (RO/UA) after the inlet of

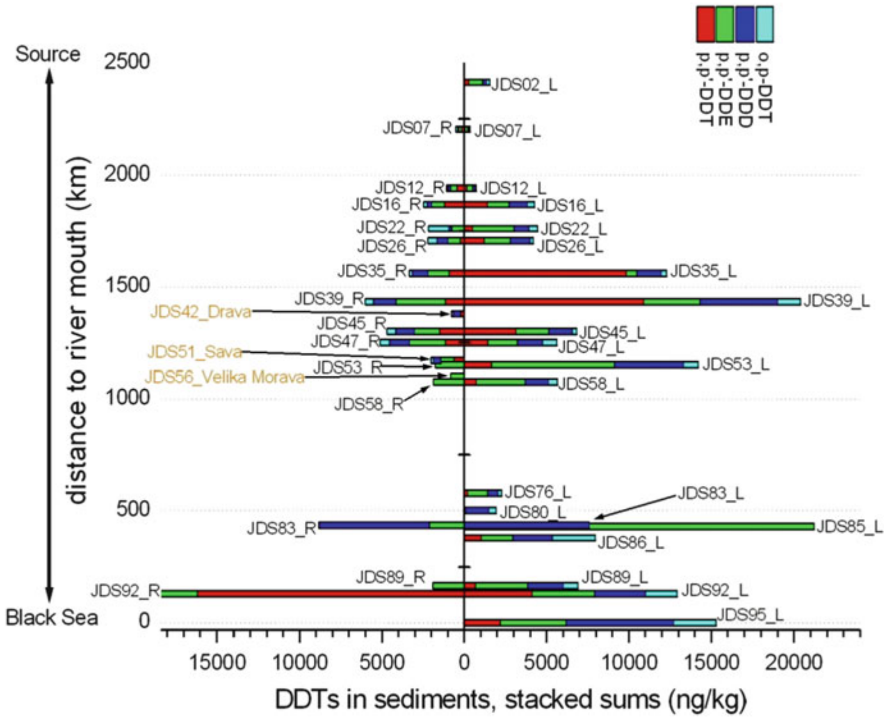

Fig. 13 Downstream concentration profile of p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT in sediments

the tributaries Siret and Prut entering from the right-hand side. This site displayed also the maximum abundance in SPM-associated DDT (not reported here) and in the water column, thus confirming the high p,p'-DDT concentrations reported from this site in SPM during the JDS 1 cruise. In contrast, the other tributaries entering from the right-hand side (Drava, Sava and Velika Morava) displayed low concentrations in their sediments. Historic (since not visible in the water column) intakes from the left-hand side are indicated at sites JDS 35, JDS 39, JDS 53 and JDS 85. However, none of these left-hand sites showed a significant signal in the water column,

Water (Fig. 14)

In water only JDS 92 and JDS 95 appear as sites of considerably enhanced concentrations. The sites in the middle stretch that had displayed higher DDT concentrations in the sediments do not result in high concentrations in water. This suggests that for DDT and metabolites the only significant current sources are in

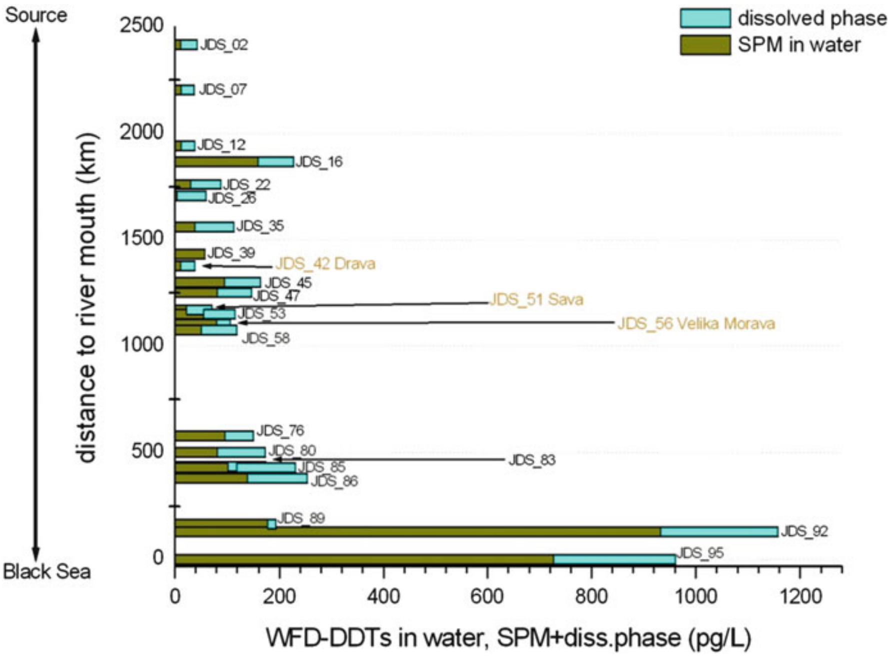

Fig. 14 Downstream concentration profile of $\sum p,p'$-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT in water

between JDS 89 (upstream tributaries Siret and Prut) and JDS 92 (downstream tributaries Siret and Prut).

In the water column, the share of SPM-associated DDT and metabolites in general rises towards the Black Sea.

3.6 *Cyclodiene (∑Aldrin, Dieldrin, Endrin, Isodrin)*

3.6.1 Overview on All Matrices

Average concentrations in sediments were 0.046 μg/kg, while SPM displayed higher average concentrations of 0.090 μg/kg. In sediments isodrin and endrin were < LOD in all samples. In SPM isodrin was < LOD in all samples.

In the water column, ∑aldrin, dieldrin, endrin, 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 the ∑cyclodiene, endrin concentrations were always dominant. In the statistics and the figure below, only quantified concentration data are included.

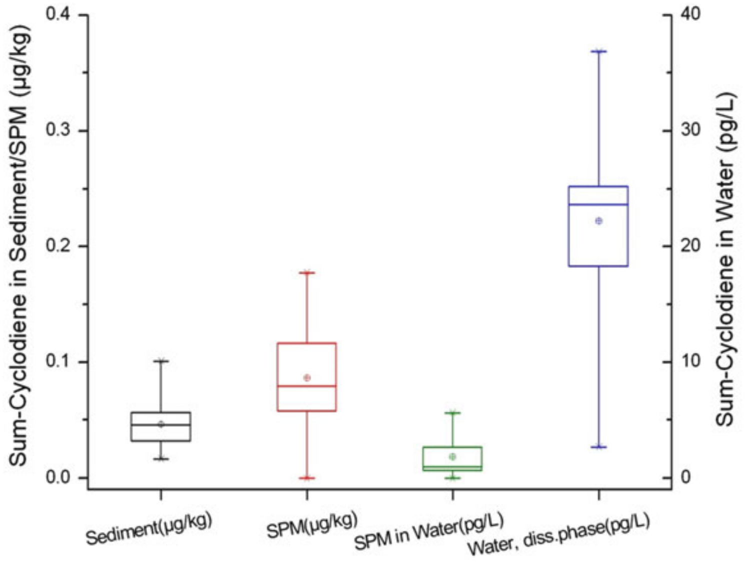

Fig. 15 ∑Aldrin, dieldrin, endrin, isodrin concentrations in all abiotic compartments

Table 6 ∑Aldrin, dieldrin, endrin, isodrin concentrations in all abiotic compartments, dissolved phase upper bound in brackets

	Sediment (µg/kg)	SPM (µg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	0.046	0.090	1.9	22 (29)
Median	0.046	0.080	0.98	24 (28)
Min	0.017	0	0	2.7 (15)
Max	0.10	0.18	5.6	37 (61)
25-Percentile	0.032	0.062	0.64	19 (22)
75-Percentile	0.055	0.12	2.7	25 (33)

Even when calculating upper bound concentrations in water, the ∑aldrin, dieldrin, endrin, isodrin remain more than two orders of magnitude below the respective AA-EQS of 10 ng/L (Fig. 15, Table 6).

3.6.2 Downstream Concentration Profile

Sediment (Fig. 16)

The downstream profile in sediments displays an influence of the tributary Altmuehl visible in the sediments of site JDS 02 (DE). Concentrations decrease then downstream JDS 02, suggesting a dilution from the tributary Inn confluence at

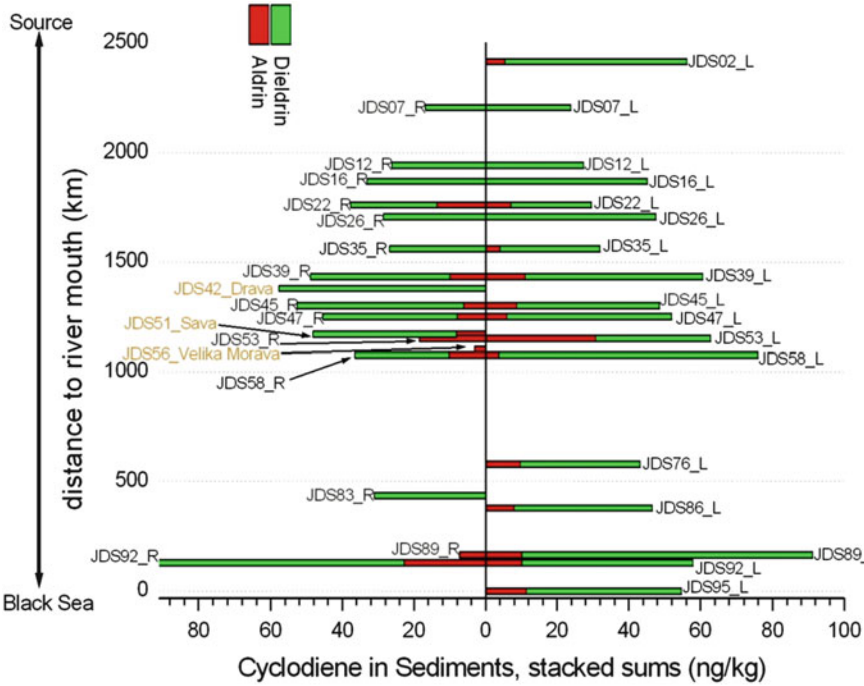

Fig. 16 Downstream concentration profile of ∑cyclodiene in sediments

km 2,225. A slight rise in concentration becomes visible along the middle stretch of the Danube. After the Iron Gate concentrations are somewhat lower except at JDS 89 and JDS 92 in Romania.

In SPM (not displayed here) the gradient is similar, however, with concentration maxima more upstream around JDS 85 (RO/BG).

In all sediment samples, the values for endrin and isodrin were < LOD.

Water (Fig. 17)

The downstream profile in the dissolved-phase water displays a slight trend of higher concentrations towards the Black Sea. As in the sediments, mainly Dieldrin was detected. The dissolved-phase water samples from the tributaries Drava (JDS 42, HR), Sava (JDS 51, RS) and Velika Morava (JDS 56, RS) display lower concentrations than the Danube itself.

Note: all samples < LOD are set to 0 in the figures.

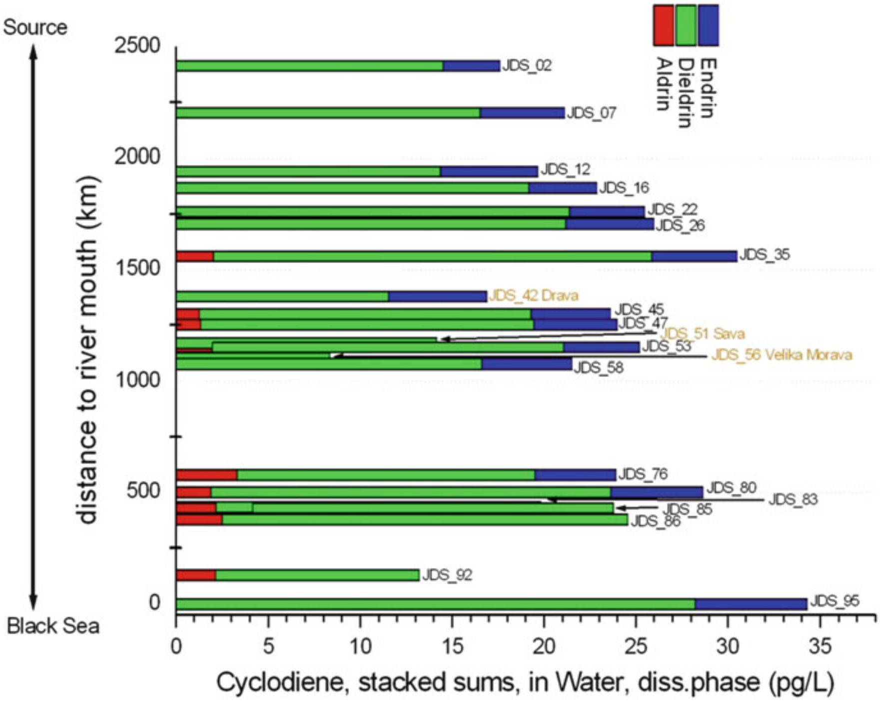

Fig. 17 Downstream concentration profile of ∑cyclodiene in dissolved phase

3.7 *Endosulfan (∑α,β-Endosulfan)*

3.7.1 Overview on All Matrices

Due to very low concentration levels, a series of sites displayed non-detectable concentrations.

In sediments only at site JDS 12_R, one value above LOD was detected for α-endosulfan, with 0.20 μg/kg.

In SPM only site JDS 56 in the Velika Morava Tributary (RS) was positive, with levels of 0.53 μg/kg for α-endosulfan and 0.11 μg/kg for β-endosulfan.

In the water column, ∑α,β-endosulfan was detected only in the dissolved phase except at site JDS 56 (Velika Morava Tributary, RS), with concentrations typically below 0.02 ng/L, more than two orders of magnitude below EQS (Table 7).

3.7.2 Downstream Concentration Profile

α- and β-Endosulfan were not detected in sediments besides site JDS 12_R where a value for α-endosulfan was detected above the LOD with 0.20 μg/kg, and in SPM

Table 7 Sum-endosulfans in all abiotic compartments

	Sediment (μg/kg)	SPM (μg/kg)	SPM in water (pg/L)	Water dissolved (pg/L)
Average	0.20	0.64	16	10
Median	0.20	0.64	16	8.1
Min	0.20	0.64	16	3.2
Max	0.20	0.64	16	39
25-Percentile				6.4
75-Percentile				11

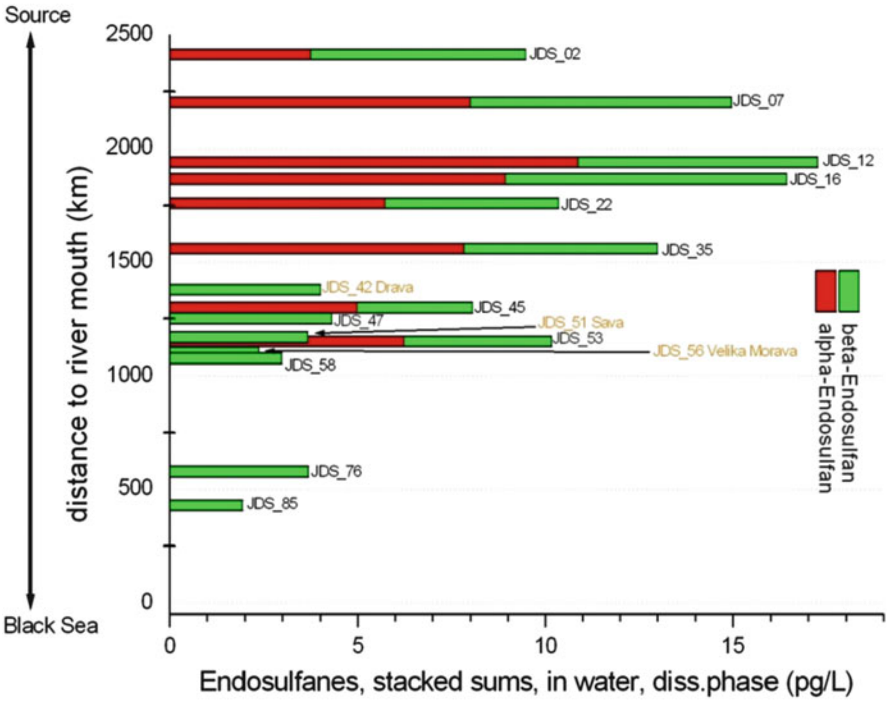

Fig. 18 Downstream concentration profile of ∑α,β-endosulfan, dissolved

only site JDS 56 (Velika Morava Tributary, RS) was positive at a level of 0.53 μg/kg for α-endosulfan and 0.11 μg/kg for β-endosulfan.

The downstream profile in the dissolved phase displays a decreasing trend downstream JDS 12 (AT) towards the Black Sea (Fig. 18).

3.8 Cis- *and* Trans-*Chlordanes*

3.8.1 Overview on All Matrices

Sediments displayed average values around 0.033 μg/kg. In SPM, due to some isolated maxima, the average concentration was around 0.084 μg/kg. In the water column, the chlordanes were detected both in the dissolved phase and associated with SPM with average level of around 2.3 pg/L each (Fig. 19, Table 8).

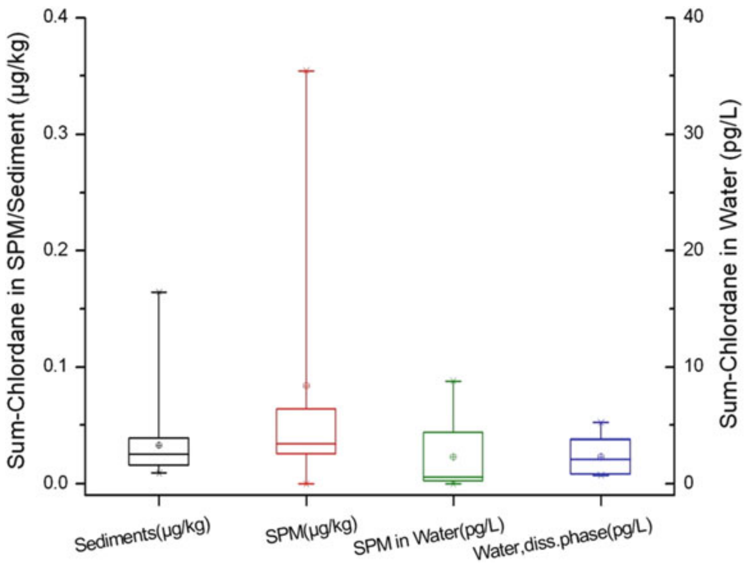

Fig. 19 *Cis*- and *trans*-chlordane concentrations in all abiotic compartments

Table 8 *Cis*- and *trans*-chlordane concentrations in all abiotic compartments

	Sediment (μg/kg)	SPM (μg/kg)	Water SPM (ng/L)	Water dissolved (ng/L)
Average	0.033	0.084	2.3	2.3
Median	0.026	0.035	0.58	1.9
Min	0	0	0	0.74
Max	0.16	0.35	8.8	5.2
25-Percentile	0.016	0.025	0.26	0.90
75-Percentile	0.039	0.062	4.2	3.8

3.8.2 Downstream Concentration Profile

Sediment (Fig. 20)

In the sediments the downstream profile displays a marginal trend of rising concentrations towards the Black Sea with no clear differentiation between left- and right-hand side samples. One distinct higher level was found in the sediments around the site JDS 85 (RO/BG), in particular on the left-hand side downstream the Arges Tributary entering from left. The share of *trans*-chlordane in sediments rises slightly towards the Black Sea.

In SPM (not displayed here) concentrations were again higher around JDS 85 but also in the sample JDS 56 (RS) taken in the tributary Velika Morava.

Water (Fig. 21)

The water column displays higher concentrations in the tributary Velika Morava (RS) and again in the lower Danube from JDS 83, to a large extent caused by the presence of SPM-associated chlordane.

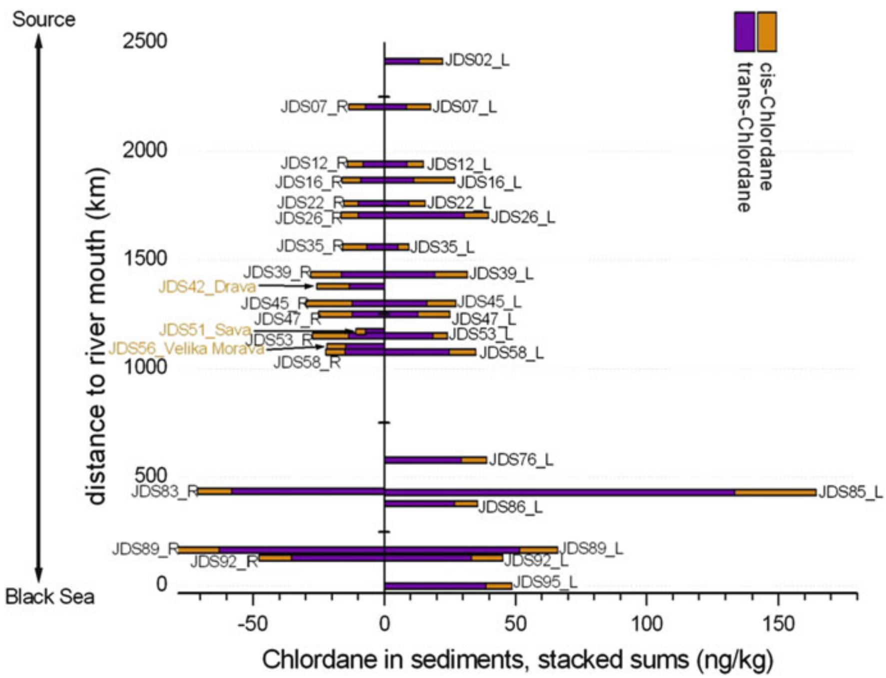

Fig. 20 Downstream concentration profile of chlordane in sediments

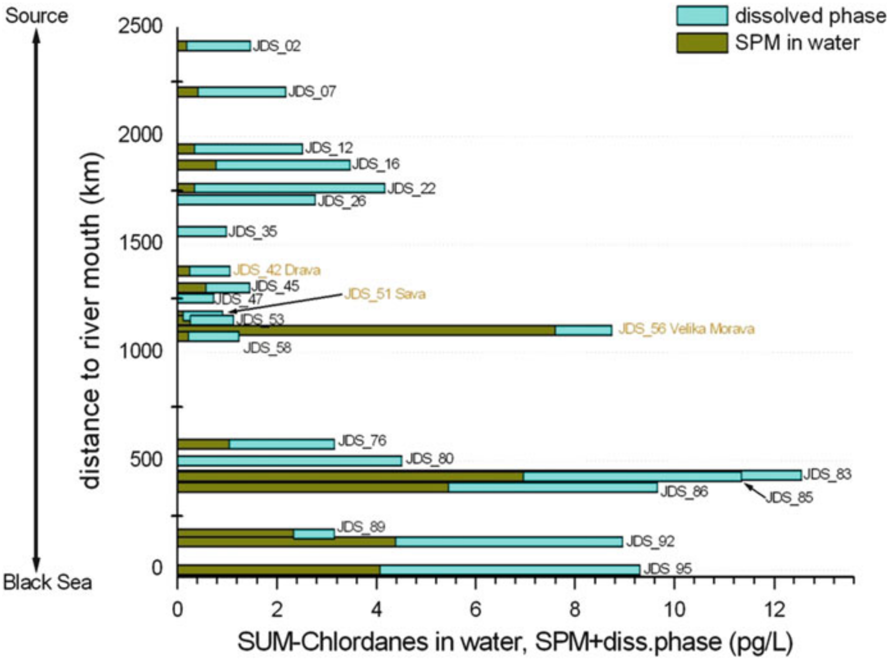

Fig. 21 Downstream concentration profile of chlordane in water

3.9 Mirex

In sediment, SPM and the dissolved phase, all samples were < LOD, which was 3.3 pg/L for the dissolved phase, 6.7 ng/kg for SPM and 17 ng/kg for sediments.

3.10 Heptachlor

Heptachlor and its *exo*- and *endo*-epoxides were not detected in sediments apart from some isolated signals for heptachlor-*exo*-epoxide not exceeding 0.1 μg/kg.

3.10.1 Downstream Concentration Profile in Water

The detected concentrations in the dissolved phase and SPM were close to the LOD and shall only be considered as an indication. The downstream profile in SPM and in the water column displays some distinct signals at JDS 22, JDS 56 and zone of higher concentration between JDS 80 and JDS 86 (Fig. 22).

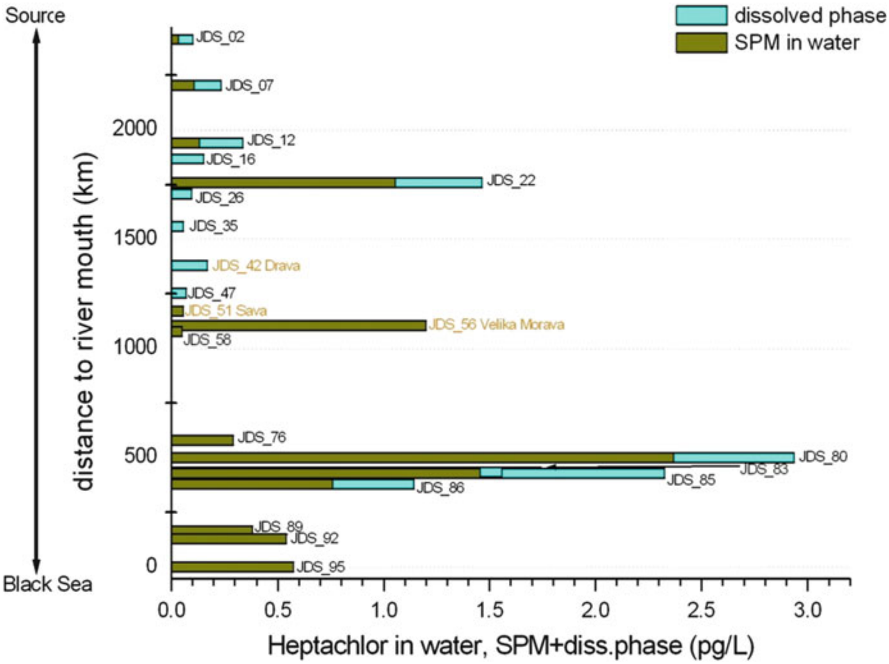

Fig. 22 Downstream concentration profile of heptachlor in water (SPM + dissolved phase)

3.11 Indicator Polychlorinated Biphenyls (EC-6 PCBs)

3.11.1 Overview on All Matrices

Indicator PCBs, also referred to as EC-6 PCBs in the Water Framework Directive, are the sum of PCB 28, 52, 101, 138, 153 and 180 and were analysed in sediment, SPM, dissolved phase and mussels (Fig. 23, Table 9).

EC-6 PCBs in sediments were at average 6.4 μg/kg with a maximum of 46 μg/kg at JDS 85 (RO/BG).

None of the individual EC-6 PCBs exceeded the chemical quality standard of 20 μg/kg for the individual EC-6 PCBs in sediments applied in Germany [14]. SPM samples display similar, somewhat lower median/average concentrations of 4.6 μg/kg also with a lower maximum of 9.1 μg/kg at JDS 92 (DE).

The observed data range fits into the lower end of the concentration ranges observed in fresh SPM from the River Elbe, where annual averages of SPM-derived fresh sediments were 2, 8 and 6.5 μg/kg in Hamburg, the highest annual average for the EC-6 PCBs of 1200μg/kg was found at Magdeburg during 2006 [14].

In the Seine estuary, typical PCB contents in SPM are one order of magnitude higher; 12 SPM samples of EC-6 PCBs without PCB 28 displayed an average of 183 μg/kg with a maximum of 380 μg/kg [15].

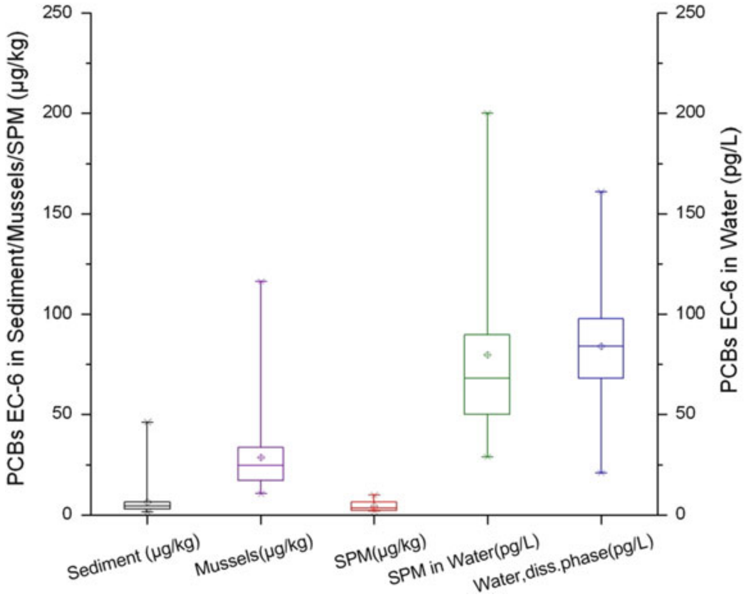

Fig. 23 EC-6 PCB concentrations in all compartments

Table 9 EC-6 PCB concentrations in all compartments

	Sediment (µg/kg)	Mussels (µg/kg)	SPM (µg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	6.4	29	4.6	80	84
Median	4.3	25	3.6	68	84
Min	1.5	11	1.9	29	21
Max	46	116	9.9	200	161
25-Percentile	3.0	17	2.2	50	68
75-Percentile	6.3	34	6.4	90	98

In the water column, the average concentrations were around 150 pg/L, which is low compared to typical annual averages of the River Elbe and individual samples from the River Seine (River Elbe, 1.6 ng/L at Zehren in the stretch after Dresden [14]; River Seine estuary, 12 water samples of EC-6 PCBs without PCB 28 = 20 ng/L with a maximum of 47 ng/L [15]).

In mussels the ∑EC-6 PCB concentrations were about an order of magnitude higher as in the solids with an average of 29 µg/kg and a range of 11–116 µg/kg dry weight. For comparison Covaci et al. [18] report for freshwater mussel species from Flanders (BE) a range of 6.2–102 µg/kg wet weight, which corresponds approximately to 62–1,020 µg/kg dry weight.

Mussel/sediment bioconcentration would average around a factor of 5 on a dry weight base within the zones where a spatial overlap between sediment and mussel sampled could be obtained.

3.11.2 Downstream Concentration Profile

Sediment (Fig. 24)

The overall picture of the downstream concentration profile of EC-6 PCBs in sediments suggests some distinct historic (historic because the distinction is not visible in the SPM and water data) inputs form the left-hand side of the Danube.

The important tributary Inn apparently has a diluting influence as indicated by the lower concentration in the sediments on the right-hand side at JDS 07 (AT), 20 km downstream the inlet and further on lower concentrations downstream at JDS 12.

At JDS 16, downstream the tributary Morava (SK) from left, higher concentration with a high abundance of PCB 28 was observed on the left-hand side, pointing to an input from tributary Morava

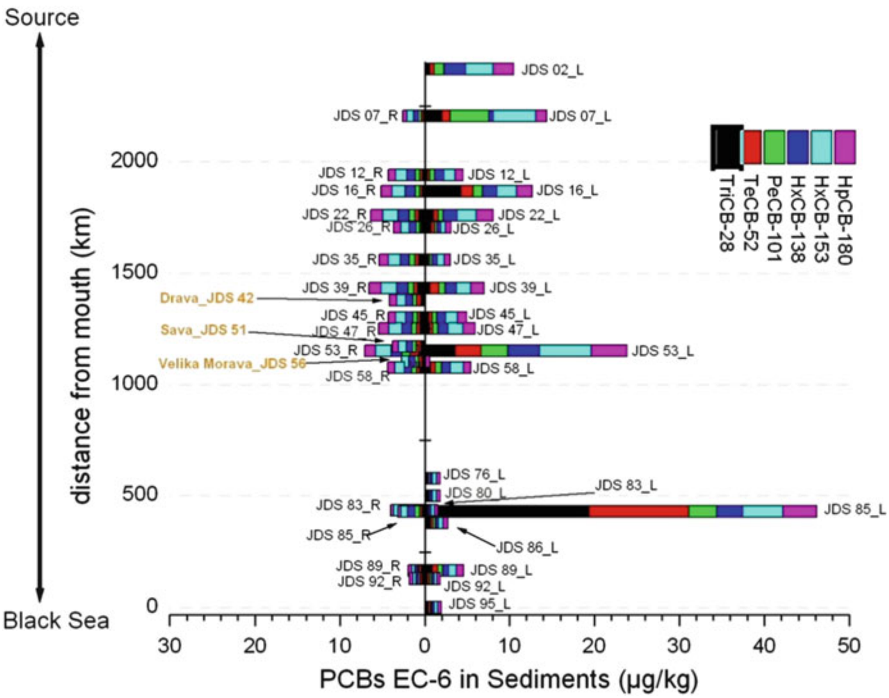

Fig. 24 Downstream concentration profile of EC-6 PCBs in sediment

The samples from the tributaries Drava, Sava and Velika Morava (JDS 42, JDS 51 and JDS 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.

At site JDS 53, downstream the city of Pancevo[1] (RS, left-hand shore of the Danube), with tributary Tamis from entering from left, a significant concentration rise was observed (JDS 52 is also the site with the maximum concentration of PCDD/Fs in sediments).

The highest PCB concentrations in sediments were detected in the left-hand side sediments of site JDS 85 (RO/BG), again with a strong abundance of PCB 28 and also PCB 52. This suggests a strong historic influence of the tributary Arges entering 2 km upstream of site JDS 85. The impact from River Arges is supported by the comparable low concentrations detected in the sediments of site JDS 83 taken in the Danube at 3 km upstream the confluence.

SPM (Fig. 25)

The downstream profile in SPM appears more equilibrated when compared to the sediments above. 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 reservoir through sedimentation.

The high PCB levels found in the sediments downstream of the tributary Arges (JDS 53) and downstream Pancevo (JDS 85) are not visible in the SPM samples, which supports the historic character of the sediment contamination of these sites.

Differences in congener distribution in SPM are less obvious than in the sediments.

Similar to the sediments, the SPM samples taken in the tributary Drava (JDS 42) show low levels when compared to the Danube itself.

Water (Fig. 26)

In the water columns, the downstream concentration profile is more equilibrated when compared to sediments and SPM. This suggests that the Danube is currently affected rather by diffuse impacts from environmental sinks rather from distinct PCB releases from urban activities. Historic impacts, still reflected in the sediments, are no longer visible in the water column. A considerable portion of the EC-6 PCBs present in water is associated with SPM.

[1] In 1999 the city of Pancevo (left-hand side of the Danube) was heavily bombed by NATO forces. Targets included an oil refinery, the airplane factory Lola-Utva and chemical plants.

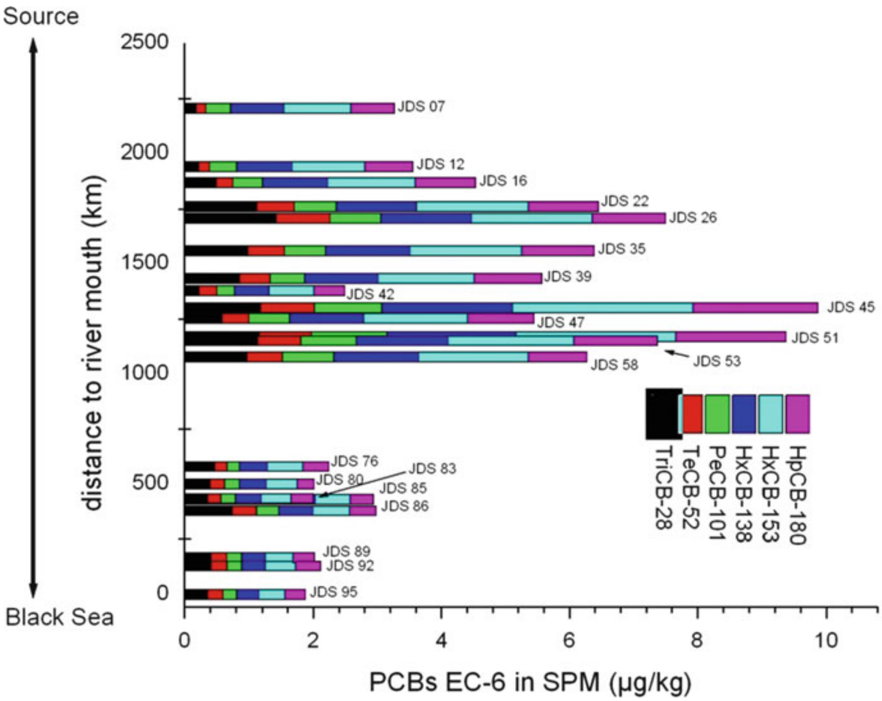

Fig. 25 Downstream concentration profile of EC-6 PCBs in SPM

EC-6 PCB Fingerprint

At average the PCB pattern in the sediments shows the typical ‘aged’ environmental fingerprint dominated by the higher boiling isomers of the technical mixtures. Sediments from the River Elbe [14] and the River Seine [15] show a similar distribution.

As discussed above the variability of the pattern in the sediments is much higher than in SPM. This suggests that the SPM reflects the current situation of diffuse, secondary PCB releases into the Danube, whereas the sediments reflect the historic primary inputs from different types of industrial effluents that displayed a high variability in PCB composition.

The fingerprint in mussels follows that of SPM, except for a lower abundance of PCB 28.

Mussel (Fig. 27)

For 8 sites where corresponding concentrations were available, no correlation with dissolved phase or SPM was observed for selected isomers. A slight coherence of

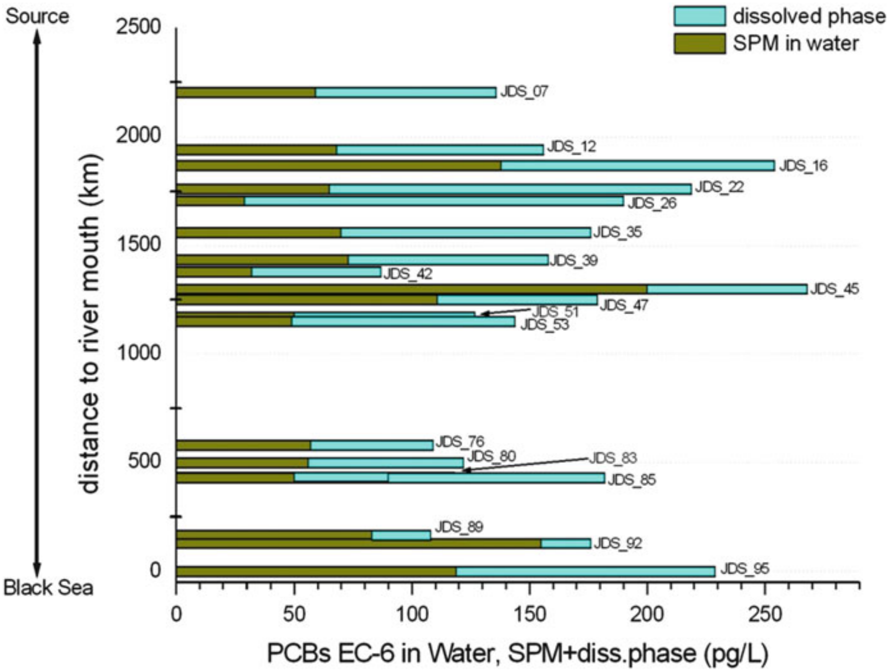

Fig. 26 Downstream concentration profile of EC-6 PCBs in water

the spatial trends was observed between *Unio tumidus* and sediment, however at a R^2 of typically below 0.2. The spatial EC-6 PCB pattern in mussels follows to some extent the concentration decrease in the sediments between the sites JDS 15 and JDS 35, as well as the subsequent concentration rise in sediment until maximum concentration at JDS 53. Subsequently the concentrations decrease both in mussels and sediment.

*3.12 Polychlorinated Dibenzo-*p*-Dioxins and Dibenzofurans*

3.12.1 Overview on All Matrices

PCDD/Fs were quantified at all sites (Table 10, Fig. 28). Most sediment samples display moderate TEQs at an average 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 JDS 53 on the left-hand side downstream Pancevo (RS). This has been the only site where the safe sediment level of 20 ng/kg I-TEQ was exceeded.

Similar concentration ranges in sediments were reported for the River Po showing PCDD/F concentrations between 1.3 and 13 ng/kg WHO-TEQ [19].

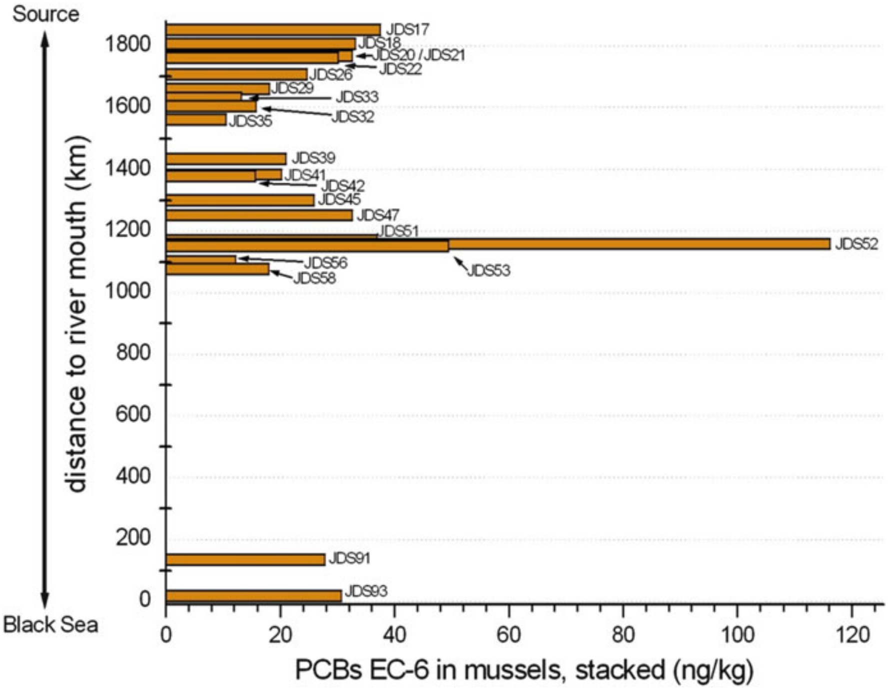

Fig. 27 Downstream concentration profile of EC-6 PCBs in mussels (all species)

Table 10 PCDD/Fs (WHO-TEQ) in all compartments

	Sediment (ng/kg)	Mussels (ng/kg)	SPM (ng/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	2.8	1.4	2.0	0.037	0.077
Median	1.9	1.3	1.6	0.032	0.072
Min	0.97	0.61	0.83	0.0094	0.049
Max	21	4.5	8.2	0.17	0.21
25-Percentile	1.4	0.94	1.1	0.021	0.061
75-Percentile	3.3	1.7	2.4	0.041	0.081

Levels in sediments of the River Elbe are typically around 40–80 ng/kg WHO-TEQ in the more industrialised stretches and around 5–10 ng/kg WHO-TEQ along stretches with diffuse inputs [20–23].

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 (HR/RS) downstream the confluence of the River Drava.

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 at the range of the average concentration in water associated with SPM. In the water

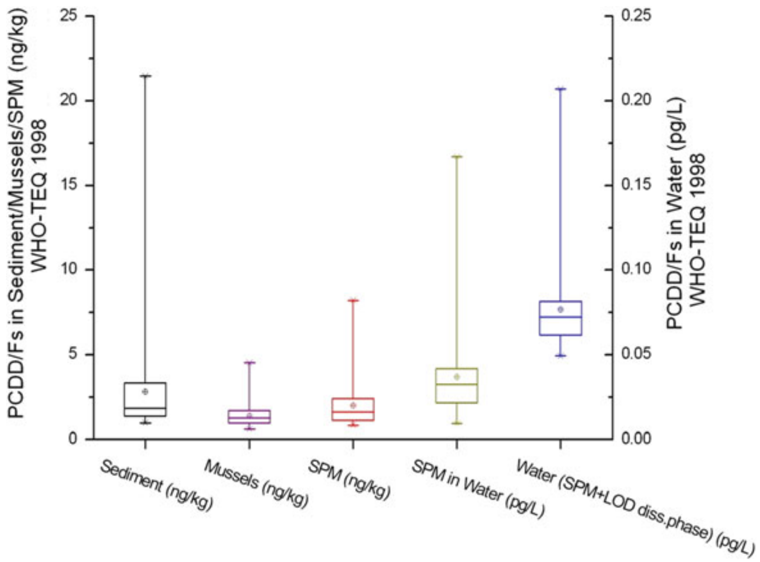

Fig. 28 PCDD/F concentrations in all compartments

phase, PCDD/Fs are predominantly associated with SPM [24], which means that the average value 0.037 pg/L WHO-TEQ derived from the quantification based on SPM should fairly reflect the total concentration in the water column.

However, a theoretical upper bound calculation for the total PCDD/F TEQ concentration in water taking into consideration the LODs in the dissolved phase is given in Fig. 28.

In the mussels the PCDD/F concentration on a TEQ base was lower compared to SPM and sediments suggesting a lower bioavailability as observed for the ∑EC-6 PCBs above.

3.12.2 Downstream Concentration Profile

Sediment (Fig. 29)

The downstream concentration profile of PCDD/Fs in the sediments shows only few extremes and in most cases no interpretable differences between left- and right-hand side samples, which suggest input coming from various diffuse sources.

Comparably high concentrations at Site JDS 02 point again to an input from the tributary Altmuehl as observed for PAHs above. Another site with somewhat higher PCDD/F concentrations on both sides of the Danube was at JDS 39 (HU), which had displayed highest PCP (known for containing impurities of PCDD/Fs) result during JDS 1. Maximum TEQ concentrations in sediment of 21 ng/kg were detected

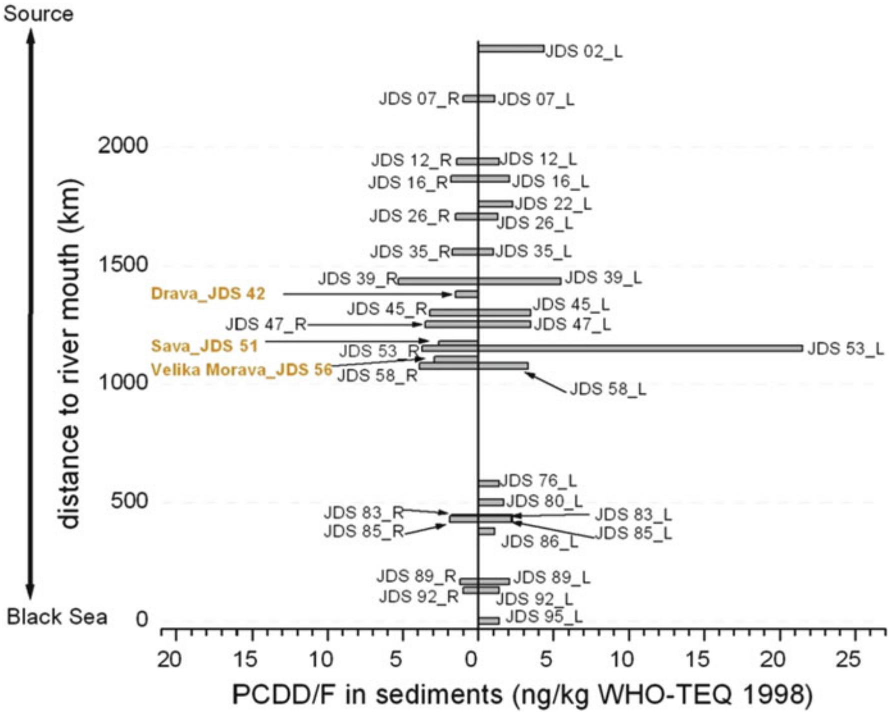

Fig. 29 Downstream TEQ profile of PCDD/Fs in sediments

at JDS 53 (RS) on the left-hand side downstream of Pancevo and River Sava. The site had shown a high abundance of EC6- and DL-PCBs as well.

As for the EC-6 PCBs, the samples taken in the 3 tributaries Drava, Sava and Velika Morava (JDS 42, JDS 51 and JDS 56, respectively) show lower levels both in sediments and SPM when compared to the Danube itself.

SPM (Fig. 30)

The downstream concentration profile in SPM shows a tendency of higher concentrations in the upper and middle stretch and lower concentrations at all sites after the Iron Gate, similar to what could be seen for PAHs and PCBs.

Noticeable is site JDS 45 (Bačka Palanka, HR/RS) where the maximum TEQ concentration of 8.2 ng/kg WHO-TEQ was detected. An influence from the tributary Drava (site JDS 42) entering 79 km upstream that site can be excluded, also due to the low PCDD/F contents in SPM measured there. The NATO air strike in 1999 was limited to The Bridge of Yough or Ilok–Bačka Palanka Bridge; therefore an impact from damaged industrial installation seems unlikely, especially since this should have left a signal in the sediment as well. The question whether the

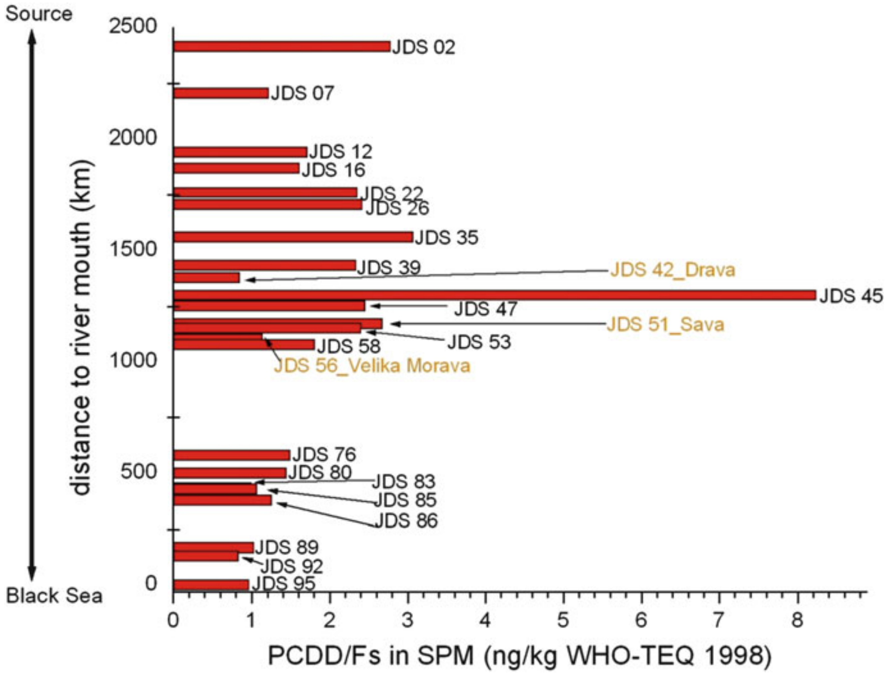

Fig. 30 Downstream TEQ profile of PCDD/Fs in SPM

local metallurgy, textiles and electronic and machine industry might release PCDD/Fs remains. Especially the metallurgic sector is known for diffuse PCDD/F emissions [25, 26].

Water (Fig. 31)

In the water column, PCDD/Fs were detected only in SPM. A slight tendency of rising concentrations towards the Black sea can be observed, as a result of higher SPM contents in the water column. However, a single maximum appears – as seen above in SPM on a dry weight base – at site JDS 45, which seems the only sampling station affected by current releases of PCDD/Fs.

Left-hand side upstream of JDS 45 is Bačka Palanka, an agricultural and industrial centre. Main industries there are food, metallurgy, textiles and electronic and machine industry.

However, the concentration at site JDS 47 only 50 km downstream of JDS 45 does not show abnormalities in PCDD/F, suggesting only a local impact of the higher PCDD/F levels around JDS 45. Also the PCDD/F contents in mussels from site JDS 45 are not peculiar (Fig. 36).

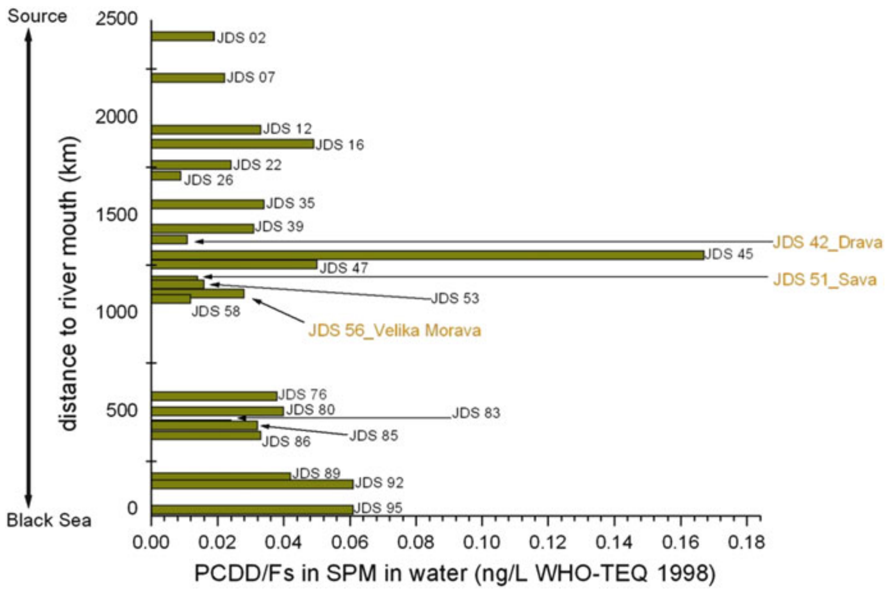

Fig. 31 Downstream TEQ profile of PCDD/Fs in water (SPM only)

PCDD/F Fingerprint

The congener 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 transport/deposition [27]. It can be found worldwide in background soils and sediments at the absence of the influence of direct emissions. Taking also into consideration the comparably low PCDD/F concentrations as discussed above, current PCDD/F emissions do not seem to affect the Danube.

3.13 Dioxin-Like Polychlorinated Biphenyls

3.13.1 Overview on All Matrices

DL-PCBs were quantified at all sites (Table 11, Fig. 32). Most sediment samples display low TEQs with an average value of 0.6 ng/kg WHO-TEQ, with maximum levels of 2.6 ng/kg at site JDS 85 on the left-hand side (downstream tributary Arges, RO) and 2 more distinctive input spots at JDS 53 (downstream tributary Tamis, RS) and JDS 02 (downstream tributary Altmuehl, DE), both on the left-hand side.

SPM samples displayed lower values with highest concentration of 1.5 ng/kg WHO-TEQ at site JDS 02 downstream tributary Altmuehl.

Table 11 DL-PCBs (WHO-TEQ) in all compartments

	Sediment (ng/kg)	Mussels (ng/kg)	SPM (ng/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	0.59	1.9	0.46	0.0091	0.0019
Median	0.49	1.5	0.42	0.0081	0.0016
Min	0.17	0.79	0.16	0.0033	0.00083
Max	2.63	8.1	1.53	0.021	0.0042
25-Percentile	0.29	1.2	0.22	0.0061	0.0012
75-Percentile	0.64	2.2	0.60	0.011	0.0024

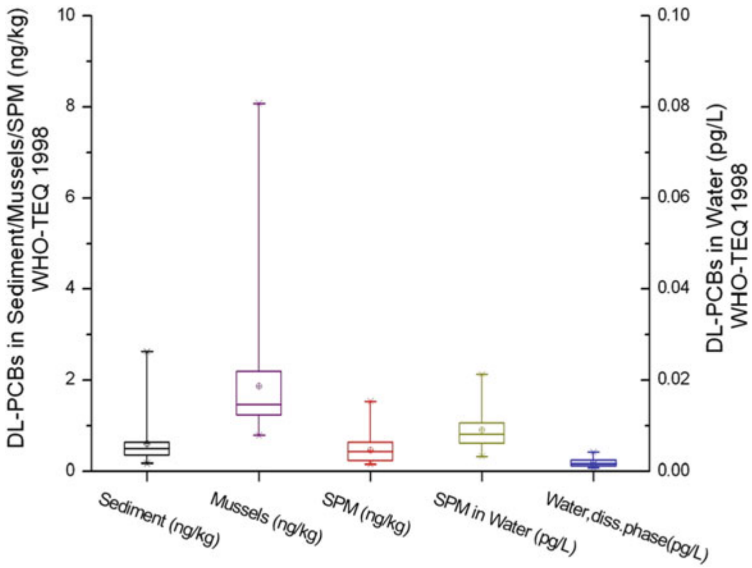

Fig. 32 DL-PCB concentration in all compartments

The low overall contribution of DL-PCBs of less than 20% to the combined PCDD/F and DL-PCB-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.

In the water column, DL-PCBs were detected predominately associated with SPM at an average TEQ level of around 10 fg/L. In the dissolved phase, the average WHO-TEQ was five times lower.

In mussels the average concentration of DL-PCBs was close to 2 ng/kg. DL-PCBs in mussel contributes a higher share to the combined TEQ of PCDD/Fs and DL-PCBs than in the sediments and SPM samples. In some cases the TEQ contribution from the DL-PCBs was even higher (compare section on PCDD/Fs).

DL-PCBs bioconcentrate in mussel (this observation is mainly based on PCB 126, which dominates the PCB-TEQ). Bioconcentration factors for sediment/mussel were typically around 4 on a dry weight basis, similar to those observed for the ∑EC-6 PCBs

3.13.2 Downstream Concentration Profile

Sediment (Fig. 33)

The downstream concentration profile of DL-PCBs (on a TEQ basis), dominated by inputs from the left-hand side of the catchment, is very similar to those of the EC-6 PCBs discussed above, except for a stronger signal at JDS 2 (DE) under the influence of the tributary Altmuehl. On a concentration basis, the maximum in sediments was found at site JDS 7.

Two more noticeable sites with higher TEQs were the left-hand side sediments from JDS 53 (RS, downstream Pancevo) and JDS 85 (RO, downstream the confluence of the Arges tributary from the left-hand side).

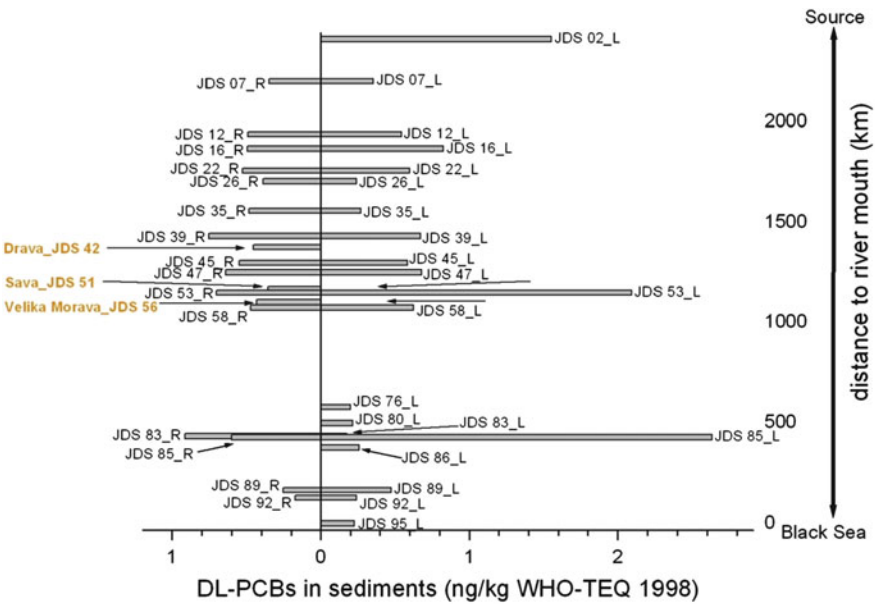

Fig. 33 Downstream TEQ profile of DL-PCBs in sediments

SPM (Fig. 34)

As seen for the PCDD/F and EC-6 PCBs, the concentration in DL-PCBs in SPM and water does not follow the spatial pattern in the sediments.

Higher concentrations up to 1.5 ng/kg WHO-TEQ appear upstream river km 1,000 while the concentrations downstream the Iron Gate are constantly below 0.25 ng/kg WHO-TEQ. The maximum concentration at JDS 02 (DE) under the influence of the tributary Altmuehl was at the concentration level of the corresponding sediment sample.

Water (Fig. 35)

In water the SPM-associated portion of the DL-PCBs dominates the TEQ. Low impacts can be seen from the tributaries Drava and Sava, while the River Velika Morava displayed higher concentrations. The high TEQ at site JDS 45 (SR) corresponds to the maxima in water observed for PCDD/Fs and EC-6 PCB. Since the upstream tributary Drava displayed low concentrations of PCDD/Fs and PCBs, the sudden rise at JDS 45 (HR/RS) suggests an influence from Bačka Palanka, an agricultural and industrial centre located on the left-hand side upstream

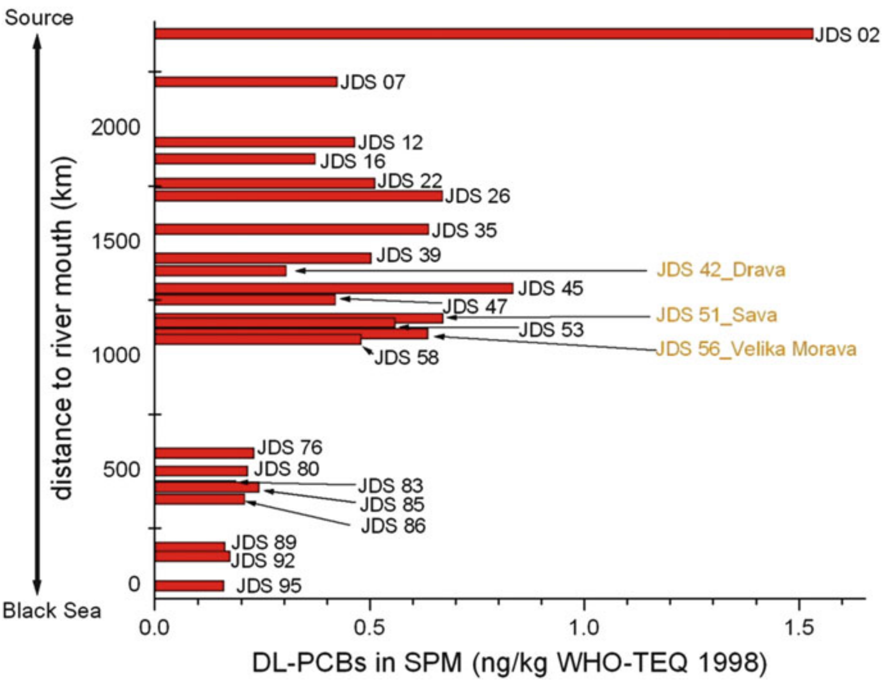

Fig. 34 Downstream TEQ profile of DL-PCBs in SPM

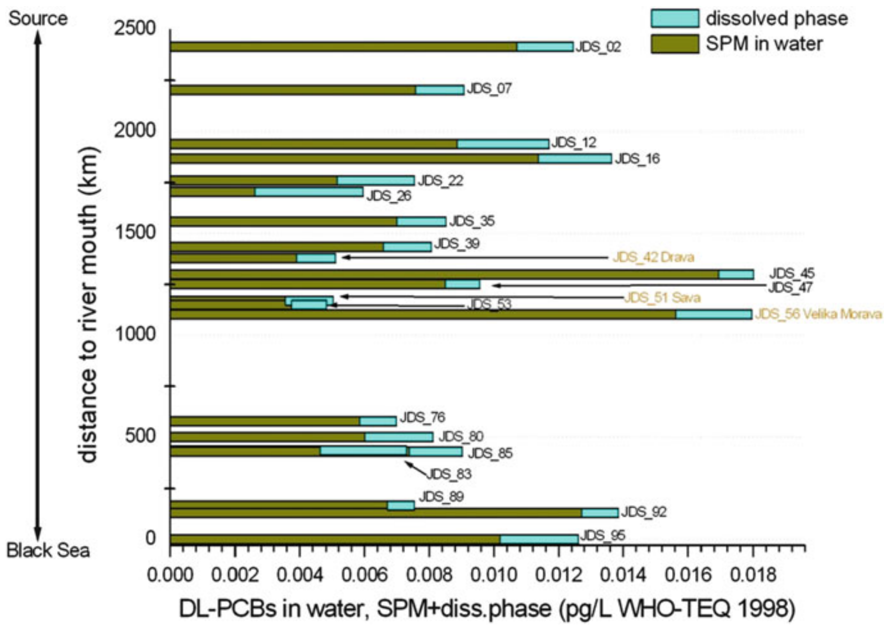

Fig. 35 Downstream TEQ profile of DL-PCBs in water

of JDS 45. Main industries there are food, metallurgy, textiles and electronic and machine industry.

3.13.3 Combined PCDD/Fs and DL-PCB-TEQ in Mussels

Although DL-PCBs displayed TEQs lower than PCDD/Fs in all abiotic matrices (Figs. 28 and 32), they contribute a significant portion to the combined TEQ in mussel (Fig. 36).

At sites JDS 52 and JDS 53, the sites with the highest combined TEQ, the toxicity arising from the DL-PCBs dominates.

According to our information, mussel products from the Danube are not marketed. It is noticeable, however, that at JDS 53, a stretch where higher PCDD/F and DL-PCB-TEQs were observed, the mussels exceeded the EU maximum level of 8 pg/g WHO-PCDD/F-PCB-TEQ for fish products [28].

Unfortunately it had not been possible to obtain mussel samples for most of the sites where abiotic samples were taken. For eight sites where corresponding concentrations were available, no correlation with dissolved phase or SPM was observed. A slight coherence of the spatial trends was observed between *Unio tumidus* and sediment, however at a R^2 of typically below 0.3.

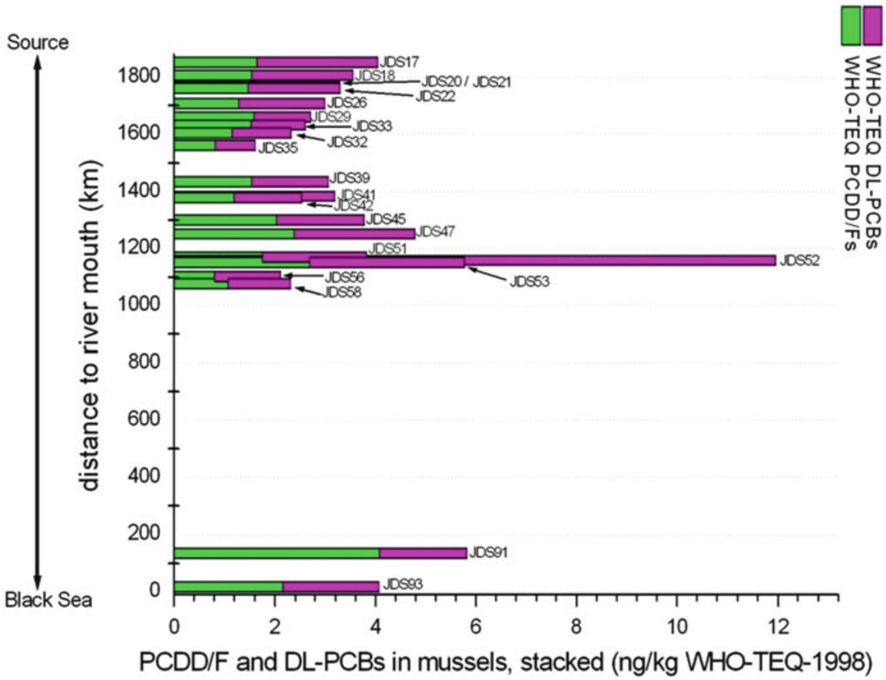

Fig. 36 Downstream TEQ profile of combined PCDD/F and PCB-TEQ in mussels (all species)

3.14 Polybrominated Diphenyl Ethers

PBDEs were quantified at all sites. Among the PBDEs measured in sediments, SPM and in the water samples, Deca-BDE dominated the pattern by far.

In the downstream profile, PBDEs in general displayed bigger and more consistent concentration gradients than PAHs and PCDD/Fs, suggesting a more recent emission history for this compound class.

3.14.1 Overview on All Matrices

Commercial Penta BDE (cPenta-BDE) (Fig. 37, Table 12)

The cPenta-BDE mixture is reported below as ∑BDE 28, 47, 99, 100, 153 and 154. In sediment cPenta-BDE concentrations averaged at 0.47 μg/kg. Average cPenta-BDE concentrations in SPM were somewhat higher at 0.60 μg/kg with a maximum level of 1.8 μg/kg.

In water cPenta-BDE was mainly associated with the dissolved phase. Among the PBDEs, only the cPenta mixture is regulated by the Water Framework Directive. Average cPenta-BDE concentrations in water (dissolved phase + SPM) were

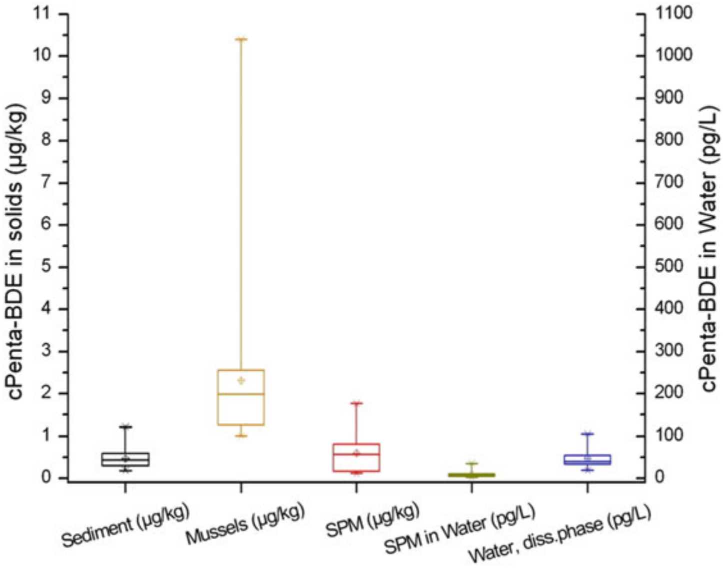

Fig. 37 cPenta-BDE concentrations in all compartments

Table 12 cPenta-BDE concentrations in all compartments

	Sediment (µg/kg)	Mussels (µg/kg)	SPM (µg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	0.47	2.3	0.60	9.0	47
Median	0.43	2.0	0.54	7.5	40
Min	0.19	1.0	0.12	2.8	19
Max	1.2	10	1.77	36	105
25-Percentile	0.30	1.3	0.17	5.1	31
75-Percentile	0.59	2.5	0.80	10	54

57 pg/L with a maximum level of 121 pg/L, which is still fairly below the EQS of 500 pg/L. However, the PBDEs being among the ‘emerging POPs’ require future surveillance in the Danube, since future releases into the environment can be expected from many products.

cPenta-BDE in water was more associated with the dissolved phase when compared with PAHs and PCDD/Fs having similar Ko/w values, which suggests release from products and process effluents rather than from atmospheric sources where the association with carbon-containing particulates reduces the availability for redistribution in the environment.

The bioconcentration factor for mussels/solids is in the range observed for the EC-6 PCBs (Fig. 23) and DL-PCBs (Table 9).

Commercial Octa-BDE Mixture (cOcta-BDE) (Fig. 38, Table 13)

The cOcta-BDE mixture is reported below as ∑of BDE 183, 196, 197, 203.

Average concentrations of cOcta-BDE in SPM were 0.17 μg/kg with maximum levels of 0.49 μg/kg at site JDS 45 (HR/RS). Sediments displayed almost identical values.

In the water column, cOcta-BDE SPM is more strongly associated with SPM than the cPenta mixture.

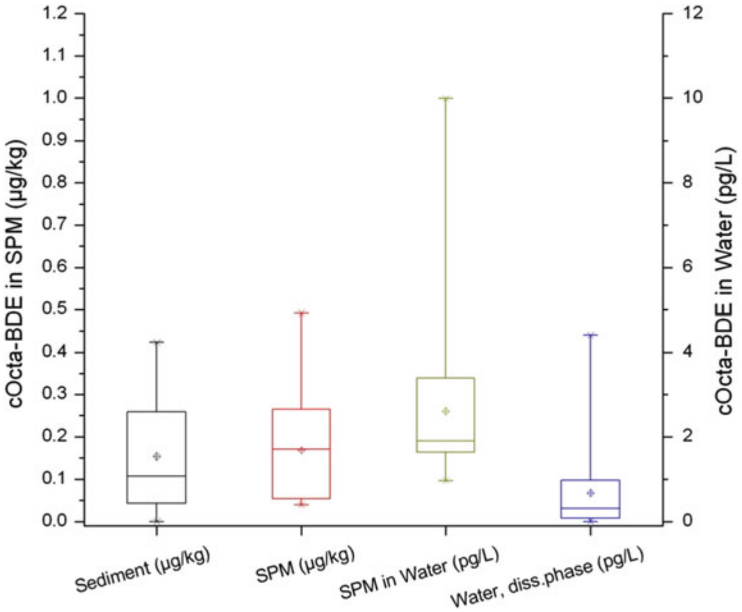

Fig. 38 cOcta-BDE concentrations in all abiotic compartments

Table 13 cOcta-BDE concentrations in all abiotic compartments

	Sediment (μg/kg)	SPM (μg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	0.15	0.17	2.6	0.68
Median	0.11	0.15	1.8	0.31
Min	0	0.04	0.97	0
Max	0.42	0.49	10	4.4
25-Percentile	0.042	0.06	1.6	0.04
75-Percentile	0.26	0.26	3.2	0.84

Commercial Deca-BDE Mixture (cDeca-BDE) (Fig. 39, Table 14)

The cDeca-BDE mixture is reported below as ∑BDE 206, 207, 208 and 209.

Average concentrations of cDeca-BDE in SPM were 15 μg/kg with maximum levels of 56 μg/kg at site JDS 45 (HR).

In the sediment samples, average and maximum concentrations were slightly lower as for SPM. The concentration levels observed in this study are around one order of magnitude lower than in SPM collected in various rivers in the Netherlands, where a median of 71 μg/kg and a range of 9–4,600 μg/kg were reported by [29].

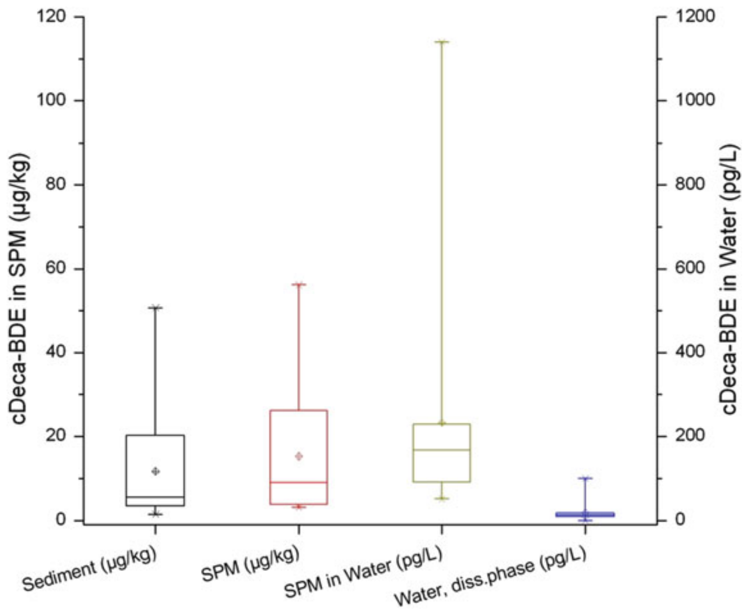

Fig. 39 cDeca-BDE concentrations in all abiotic compartments

Table 14 cDeca-BDE concentrations in all abiotic compartments

	Sediment (μg/kg)	SPM (μg/kg)	Water SPM (pg/L)	Water dissolved (pg/L)
Average	12	15.3	232	19.1
Median	5.6	7.6	162	12.5
Min	1.5	3.1	51	0.0
Max	51	56.2	1,140	100.2
25-Percentile	3.5	3.9	94	8.4
75-Percentile	18	26.1	224	17.1

In water the average concentration of cDeca-BDE was 251 pg/L, and the maximum was 1,163 pg/L at site JDS 45 (HR). In the water column cDeca-BDE was almost exclusively associated with SPM.

3.14.2 Downstream Concentration Profile

Sediment (Fig. 40)

The zone of comparably high PBDE concentrations in sediment appears on the right-hand side in the stretch between km 1,560 (JDS 35, HU) and km 1,077 (JDS 58, RS), with a maximum in the tributary Drava.

The downstream sediment data suggests PBDEs are entering from the right-hand side of the catchment, the tributaries Drava and Velika Morava being important contributors.

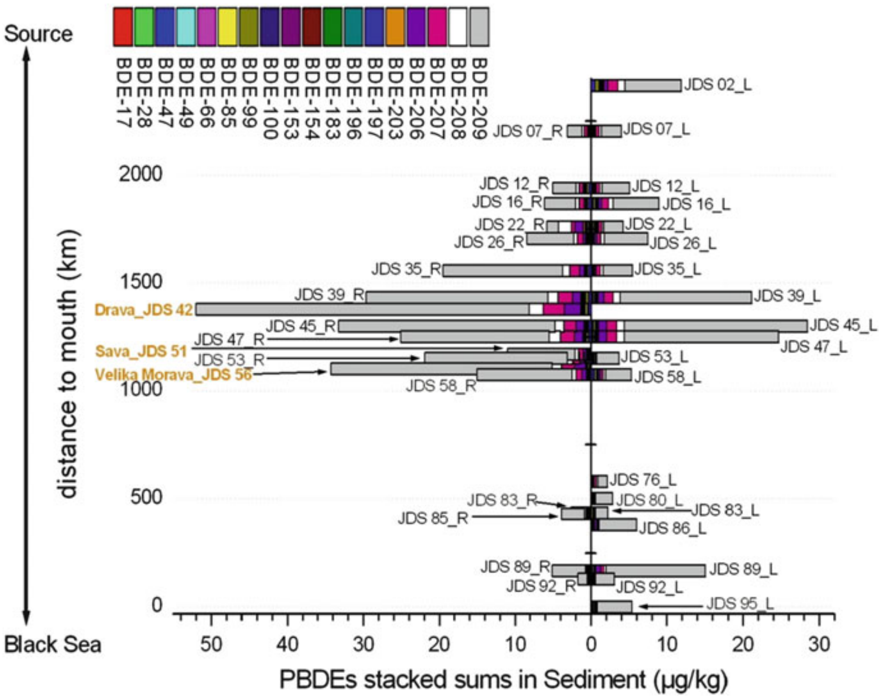

Fig. 40 Downstream concentration profile of PBDEs in sediments

SPM (Fig. 41)

In SPM the ∑PBDEs is agglomerated along the same stretch where high values in the sediment were detected. Highest concentrations were found at site JDS 45 (HR/RS) downstream Bačka Palanka and the confluence of River Drava. Compared to the sediment data, the PBDE composition in SPM displays some more contribution from lower boiling PBDEs.

Water (Fig. 42)

Similar as seen for SPM and sediment, the zone of maximal PBDE concentration in water is agglomerated in the middle stretch between km 1,252 (JDS 47, downstream Novi Sad, RS) and km 1,077 (JDS 58, RS). No particular impact from the River Drava (JDS 42) occurred during the sampling of the water, most probably due to the overall low water levels (and consequently low SPM mobilisation) during the sampling campaign.

The PBDE analysed in water is dominated by BDE 209, and consequently the major share of the ∑PBDE is associated with SPM, except in the stretch between JDS 35 and JDS 07 where the dissolved phase dominates the total concentration in water and where the highest absolute concentrations in the dissolved phase were detected (Fig. 43).

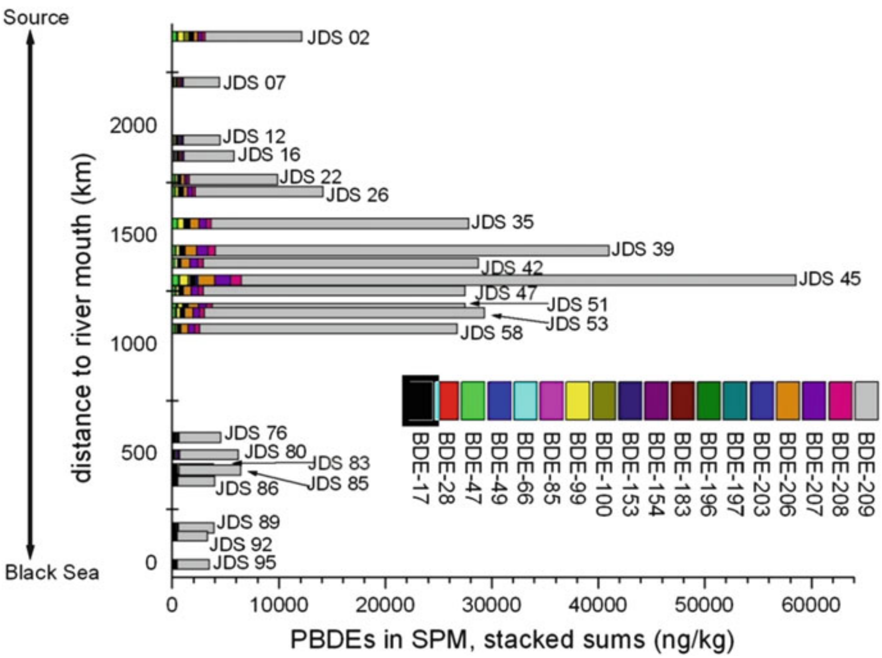

Fig. 41 Downstream concentration profile of PBDEs in SPM

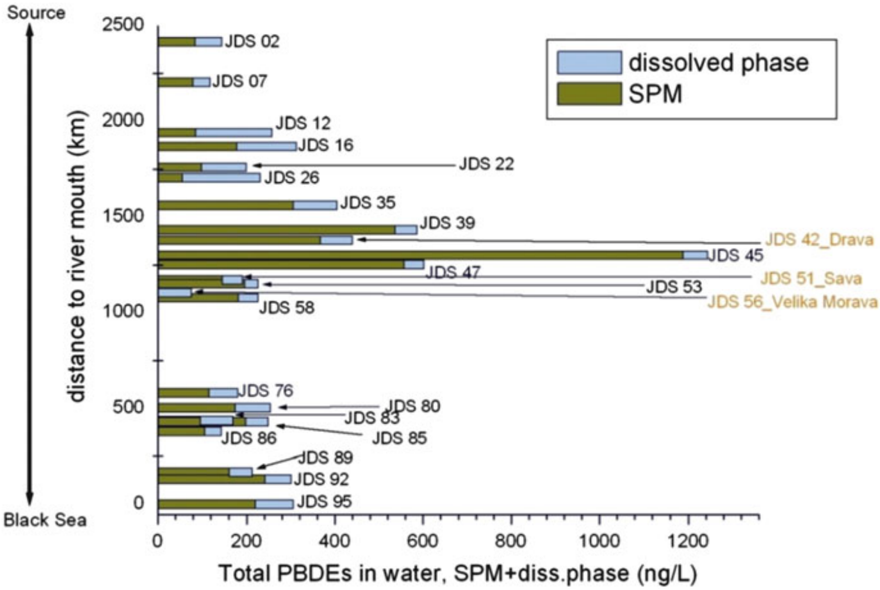

Fig. 42 Downstream concentration profile of PBDEs in water

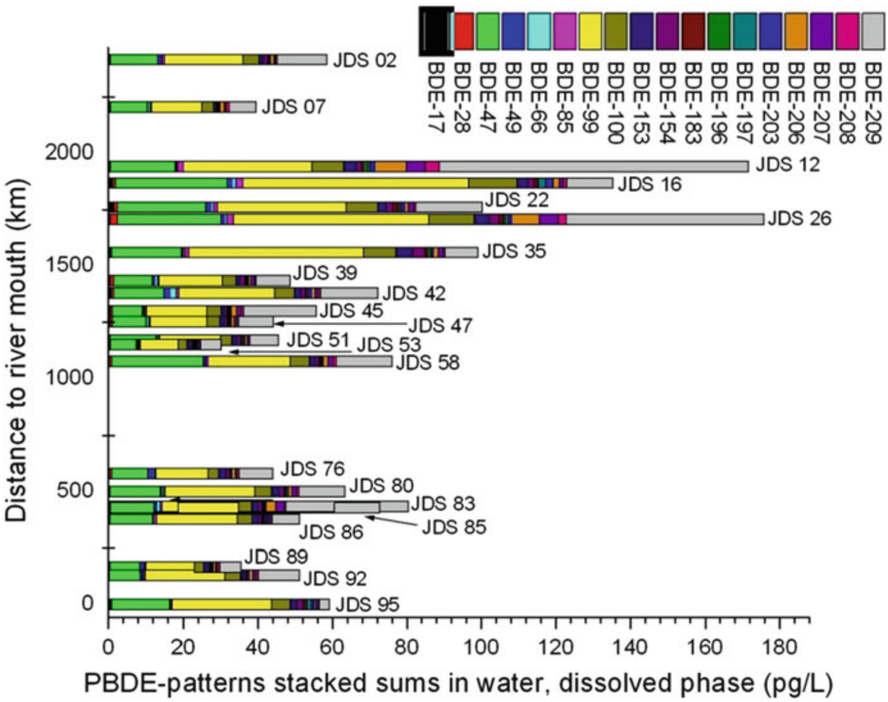

Fig. 43 Downstream concentration profile of PBDEs in dissolved phase

The dissolved-phase isomer pattern is dominated by BDE 47, BDE 99 and BDE 209. The high Ko/w of the Deca-BDE suggests that its presence in the apparent dissolved-phase fraction is not a truly dissolved fraction but adsorbed to colloidal organic matter [30].

The PBDE concentrations detected in sediments, SPM and in the water column suggest an important impact from the catchments of the tributaries Drava, Sava and Velika Morava all entering River Danube from the right-hand side. These tributaries displayed a diluting effect instead for PAHs, PCBs and PCDD/Fs. The zone of maximal PBDE concentration is agglomerated around a 500 km stretch. In contrast to PCBs, PAHs and PCDD/Fs, we got a clear spatial signal for PBDE and a good spatial overlap between sediments (historic signal) and the water column (current signal). This suggests recent and ongoing emissions for PBDEs in this region.

Mussel (Fig. 44)

The downstream concentration pattern of the cPenta-BDE mixture in the mussel samples does not reflect the situation in the sediments, SPM and water except for a general trend of lower concentrations in the lower Danube.

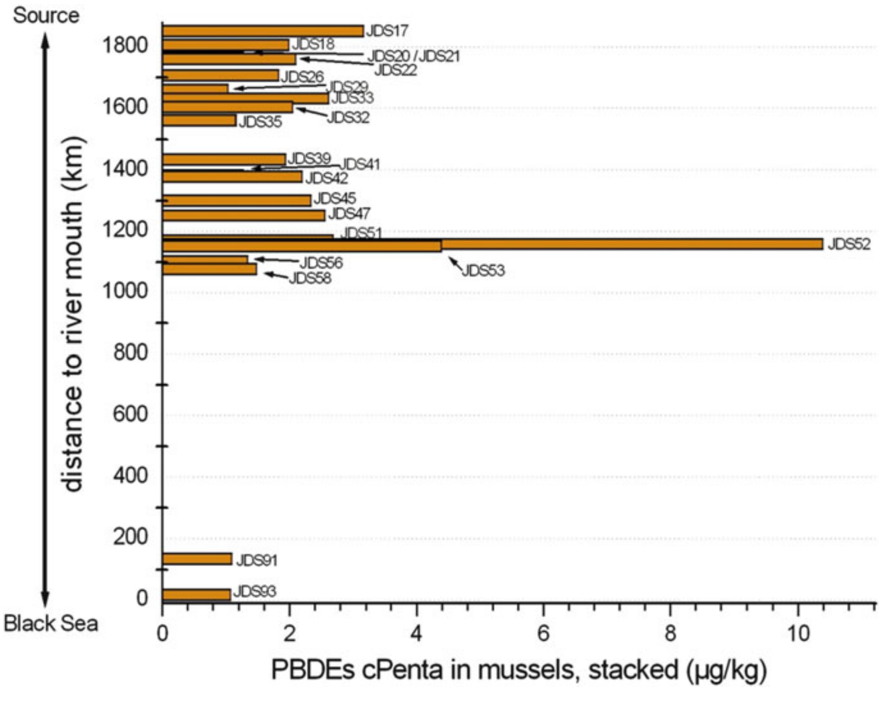

Fig. 44 Downstream concentration profile of cPenta-BDE in mussels (various species)

The isolated concentration maximum in mussel at site JDS 52, followed by the second highest concentration further downstream at JDS 53, lies within the zone where high PBDE levels were detected also in the sediments. But mussel samples taken more upstream do not reflect the high PBDE releases in this zone.

For eight sites where corresponding concentrations were available, no correlation with dissolved phase, SPM or sediment was observed except for BDE 47 in the sediments that correlated with the *Unio tumidus* at R^2 of 0.47.

4 Summary

4.1 Indication of the Chemical Status of the Water Column During the JDS 2 Cruise

From the available data of the 23 sites analysed, EQS set by the Directive 2008/105/EC were not exceeded for most of the following compound classes:

PAHs, where most of the PAHs in water samples of all 23 sites 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. Only for the ∑benzo(g,h,i) perylene and indeno(1,2,3-cd)pyrene concentrations at most sites were close to the EQS of 2 ng/L. In five sites the EQS was exceeded, namely, at sampling stations JDS 02, 16, 39, 92 and 95.

OCPs, where most compounds in the water column were orders of magnitude below the EQS and only HCH displayed some isolated maxima in the lower stretch, which however were still a factor of 4 below the MAC-EQS.

PCDD/Fs and dioxin-like PCBs, which were more than one order of magnitude lower in all compartments compared to River Elbe and in which only one site exceeded slightly the ‘safe sediment value’ for PCDD/Fs.

EC-6 PCBs, which were not exceeding the related German quality standards in sediment.

PBDEs, where concentrations in SPM were an order of magnitude lower than in Dutch rivers for c-Deca-BDE and where cPenta-BDE was around a factor of 5 below the EQS value in all water samples.

4.2 Spatial Distribution: Downstream Concentration Profiles

The concentration profiles in the sediments downstream the Danube suggest that PAHs and PCDD/Fs arise from diffuse sources, whereas PBDEs (currently) and PCBs (historically) 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 expressed spatial trend, and endosulfan concentrations decreased downstream the Danube.

The comparison of left and right bank sediment data suggests a diffuse emission from both sides of the catchment for PAHs. PCDD/Fs and PCBs and OCPs (except DDT and metabolites) show some distinct signals from the left bank while the PBDEs are emitted from the right bank of the catchment.

Only PBDEs show a clear impact from the tributaries Drava, Sava and Velika Morava all entering River Danube from the right bank, whereas for the other compound classes reported here, these tributaries displayed a diluting effect.

For most compounds, the memory contained in the sediments is scarcely reflected by the data in the water column, where the spatial gradients are less pronounced and maxima appear often at different sites than in the sediments. This underlines the historic character of many of the findings in the sediments. Exceptions were PBDEs, the most recent class of chemicals investigated in this study, and DDT and metabolites.

In order to assess the current situation of pollutant releases into the River Danube and to localise their current sources, temporarily resolved water column data from the whole watershed are desirable.

4.3 Mussels

For EC-6 PCBs, dioxins, DL-PCBs and cPenta-BDE, the downstream concentration profiles in the mussels do not show particular gradients that would exceed the inner- and interspecies deviations. The only exception with higher levels that exceeds the inner- and interspecies variability was at JDS 52, where all compound classes displayed a distinct maximum. However, from this site, no samples from the other compartments were available for this study.

The lack of correlation between the concentration in mussels and the other compartments at the sites where all matrices were sampled suggests a poor suitability of mussels as an indicator for spatial trends of SOCs in the Danube.

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Alkylphenolic Compounds in the Danube River

Vesna Micić and Thilo Hofmann

Abstract The occurrence of alkylphenolic compounds along the Danube River revealed a ubiquitous fingerprint of wastewater impact, recorded in various extents and being the most prominent in the main tributaries and side arms, as well as in vicinity of industrial areas and some Danubian capitals.

As revealed by the Joint Danube Survey 2 (JDS2) in 2007, there was a significant decrease in nonylphenol and octylphenol levels in both sediments and suspended particulate matter (SPM) compared to the findings of the Joint Danube Survey 1 (JDS1) in 2001, validating the effects of the EU regulations.

Nevertheless, the JDS2 results showed that the inputs of untreated or insufficiently treated wastewater mostly from metropolitan and industrial areas are still large enough to (occasionally) cause nonylphenol concentrations above environmental quality standards (EQS) for freshwater sediments.

Nonylphenol mono- and diethoxylates (NP1EO and NP2EO) often coexist with nonylphenol in sediments and SPM in comparable concentrations, which may induce additive mixture effects on Danube biota.

Given that there are no EQS for alkylphenolic compounds in SPM, it is difficult to estimate potential risks that SPM-linked contamination may exert on Danube biota. Slight nonylphenol accumulation in mussels was evident at the sites where nonylphenol levels in SPM were continuously high.

Based on the JDS2 findings, octylphenol and its lower ethoxylates rarely occur and in low concentrations, thus appear to be of no concern for the Danube environment.

Nonylphenol and nonylphenoxyacetic acid (NPE1C) were frequently found in water during the JDS2, exceeding the valid (or proposed) EQS for freshwater in

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some tributaries. Which possible additive or synergic effects these two compounds may have on aquatic organisms remains however unclear.

The results of the Danube surveys highlighted the necessity of reduction of untreated wastewater discharges, especially in areas where alkylphenolic compounds exceeded EQS, in order to protect quality and environmental conditions of the Danube River.

Keywords Alkylphenolic compounds, Danube River, Mussels, Sediments, Suspended particulate matter, Water

Contents

1 Introduction

Danube River flows through many different landscapes; the natural variations in topography, changes in land use, and human impacts causing pollution all affect the overall environmental quality of the river and restrict the use of water resources. Most river pollution is caused by wastewater that contains liquid waste from household, industrial, and agricultural practices. Wastewater largely contains surfactants and their metabolites, which inevitably enter rivers either through effluents from wastewater treatment plants (WWTPs) or through direct discharges and runoffs.

Some of the cost-effective surfactants widely used in industrial, institutional, and household applications, as well as in pesticide formulation, are alkylphenol polyethoxylates (APEOs) [1]. APEOs are manufactured from alkylphenols, which in addition have other industrial usages, such as in the preparation of phenolic resins, polymers, heat stabilizers, and antioxidants [2]. Approximately 80% of APEOs is built of nonylphenol polyethoxylates (NPEOs), while the remaining 20% are attributed to octylphenol polyethoxylates (OPEOs) [3]. In WWTPs as well as in rivers, both NPEOs and OPEOs biodegrade by a stepwise loss of ethoxy groups, resulting in the formation of shorter chain hydrophilic alkylphenoxy carboxylic acids and shorter chain hydrophobic alkylphenol ethoxylates. All these APEO metabolites ultimately degrade back to alkylphenols [2].

Either as constituents of WWTP effluents or of untreated wastewater, residual surfactants and their degradation products are discharged into surface water and then dispersed into different environmental compartments. Due to their physical/chemical properties, such as low water solubility and high hydrophobicity ($\log K_{OW} = 4.0–4.5$, [4]), octylphenol, nonylphenol, and their mono- and diethoxylates accumulate in environmental compartments that are characterized by high organic content (sediments, suspended particulate matter (SPM), biota), where they persist. The acidic, more hydrophilic metabolites of APEOs remain in water and (depending on hydraulic conditions) may infiltrate into ground- and drinking water [5, 6]. All APEO metabolites with 0–2 ethoxy groups are hereafter called alkylphenolic compounds.

Alkylphenolic compounds are more toxic to aquatic life than their precursors and may have carcinogenic as well as estrogenic effects [1, 7–10]. Octyl- and nonylphenol are therefore defined as priority pollutants by the EU Water Framework Directive (EU WFD). Similar behavior may be expected for the earlier APEO metabolites, such as alkylphenoxy(ethoxy) acids and alkylphenol mono- and diethoxylates, regarding their physical, chemical, and structural characteristics [2, 11]. Therefore, they are frequently discussed as potential emerging pollutants by the network of reference laboratories for the monitoring of emerging environmental pollutants [12].

Taking into account the emissions and potential risks of alkylphenolic compounds, it is in the interest of river management to monitor the occurrence and spatial distribution of these compounds in the Danube River, to identify their sources, and to support pollution control and prevention, as well as the overall protection of the Danube River environment. This report summarizes the findings of the investigations of alkylphenolic compounds in the Danube River carried out during the two Joint Danube Surveys (JDS1 and JDS2) organized by the International Commission for the Protection of the Danube River (ICPDR).

2 Sampling Sites and Sample Collection

In the course of the Danube surveys, the core team collected environmental samples along a 2,600 km long river stretch at close to a hundred sites from the main river channel and the main tributaries and side arms.

Surface sediments were taken with a sampling net from the left and right sides of the main river channel. Either there was only one sediment sample taken per tributary (from the middle or from one side of the channel cross section) or two samples from the left and right sides of the channel were combined into a mixed sample prior to analysis. Sediments were wet-sieved shipboard through a 0.063 mm sieve, and the fine sediment fraction was preserved at 4°C for further analysis.

SPM samples were collected with a continuous flow centrifuge in the middle of the river, usually while underway between two neighboring sites (due to the long time required for collection of a sufficient amount, but also in order to minimize the collection of re-suspended sediments). The exceptions were only a few sites where a stationary sampling was carried out. SPM was deep frozen shipboard and freeze-dried and homogenized in the laboratories onshore along with the fine sediment fraction.

Alkylphenolic compounds in water and mussels were analyzed during the JDS2 only. Water samples were taken with a grab sampler in the middle channel of the river below the water surface. Different mussel species were collected from the selected locations. The whole soft tissues were used, deep frozen shipboard and then freeze-dried and homogenized in the laboratory onshore.

3 Laboratories and Methodologies

Nonylphenol and octylphenol in the fine sediment fraction collected during the JDS1 and JDS2, as well as in the SPM samples collected during the JDS1, were analyzed in the laboratories of the Water Technology Center (Karlsruhe, Germany). Samples were ultrasonic extracted in a cyclohexane–acetone (9:1) mixture, followed by centrifugal separation of the liquid extract. After derivatization by a mixture of (trimethylsilyl)trifluoroacetamide (MSTFA) and trimethyliodosilane (TMIS) (1,000:2), the extracts were analyzed by means of gas chromatography/mass spectrometry (GC/MS). The compounds were quantified using 4-*n*-nonylphenol as internal standard, with quantification limits of 0.01 and 0.005 mg/kg for nonylphenol and octylphenol, respectively. More details on the analytics are given in the JDS1 Technical Report [13].

Nonylphenol and octylphenol in the SPM samples collected during the JDS2 were analyzed in the laboratories of the Bavarian Environment Agency (Munich, Germany). Samples were Soxhlet extracted in a hexane–dichloromethane (1:1) mixture, followed by extract purification via column chromatography with silica gel as stationary phase. After derivatization by MSTFA, the extracts were analyzed with a GC/MS and compounds were quantified using $^{13}C_6$-ring-labeled 363-nonylphenol and 4-octylphenol, with the quantification limits of 0.01 and 0.005 mg/kg, for nonylphenol and octylphenol, respectively. More details on analytics are given in the JDS2 Technical Report [14].

Nonylphenol and octylphenol in water were analyzed in the laboratories of the TG Masaryk Water Research Institute (Prague, Czech Republic). Non-filtered water samples were liquid–liquid extracted and purified via column chromatography using silica gel as stationary phase. Without derivatization, samples were analyzed with a GC/MS, and compounds were quantified using $^{13}C_6$-ring-labeled 363-nonylphenol, following the ISO 18857-1 protocol [15], with quantification limits of 0.02 μg/L for nonylphenol and 0.005 μg/L for octylphenol.

Table 1 Locations of the 23 sites selected during the JDS2 by the MA EG for detailed studies, with the distance from the Danube Delta (in river km), ISO 3166-1 alpha-2 country code, and mussel species

River km	Site name	Country code	Mussel species
2,412	Kelheim	DE	n.a
2,205	Jochenstein	DE/AT	n.a
1,942	Klosterneuburg	AT	n.a
1,869	Bratislava	SK	n.a
1,761	Iža/Szőny	SK/HU	*Unio tumidus* (25)
1,707	Szob	HU	*Unio pictorum* (18)
1,580	Dunaföldvár	HU	*Unio tumidus* (17) *Unio pictorum* (11)
1,434	Hercegszántó	HU	*Unio tumidus* (20) *Anodonta anatina* (8)
1,379	Drava*	HR/RS	*Sinanodonta woodiana* (6)
1,300	Ilok/Bačka Palanka	HR/RS	*Unio tumidus* (22)
1,252	Ds. Novi Sad	RS	*Unio tumidus* (?) *Anodonta anatina* (20)
1,170	Sava*	RS	*Unio tumidus* (30)
1,151	Ds. Pančevo	RS	*Unio tumidus* (21)
1,103	Velika Morava*	RS	*Unio tumidus* (27)
1,077	Stara Palanka/Ram	RS	*Unio tumidus* (35)
579	Ds. Turnu Măgurele/ Nikopol	RO/BG	n.a
500	Us. Ruse	RO/BG	n.a
434	Us. Argeş*	RO/BG	n.a
429	Ds. Argeş*	RO/BG	n.a
378	Chiciu/Silistra	RO/BG	n.a
167	Brăila	RO	n.a
130	Reni	RO/UA	n.a
0	Sulina arm	RO	n.a

The numbers in brackets show the number of mussels collected per site. Tributary names are marked with an *asterisk*
Ds downstream, *Us* upstream, (?) unknown, *n.a.* not available

Nonylphenoxy acetic acid in water was analyzed in the laboratories of Joint Research Centre (Ispra, Italy). Non-filtered water samples were extracted by solid-phase extraction, followed by elution with methanol. The analyses were carried out on a liquid chromatography coupled to tandem mass spectrometry (LC–MS^2). The quantification was performed using deuterated 4-*n*-nonylphenol (4-*n*-NP-D8), with the quantification limit of 0.002 μg/L. More details on the analytics are given in Loos et al. [5].

During the JDS2, the Monitoring and Assessment Expert Group of the ICPDR (MA EG) has selected 23 sampling sites for a more detailed investigation (Table 1). Using the samples from these sites, a cross-matrices study (including fine sediments, SPM, mussels, and water) was carried out at the laboratories of the Department of Environmental Geosciences, University of Vienna (Vienna, Austria). A suite of six alkylphenolic compounds including nonylphenol, nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), octylphenol, octylphenol monoethoxylate (OP1EO), and octylphenol diethoxylate (OP2EO) was simultaneously investigated in all matrices. Sediments and SPM samples were extracted by an accelerated solvent extractor (ASE) using methanol as extraction solvent and partitioned in *n*-hexane. Mussels were extracted by an ASE with an acetone–*n*-hexane (1:1) mixture. After the partitioning in *n*-hexane, the mussel extracts were purified using an open column chromatography with Florisil as stationary phase. All sediment, SPM, and mussel extracts were derivatized by a mixture of MSTFA and TMIS (1,000:2) and analyzed on a GC/MS with 4-*n*-nonylphenol and 4-*n*-NP2EO as quantification standards. Water samples provided by the JRC laboratory were spiked with the same internal standard mixture, derivatized, and further analyzed in the same way as the solid matrices. The quantification limits in solid matrices were as follows: 0.02 mg/kg for nonylphenol, NP1EO, and NP2EO; 0.0015 mg/kg for octylphenol; 0.0025 mg/kg for OP1EO; and 0.003 mg/kg for OP2EO. Quantification limits in water were 0.1 μg/L for nonylphenol, NP1EO, and NP2EO and 0.005 μg/L for octylphenol, OP1EO, and OP2EO. More details on the analytics are given in Micić and Hofmann [16] and Micić et al. [17].

4 Results and Discussion

4.1 Alkylphenols in Surface Sediments

During the JDS1 nonylphenol was identified in almost all sediment samples, both from the main Danube channel (gray-filled triangles) and from its tributaries and side arms (gray hollow triangles, Fig. 1).

The concentrations were evidently higher in the tributaries and in the side arms than in the main channel (Fig. 1). The peak concentrations of 160 mg/kg in the Bulgarian tributary Rusenski Lom and 46 mg/kg in the Romanian tributary Argeş were a clear sign of an extended use of NPEO-based surfactants in these areas and a lack of wastewater treatment.

The elevated nonylphenol concentrations in Schwechat (AT), Váh (SK), Drava (HR), Timok (BG), Tisa, and Velika Morava (RS) and in side arms such as Kelheim (DE) and Chilia arm (UA/RO) evidenced that also these tributaries and arms were among the main receivers of untreated NPEO surfactant-containing wastewaters.

In the main Danube channel, the nonylphenol concentrations were significantly lower, often below 0.1 mg/kg. Levels above this threshold were commonly recorded in the middle river stretch, 1,700–1,000 km from the Danube Delta and at a few downstream locations. The highest concentrations were mostly found downstream of the confluences with the biggest tributaries, such as downstream

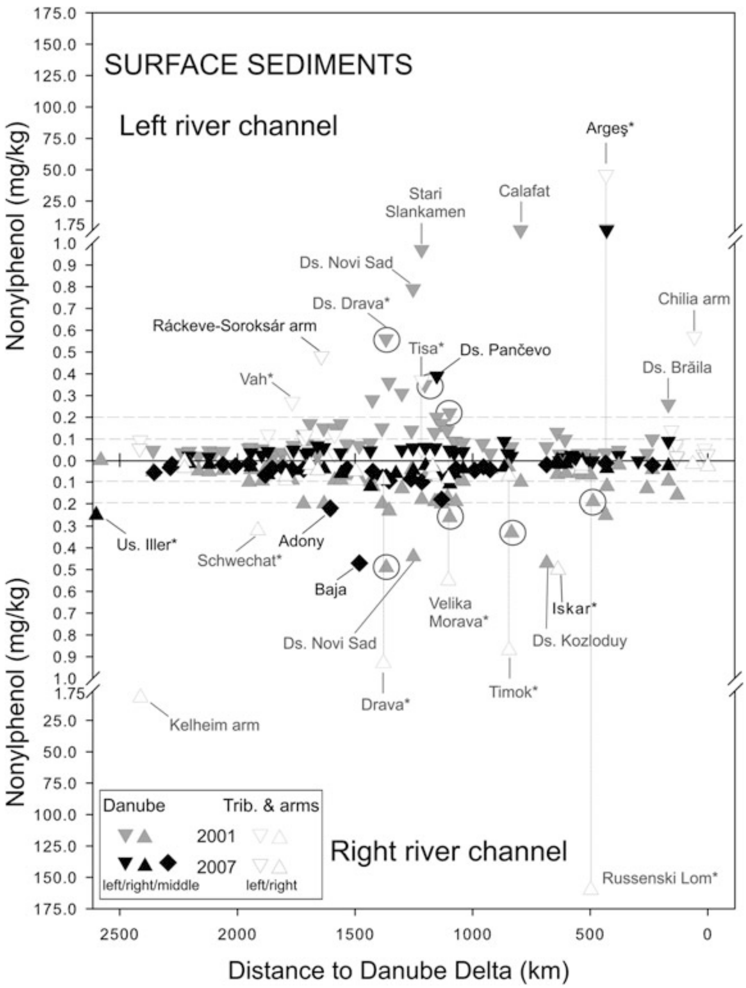

Fig. 1 Spatial distribution of nonylphenol along the main Danube channel and in its main tributaries and side arms, as revealed during the JDS1 (2001) and JDS2 (2007). Note that during the JDS2, sediments from the left and the right sides of the river channel were combined at some sites and the results are reported as from the middle river channel. *Ds* downstream, *Us* upstream. Tributary (trib.) names are marked with an *asterisk*

of Argeş (RO, 2.8 mg/kg) and Drava (~0.5 mg/kg) and also downstream of Tisa, Velika Morava, and Timok (gray-filled triangles marked with cycles, Fig. 1). Other locations with elevated nonylphenol concentrations were downstream of bigger and industrial cities, such as Novi Sad (RS), Brăila (RO), and Kozloduy (BG), but also upstream of the Tisa River (at Stari Slankamen, RS) and at Calafat (RO).

Octylphenol was present only in half of the sediments at levels above the quantification limit. As for nonylphenol, octylphenol was generally higher in the middle Danube stretch. Maximal concentration along the main Danube channel was recorded in the Serbian stretch, reaching 0.84 mg/kg downstream of Pančevo and 0.76 mg/kg downstream of the Velika Morava–Danube confluence. Outside of the Serbian stretch, a remarkably high octylphenol concentration (0.6 mg/kg) was recorded at Giurgeni (RO).

The main Danube tributaries exhibited peak concentrations, the highest being in Ipel (SK/HU) and Iskar (BG), with 1.7 and 1.4 mg/kg, respectively. Other elevated octylphenol levels were found in Tisa, Sava, and Velika Morava (RS), Rusenski Lom (BG), and Argeş (RO).

The occurrence of elevated alkylphenol concentrations in 2001 along the Danube revealed that tributaries and (to a smaller extent) discharges from the industries and municipalities along the main channel are the major pathways through which these compounds reached the Danube River. In approximately 20% of sediments investigated during the JDS1, the provisional environmental quality standards (EQS) for freshwater sediments for both nonylphenol and octylphenol (0.18 and 0.034 mg/kg, respectively [18, 19]) were exceeded, raising a concern about the river degradation.

Compared to the previous survey, the JDS2 revealed a significant decrease in nonylphenol levels in sediments from both the main river channel (black-filled triangles) and the tributaries and side arms (black hollow triangles, Fig. 1). Most of the recorded concentrations were below 0.05 mg/kg. Such a decrease after 6 years reflected a reduction of the NPEOs use in commonly applied detergent formulations. In the year 2003, in fact, the time between the two Danube surveys, the European Commission (EC) passed an EU-wide restriction of marketing and use of all products and product formulations that contain more than 0.1% of NPEOs or nonylphenol [20]. These restrictions, together with the natural attenuation processes, resulted in an improved status of the Danube sediment quality regarding the nonylphenol levels. Similar to the year 2001, also during the JDS2, the highest levels of nonylphenol recorded in sediments were found in the middle stretch of the river, with concentrations remaining mostly below or close to 0.1 mg/kg. Exceptions were only a few locations such as the Ráckeve-Soroksár arm downstream of Budapest (~0.5 mg/kg), in the vicinity of the Hungarian cities Baja (~0.5 mg/kg) and Adony (~0.2 mg/kg), downstream of the Serbian cities Pančevo (~0.4 mg/kg) and Grocka (~0.2 mg/kg), and upstream of the Iller–Danube confluence in Germany (~0.25 mg/kg). These increased concentrations (compared to the previous survey) were probably a consequence of an extended use of products containing nonylphenol and/or NPEOs, increased industrial activity, and more untreated wastewater discharges in these areas.

The peak nonylphenol concentration remained close to 2 mg/kg in the sediments downstream of the Argeş–Danube confluence, reflecting that the wastewater composition and the amount discharged into the Argeş River remained almost unchanged between the surveys.

Results of the surveys highlighted the necessity of nonylphenol reduction in sediments at all locations where the provisional EQS for freshwater sediments of 0.18 mg/kg dry wt. (proposed by the Common Implementation Strategy for the Water Framework Directive [18]) was exceeded, in order to protect the benthic organisms in these areas.

Moreover, no information on nonylphenol levels in the JDS1 hotspot locations (tributaries Rusenski Lom and Argeş and the Kelheim arm) was available in the year 2007. Even though there is only few data on the toxicity of sedimentary alkylphenolic compounds to benthic organisms, the nonylphenol concentrations recorded in 2001 in the Rusenski Lom and Argeş tributaries were ~2–6-fold higher than the lowest reported effect concentration for subacute toxicity of nonylphenol to shrimps, 26 mg/kg [21].

During the JDS2 (after the 6-year period), the decrease in octylphenol levels in sediments was even more prominent. In fact, octylphenol was recorded only in approximately one fifth of the samples, lying mostly in the range from 0.005 to 0.01 mg/kg. Values above this range were recorded at sites with the elevated nonylphenol levels, the highest being downstream of Pančevo (RS, 0.026 mg/kg), in the tributary Iskar (BG, 0.022 mg/kg), at Baja (HU, 0.019 mg/kg), at Klosterneuburg (AT, 0.015 mg/kg), downstream of the Argeş–Danube confluence (RO, 0.014 mg/kg), and close to Budapest (HU, 0.011 mg/kg).

All octylphenol concentrations recorded in the JDS2 were nevertheless clearly lower than the provisional EQS of 0.034 mg/kg dry wt., proposed by the CIRCA [19], and therefore did not pose any threat to the benthic organisms.

4.2 Alkylphenols in Suspended Particulate Matter

Both Danube surveys revealed the presence of nonylphenol in SPM at the majority of the sampling sites. The observed "background concentration" in the year 2001 was close to 0.05 mg/kg (grey-filled circles, Fig. 2). The values above this threshold were distributed along the Danube in the form of two bell-shaped curves. The first increase starting at ~60 km downstream from Bratislava (SK) reached its maximum of 0.1 mg/kg at ~70 km downstream from Budapest (Dunaföldvár, HU). Then the concentrations continuously decreased to the quantification limit but were rising again in the Serbian sector downstream of Novi Sad. They reached the second maximum of ~0.2 mg/kg in the main river channel downstream of the confluences with the tributaries Tisa and Sava and of 1.4 mg/kg in the tributary Velika Morava. Nonylphenol levels decreased again toward the lower river stretch and exhibited a constant but elevated value of ~0.08 mg/kg in the area downstream of the confluence with the Argeş River until the Danube Delta, with a peak downstream of the Olt–Danube confluence in Romania (0.12 mg/kg).

Octylphenol in concentrations above the quantification limit of 0.005 mg/kg was not found in any of the SPM samples collected during the JDS1.

Similarly to the sediments, a clear decrease in nonylphenol concentration was noticeable in the SPM samples taken during the JDS2, possibly also as a consequence of the EC regulations and natural attenuation. The "background values" were four- to fivefold lower compared to those from 2001, between 0.01 and 0.02 mg/kg. A double bell-shaped increase above this threshold was apparent along the same river stretches as in the year 2001, with again one of the highest

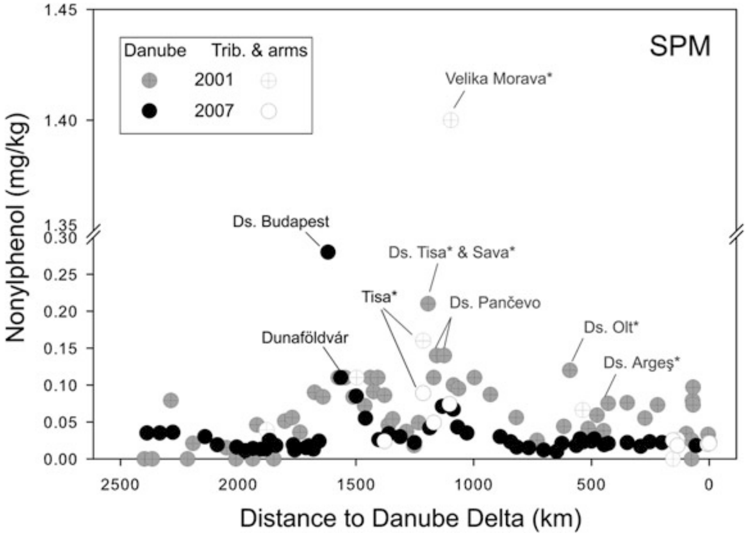

Fig. 2 Nonylphenol concentrations in SPM along the Danube and in its main tributaries and side arms in samples collected during the JDS1 and JDS2. Note that since SPM was mostly sampled while underway between two neighboring sites, nonylphenol concentrations are plotted against the middle distance to the Danube Delta between these neighboring locations. *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

(and unchanged) values at ~70 km downstream of Budapest (0.1 mg/kg at Dunaföldvár, HU). The only increase in nonylphenol concentration (compared to the the JDS1) was identified in the sample taken even closer to Budapest (~35 km downstream), therewith being the peak concentration of 0.28 mg/kg observed in 2007. This was most likely caused by an intrusion of untreated and/or insufficiently treated effluents from the city of Budapest, since at the time of the JDS2 sampling, the new Budapest central wastewater treatment plant was still under construction [14].

Octylphenol was recorded at only few locations at levels higher than the quantification limit. The highest levels were recorded along ~200 km river stretch downstream of Budapest (HU), reflecting the intrusion of wastewater from the Budapest metropolitan area. The peak concentrations recorded at the Hungarian sites Baja (0.043 mg/kg) and Dunaföldvár (0.038 mg/kg) were only slightly higher than the provisional EQS for freshwater sediments [19] but still highlighted the necessity of a reduction of the alkylphenol release from these areas.

4.3 Alkylphenols and Nonylphenoxyacetic Acid in Water

During the JDS2, nonylphenol was present in water along the whole river stretch in concentrations above the quantification limit (0.02 μg/L, Fig. 3) but rarely exhibited levels above 0.1 μg/L in the main channel. The concentrations reached this

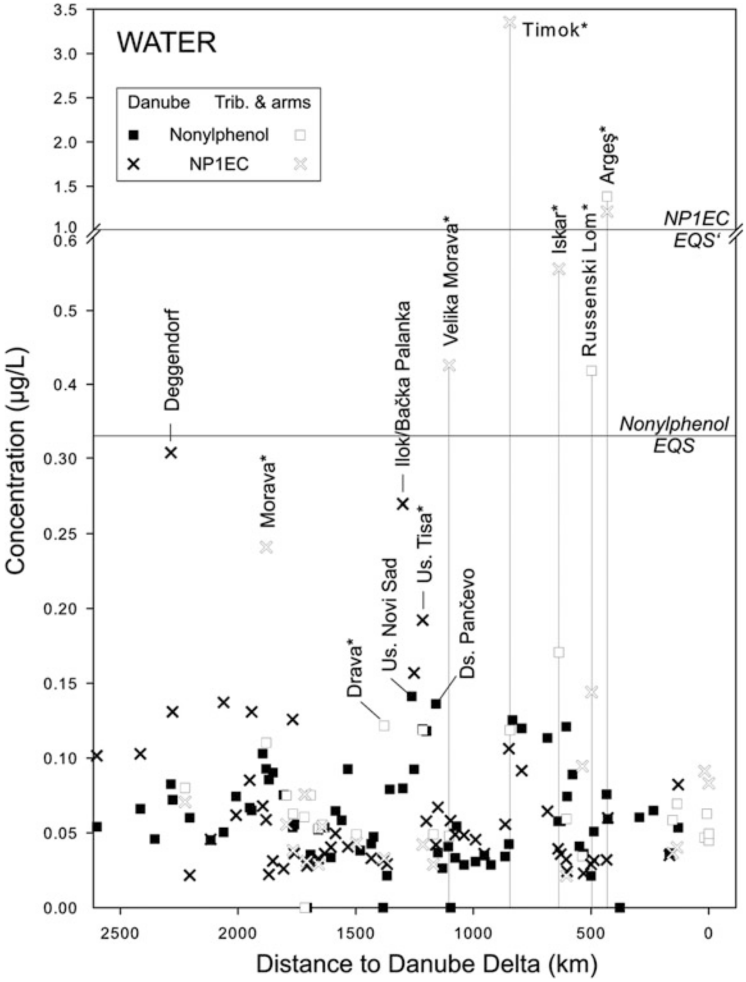

Fig. 3 Concentration and spatial distribution of nonylphenol and NPE1C in water samples collected during the JDS2. *Ds* downstream, *Us* upstream. Tributary (trib.) names are marked with an *asterisk*. EQS′ proposed EQS

threshold in the Morava tributary close to Bratislava (SK) and then again in the Serbian stretch of the river, from the tributary Drava (HR/RS) until a site downstream of the city of Pančevo, with the maximal concentration in the main channel found upstream of Novi Sad (0.41 µg/L). Downstream, nonylphenol levels remained low (mostly ~0.03 µg/L) up to the Bulgarian stretch where the concentrations once more increased in the tributary Timok (0.12 µg/L), downstream of the Timok–Danube confluence, and in the tributaries Iskar (0.17 µg/L) and Rusenski Lom (0.42 µg/L).

The highest concentrations of nonylphenol in water (1.38 μg/L) were found in the Romanian tributary Argeş. An even higher concentration of 3.28 μg/L (not shown in Fig. 3) was recorded in the Argeş sample taken closer to the Romanian capital Bucharest by a local sampling team [14]. These concentrations recorded in the tributaries Rusenski Lom and Argeş exceeded the EQS for freshwater of 0.33 μg/L [18]. The two peaks in the Argeş River are most likely caused by a significant amount of untreated and/or inadequately treated wastewater deriving from the municipality of Bucharest and its surroundings. The same three sites were the only ones where octylphenol was found in levels equal to or above the quantification limit: 0.005 μg/L (in Rusenski Lom), 0.011 μg/L (Argeş River, close to the confluence with the Danube), and 0.022 μg/L (Argeş River, closer to Bucharest, [14]), but did not exceed the water EQS for octylphenol (0.12 μg/L) [19].

Nonylphenoxyacetic acid (NPE1C) was also present in all water samples. Owing to its slightly better solubility in water compared to that of nonylphenol [22], NPE1C levels were generally higher but remained below 0.1 μg/L at the majority of sites. In the main river channel, levels above this threshold were found mostly at sites where nonylphenol was elevated: in the area around Bratislava and in the Croatian and Serbian stretch between Ilok (HR) and Bačka Palanka (RS) (0.27 μg/L), as well as close to Novi Sad (RS) and the Tisa–Danube confluence (HR/RS). The highest NPE1C concentration in the main channel (0.31 μg/L) was however recorded in the upper course of the Danube, close to Deggendorf (DE).

Similarly as for other alkylphenolic compounds, the tributaries exhibited generally higher NPE1C concentrations compared to the main channel. The highest concentrations were recorded in Timok (BG, 3.35 μg/L), Argeş (RO, 1.21 μg/L), Iskar (BG, 0.56 μg/L), Velika Morava (RS, 0.43 μg/L), Morava (SV, 0.24 μg/L), and Rusenski Lom (BG, 0.14 μg/L). In the Timok and in the Argeş, the proposed EQS of 1 μg/L [23] had been exceeded.

4.4 Alkylphenols and Their Lower Ethoxylates at the Selected Sites Along the Danube

During the JDS2, it was revealed for the first time that NP1EO generally and NP2EO sporadically co-occur with nonylphenol in the Danube sediments. The abundance of these NPEO metabolites in sediments was found to decrease in the following order: nonylphenol > NP1EO > NP2EO at the majority of the 23 selected sites (Fig. 4).

The highest concentrations of all target compounds in sediments detected downstream from the confluence with the Argeş River (RO, 2.83, 2.10, and 0.28 mg/kg for nonylphenol, NP1EO, and NP2EO, respectively) were among the highest reported in European sediments [16]. In the upper and the middle Danube stretch, nonylphenol mostly dominated over its lower ethoxylates. This indicated that (1) nonylphenol discharge may be higher compared to other compounds due to its

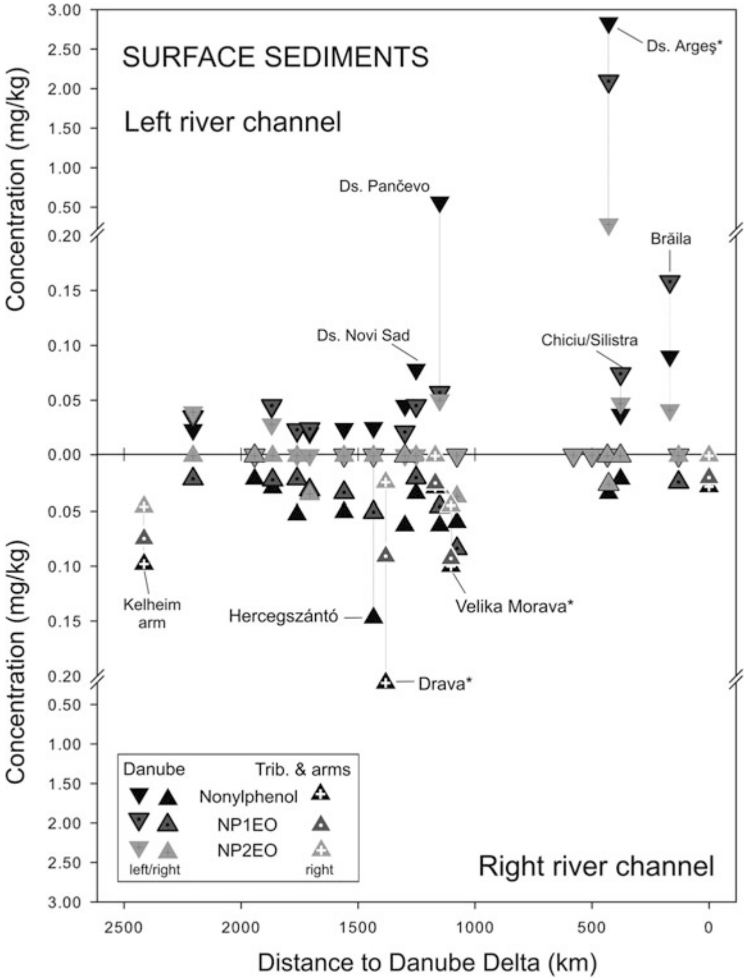

Fig. 4 Hydrophobic nonylphenolic compounds (nonylphenol, NP1EO, NP2EO) in sediments from 23 selected locations during the JDS2 (Table 1). *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

additional applications [2], (2) nonylphenol may be the most abundant NPEO metabolite in WWTP effluents [24], or (3) nonylphenol prevalence is additionally caused by in situ production from its precursors (NPE1C, NP1EO, and NP2EO) [25, 26]. All three nonylphenolic compounds were found elevated in sediments downstream of the Serbian cities Novi Sad and Pančevo, in the tributary Drava (HR/RS), and close to Kelheim in Germany (Fig. 4).

Moreover, for the first time it was revealed that NP1EO was occasionally present in comparable concentrations with nonylphenol and that in the middle and lower

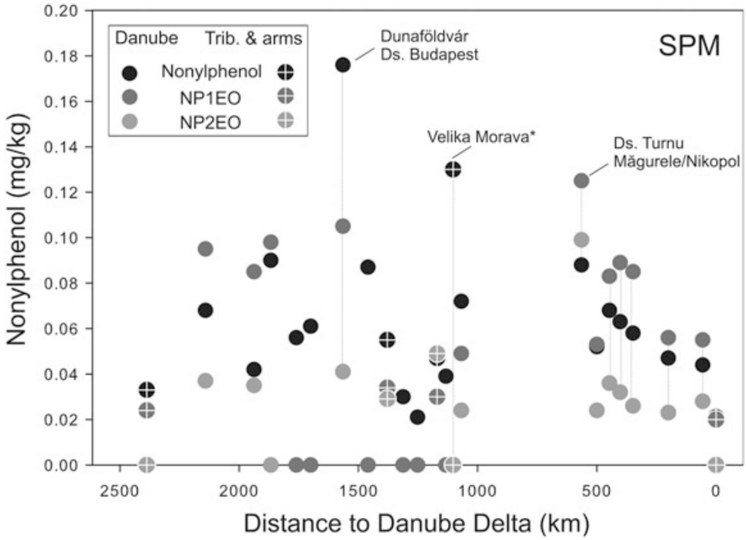

Fig. 5 Hydrophobic nonylphenolic compounds (nonylphenol, NP1EO, NP2EO) in SPM from 23 selected sites during the JDS2 (Table 1). *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

river stretch, it sporadically dominated (i.e., in Velika Morava tributary (RS), close to Chiciu/Silistra (RO/BG), and at Brăila (RO)). At these sites NP1EO was found in concentrations of 0.09, 0.16, and 0.07 mg/kg, respectively (Fig. 4), therewith being approximately twofold more abundant than nonylphenol. The NP1EO dominance suggested a fresh input of NPEO-containing untreated wastewater.

Octylphenol was recorded only at a few locations mostly at levels slightly higher than the quantification limit. The highest octylphenol concentrations were identified at the locations where nonylphenol was elevated; downstream from Pančevo (RS, 0.035 mg/kg, slightly above the EQS of 0.034 mg/kg) and from the Argeş–Danube confluence (RO, 0.017 mg/kg), indicating the use of the mixed surfactants in these areas. OP1EO and OP2EO were recorded at only one location, downstream of the Argeş River (RO) in concentrations of 0.005 and 0.007 mg/kg, respectively.

In SPM, nonylphenol was detected in the range from 0.02 to 0.18 mg/kg, NP1EO from 0.02 to 0.12 mg/kg, and NP2EO from below the quantification limit to 0.10 mg/kg. Even though peak concentrations in sediments were higher than peak concentrations in SPM, the most frequently found nonylphenol and NP1EO levels in SPM were higher than those found in sediments and often above 0.04 mg/kg (Fig. 5). Since SPM generally represents current and sediment historical pollution, this indicated higher recent inputs of nonylphenolic compounds. It is also possible that the SPM-associated contamination was subject to alteration before settling on the river bottom and that the sediment-associated contamination is additionally diluted by clastic, non-contaminated constituents.

The highest concentrations of nonylphenolic compounds were detected at Dunaföldvár, 72 km downstream from Budapest (HU): nonylphenol (0.18 mg/kg), NP1EO (0.10 mg/kg), and NP2EO (0.04 mg/kg), most likely resulting from untreated/insufficiently treated wastewater discharges from Budapest, as explained above. Among the highest concentrations identified were the ones in Velika Morava tributary (RS, nonylphenol: 0.13 mg/kg) and downstream from the cities of Turnu Măgurele and Nikopol (RO/BG, nonylphenol: 0.09 mg/kg, NP1EO: 0.12 mg/kg, NP2EO: 0.10 mg/kg).

In SPM octylphenol was found at only five sites in concentrations slightly higher than the quantification limit (0.002–0.003 mg/kg), while OP1EO and OP2EO were below the quantification limits. None of the alkylphenol lower ethoxylates were recorded in investigated water samples.

4.5 *Cross-Matrices Study of Nonylphenol at the Selected Sites*

A cross-matrices study of nonylphenol was carried out along the ~700 km long middle river stretch, where this compound was recorded in all environmental compartments studied: sediments, SPM, water, and mussels (Fig. 6).

Nonylphenol concentrations in sediments were lower than the ones in SPM at the majority of selected sites, except for sites downstream of Pančevo (RS) and in the Drava tributary (HR/RS). The sites with SPM peak concentrations did not

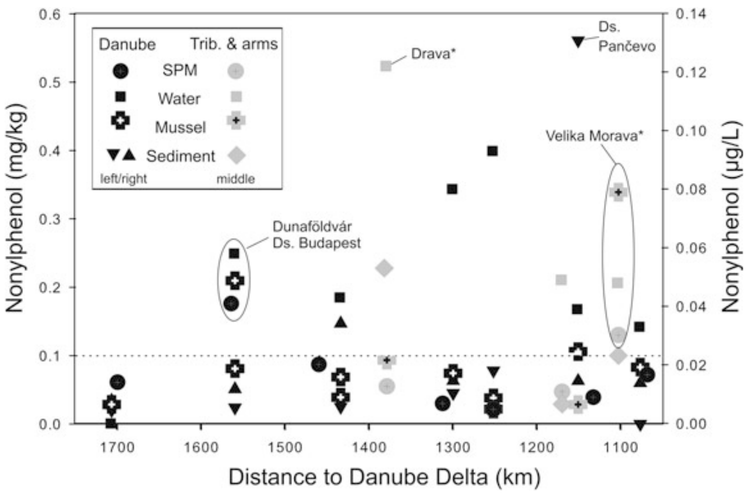

Fig. 6 Cross-matrices comparison of nonylphenol at sites where this compound was recorded in all matrices studied during the JDS2. Aqueous concentrations plotted were delivered by the TGM laboratories. *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

correspond with those with sedimentary peaks. This can be explained by the fact that SPM does not fully represent re-suspended bottom sediments but is instead a mixture of re-suspended sediments and recent inputs of particulate phase in water.

Also higher aqueous concentrations recorded were not reflected in any other matrices at these locations, indicating that water-related contaminants are subject to, e.g., dissolution and photodegradation before they finally settle at the river bottom.

It has been revealed for the first time during the JDS2 that nonylphenol was present in all mussels' tissues investigated (Fig. 6). The concentration range in the Danube River from 0.03 to 0.34 mg/kg was in accordance with the range observed in the mussel species worldwide [27, 28]. There was a similar trend of nonylphenol concentrations in mussels and in SPM, with the levels in mussels being slightly higher than those in SPM. The highest concentrations were detected in *Unio tumidus* from the tributary Velika Morava (RS, 0.34 mg/kg) and in *Unio pictorum* at Dunaföldvár (HU, 0.21 mg/kg), exactly at locations where the SPM level exceeded a threshold of 0.1 mg/kg. Since it is known that mussels can filter several liters of water per hour (and with it associated fine particles), this most likely resulted in a slight nonylphenol accumulation at sites with a higher and long-term exposure to nonylphenol in SPM.

Octylphenol was only detected in *Unio pictorum* at Dunaföldvár (HU, 0.03 mg/kg), where the nonylphenol level was also elevated, while NP1EO, NP2EO, OP1EO, and OP2EO were not detected in any of mussel samples.

5 Conclusions

The occurrence of alkylphenolic compounds along the Danube River revealed a ubiquitous fingerprint of wastewater impact.

The first Joint Danube Survey (2001) raised concerns about alkylphenol levels in Danube sediments, with up to 160 mg/kg of nonylphenol recorded in sediment from the Rusenski Lom tributary (BG). The results of the second Joint Danube Survey (2007) revealed a significant decrease in alkylphenol concentrations, thus validating the effects of the EC legislation regarding marketing and use of nonylphenol- and NPEO-containing formulations since 2003, as well as the effects of natural attenuation.

Nevertheless, results from the survey in 2007 revealed a continuous input of APEO-containing wastewater from metropolitan areas, such as Budapest (HU) and Bucharest (RO), as well as from the industrial cities close to Belgrade (RS), such as Pančevo and Grocka. In these areas, as well as at the site upstream of the Iller–Danube confluence in Germany, and in the vicinity of the Hungarian city Baja, the provisional EQS of 0.18 mg/kg for freshwater sediments was still exceeded, highlighting the necessity for improvement of wastewater treatment in these areas.

Also the presence of alkylphenolic compounds in SPM in 2007, reflecting more recent inputs into the river, revealed that despite the EC regulations, there was still an occasionally higher input of this contamination. Since there are no available EQS for SPM for none of the alkylphenolic compounds studied, it is difficult to estimate the potential risk the recorded concentration may pose to aquatic organisms. However, it was shown that in areas with peak nonylphenol concentrations in SPM (in both JDS1 and JDS2), nonylphenol tends to slightly accumulate in mussel tissues, as found in *Unio tumidus* from the Velika Morava tributary (RS, 0.34 mg/kg) and in *Unio pictorum* from the location downstream of Budapest (HU, 0.21 mg/kg). These observations revealed a need for further reduction of nonylphenol-containing discharges and monitoring of nonylphenol in mussels in the areas where high concentrations in SPM were recorded. In addition, it pointed out necessity for regulating concentrations of alkylphenols in SPM. The question of which effects these accumulations may have on mussels remains open for the future ecotoxicological studies.

The simultaneous study of alkylphenolic compounds in different riverine compartments revealed the co-occurrence of nonylphenol and its mono- and diethoxylates in sediments and SPM in occasionally comparable concentrations. This observation raises concern about potential additive mixture effects on riverine organisms, as shown for aqueous concentrations [29], which remain yet another challenge for future ecotoxicological studies.

Nonylphenol and NPE1C were frequently recorded at low concentrations along the main river course but at substantially higher levels in the tributaries. Nonylphenol concentration in tributaries Argeş (RO) and Rusenski Lom (BG) exceeded the EQS for freshwater of 0.33 μg/L. Also NPE1C levels were high in Argeş (1.21 μg/L) and in Timok (BG, 3.35 μg/L), exceeding the proposed EQS of 1 μg/L. The aqueous concentrations once again demonstrated insufficient or missing wastewater treatment in these areas and the necessity to study what possible additive or synergic effects these two compounds may have on aquatic organisms.

Since octylphenol was rarely found during the survey in 2007 (and if so, then in levels mostly lower than the provisional EQS) and its mono- and diethoxylates were recorded only at one site (at low concentrations), it is apparent that these compounds are generally of no major concern in the Danube environment any longer.

Overall, judging on the occurrence and spatial distribution on nonylphenolic compounds, it is evident that as a result of insufficient or nonexistent treatment of wastewaters, the Danube continues to show signs of degradation downstream of metropolitan and industrial areas, as well as in a number of main tributaries, and that improvement of wastewater treatment is needed.

Because of considerable lack of ecotoxicological data and estrogenic effect studies for benthic organisms, as well as scientific uncertainties regarding exposure, there are currently only provisional EQS available for freshwater sediments. Therefore, one of the priorities for the protection of benthic organisms is to carry out further ecotoxicological and estrogenic potential studies with the individual and mixed alkylphenolic compounds, in order to amend the provisional European EQS.

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PAH and Petroleum Hydrocarbon Contamination in Water, Suspended Particulate Matter, Sediments, and Biota in the Danube

Peter Literathy

Abstract Several analytical methods are used to measure petroleum hydrocarbons contamination in the environment. Each method provides different, specific information about the characteristics of the contamination. Only the results obtained with a particular analytical method can be used for a comparative study or a pollution trend analysis. The polluting aromatic hydrocarbons can be characterized in terms of fluorescence patterns; the contamination level/concentration can be calculated from the fluorescence intensity at specified excitation/emission wavelengths.

Interpretation of the fluorescence fingerprint of cyclohexane extracts of water, SPM, and bottom sediment samples, collected during the Joint Danube Surveys, as well as the results of the PAH analysis provided the following findings: (1) petroleum hydrocarbons in water were characterized by the fluorescence of gasoline; the concentrations varied in the range of 2–300 μg/L; (2) the level of oil contamination was similar in the SPM and the bottom sediment, characterized with the fluorescence of crude oil, and the concentrations varied between 5 and 500 mg/kg; (3) PAH determined in water, SPM, bottom sediment, and biota (mussels) showed similar trends in contamination as observed in the case of petroleum hydrocarbons. However, even the highest concentrations were usually below the EQS values according to the Directive 2013/39/EU, or the PELs in the Canadian Sediment Quality Guidelines.

Keywords Environmental quality standards, Fluorescence fingerprints, Fluorescence spectroscopy, Joint Danube Survey, Oil pollution

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Contents

1 Introduction

Among the organic pollutants, oil pollutants (petroleum compounds including PAHs) are one of the most common and frequently occurring organic pollutants, which are introduced into rivers, lakes, and marine waters from oil refineries, other industries, transportation, municipalities, and accidental spills. The oil pollutants, i.e., aliphatic, aromatic, cyclic, and naphthenic hydrocarbons or hetero-compounds, have mainly hydrophobic properties. They can float on the surface of the water and can be dispersed/dissolved in the water column or associated with the suspended particulate matter (SPM), and after settling of the suspended solids, they can accumulate in the bottom sediment. These compounds may undergo environmental weathering—biodegradation and/or chemical (photo-)oxidation, resulting in degradation products—and a number of the petroleum-related compounds may accumulate in aquatic organisms.

Petroleum hydrocarbons and PAHs have been studied in national research programs in several Danube countries; however, the first coordinated transnational survey, along the whole Danube, was conducted by a Cousteau team in 1991–1992 [1]. The sediment survey results indicated pollution hot spots and high variation of the oil pollution along the Danube between Vienna and Budapest. Therefore, one of the Danube Basin Applied Research Projects [2] aimed to make a collaborative study in this Danube reach. The Austrian, Slovak, and Hungarian institutions carried out this survey in 1995–1996. In 1997–1998, the MS Burgund survey [3] was carried out along the Danube reach between the confluence of the Rhein-Main channel and the Hungarian Danube section. Both of these surveys, limited to a specified Danube reach, reported about the similar level of oil pollutants as observed during the Cousteau survey.

Based on the results of these surveys, and the release of the EU Water Framework Directive (WFD) in 2000 [4], coordinated surveys, called Joint Danube Survey (JDS), were planned along the Danube to be implemented every 6 years, starting in 2001 [5].

Table 1 EQS for petroleum-related substances in surface waters and aquatic biota

	EQS as in Directive 2013/39/EC			
	Water		Biota	
Substance	AA (μg/L)	MAC (μg/L)	μg/kg wet wt.	Remarks
Anthracene	0.1	0.1		
Benzene	10	50		
Fluoranthene	0.0063	0.12	30	Crustaceans and Mollusks
Naphthalene	2	130		
Benzo[a]pyrene	0.00017	0.27	5	
Benzo[b] fluoranthene		0.017		
Benzo[k] fluoranthene		0.017		
Benzo[g,h,i]perylene		0.0082		

2 Guidelines/Standards for Assessing Petroleum Hydrocarbon and PAH Contamination in Surface Waters

Environmental quality guidelines for petroleum-related contamination are represented by aromatic hydrocarbons, particularly polyaromatic hydrocarbons, as shown in Table 1 for surface water and biota and in Table 2 for surface water sediment.

3 Methodologies

There is no single analytical method to characterize properly oil pollution due to the variable composition of complex mixture of compounds in the crude oil and its refined products. Different analytical methods have been and are being used for characterizing/estimating oil pollution in water, suspended solids (SPM), and bottom sediment. These methods are based on measuring groups of petroleum compounds or quantifying individual substances. Infrared and UV absorption and fluorescence measurements show group characteristics. Gas chromatograph with flame ionization detector (GC-FID), gas chromatograph with mass spectrometer (GC-MS), and high pressure liquid chromatograph (HPLC) methods can measure individual aliphatic hydrocarbons, volatile organic compounds (e.g., benzene), and/or polyaromatic hydrocarbons (PAHs).

Annex VIII of the WFD [4] shows the indicative list of the main pollutants, including the persistent hydrocarbons and persistent and bioaccumulable toxic organic substances. Among the petroleum hydrocarbons, the aliphatic hydrocarbons are easily biodegradable, whereas persistent hydrocarbons include usually aromatic or polyaromatic structures.

Table 2 Sediment quality guidelines for petroleum-related substances

Substances	Canadian Sediment Quality Guidelines [6] Interim sediment quality guidelines (ISQGs) (μg/kg dry weight)	Canadian Sediment Quality Guidelines [6] Probable effect levels (PELs) (μg/kg dry weight)	EU Priority Substances data sheet [7] (μg/kg dry weight)
Anthracene	46.9	245	24
Benzo[a] anthracene	74.8	693	
Benzo[a] pyrene	88.8	763	91.5
Chrysene	108	846	
Dibenz[a,h] anthracene	6.22	135	
Fluoranthene	113	1,494	2,000
Benzo[b] fluoranthene			70.7
Benzo[k] fluoranthene			67.5
Benzo[g,h,i] perylene			42
Phenanthrene	86.7	544	
Pyrene	153	1,398	

Regarding the analytical approach, infrared spectroscopy and the GC-FID methods provide information primarily on the presence of aliphatic hydrocarbons. The GC-FID chromatograms can be used to differentiate between biogenic and petrogenic hydrocarbons and between fresh and weathered oil pollution. UV and fluorescence spectrometry provides signals of the aromatic structures, indicating the persistent hydrocarbons. GC-MS and HPLC methods are used for measuring individual petroleum compounds, particularly those aromatic substances such as benzene or PAHs, which represent petroleum hydrocarbon contaminants among the priority substances and for which environmental quality standards (EQS) have been established [8].

Since the fluorescence measurements provided data/information for characterizing oil pollution of the water, suspended and bottom sediment samples during each of the three JDSs, the florescence fingerprints can be used for a comparative evaluation.

3.1 Determination and Interpretation of Fluorescence Fingerprints

Total fluorescence spectra (fingerprints) of cyclohexane extracts of water, SPM, and bottom sediment samples were recorded according to procedures described in

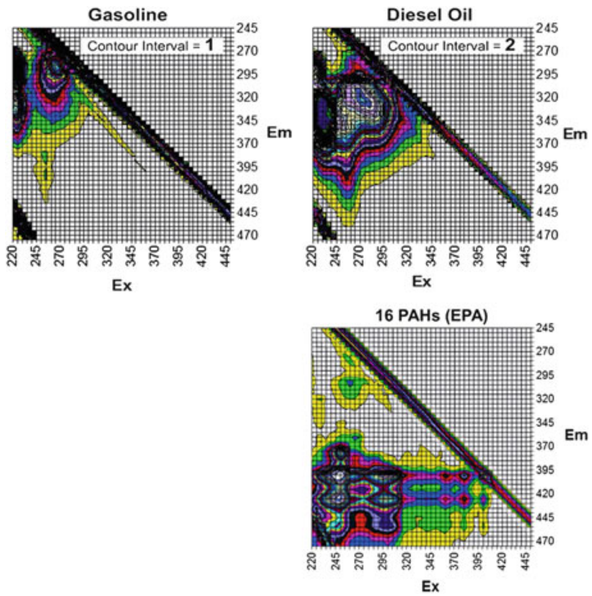

Fig. 1 Fluorescence fingerprints (contour diagrams) of arbitrary standards (gasoline, diesel, and crude oil, 1–1 μg/mL; 16 PAHs, each 3 ng/mL, in cyclohexane)

detail elsewhere [9, 10]. Fluorescence spectrophotometer (Hitachi Model 4500) was used to record the fluorescence spectra in the 220–450 nm excitation and 245–475 nm emission wavelength ranges. Figure 1 shows fluorescence fingerprints of the arbitrary standards (petroleum products) including 16-PAHs.

Determination of contamination type is based on the degree of correlation between the concatenated fluorescence spectra of the arbitrary standards and the environmental samples, which was achieved by decomposing each fingerprint into 22 emission spectra (Rayleigh scattering removed) as follows:

Spectrum number	Excitation wavelength	Emission range	Spectrum number	Excitation wavelength	Emission range
Spectrum 1	220 nm	250–365 nm	...	...	...
Spectrum 2	225 nm	255–370 nm	Spectrum 20	315 nm	345–460 nm
Spectrum 3	230 nm	260–375 nm	Spectrum 21	320 nm	350–465 nm
...	...	...	Spectrum 22	325 nm	355–470 nm

These fluorescence emission spectra were then concatenated. Examples of the concatenated spectra for the arbitrary standards are presented in Fig. 2.

After calculating the correlation between the concatenated spectra of the samples and the arbitrary standards, the standard showing the highest correlation coefficient with the samples was used as calibration standard for estimating the

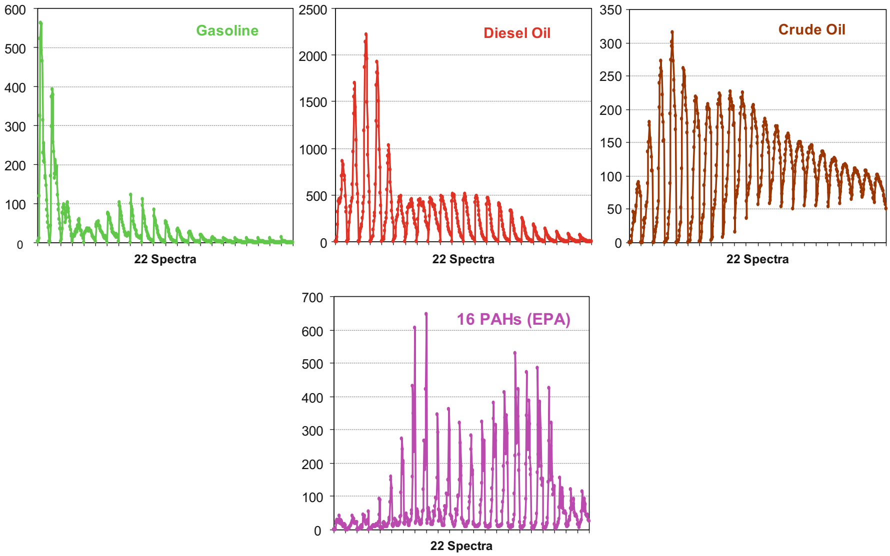

Fig. 2 Concatenated fluorescence spectra of the arbitrary standards, PAHs

concentration of the petroleum hydrocarbon contamination [9]. The fluorescence intensity at the excitation/emission (Ex/Em) wavelength, specified for each standard material, was used for this estimation.

The highest correlation was observed with the gasoline (fluorescence by monoaromatic compounds) in the case of the water and with the crude oil in both the SPM and bottom sediment samples. The specific Ex/Em wavelengths in the case of gasoline and the crude oil were 265/290 and 270/380 nm, respectively.

3.2 Determination of PAHs

PAHs were analyzed in water, SPM, and sediment samples after extraction with organic solvents and determined with HPLC-Fluo or GC-MS according to internationally accepted analytical protocols.

4 Results and Discussion

4.1 The Cousteau Survey in 1991–1992

The first coordinated survey along the Danube (excluding the then-Yugoslavian Danube reach due to the war activities) by the Cousteau team involved collection of

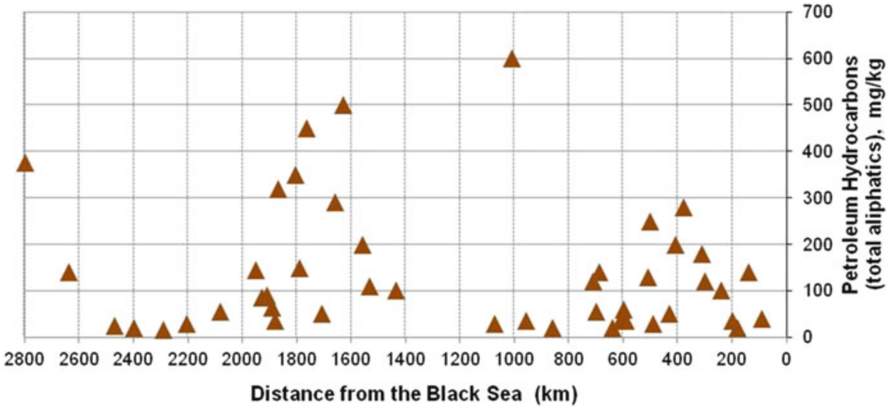

Fig. 3 Distribution of petroleum hydrocarbons in Danube sediments

sediment and bivalves samples. Petroleum-related contamination of the sediment samples was determined: (a) by analysis of *n*-alkanes as a measure of relatively fresh oil pollution using GC-FID method and (b) individual PAHs analyzed with HPLC-fluorescence detector.

Concentration of the petroleum hydrocarbons along the Danube is shown in Fig. 3, whereas Fig. 4 shows the benzo[a]pyrene concentrations.

Both figures show similar levels of petroleum hydrocarbon contamination, rather high in certain hot spot areas (e.g., the upper Danube reach in Germany, the middle reach between Austria and Hungary, and the lower Danube reach in the industrial areas of Romania and Bulgaria) but are generally inferior to similarly polluted rivers in other parts of the world. In the case of PAHs (e.g., phenanthrene, fluoranthene, benzo[a]anthracene, and benzo[a]pyrene), the concentrations were similar or slightly lower than those observed in the Lower Rhine and in the Mersey estuary in the UK.

The sediment monitoring results are very useful for detecting pollution hot spots. The multiparameter approach uses the coincidence of two pollutants associated with a given human activity. Examples of this approach are shown in Fig. 5.

Using the multiparameter approach in the case of petroleum hydrocarbons and coprostanol, the coincidence highlights those sites where petroleum hydrocarbons are discharged in association with municipal sewage. The spectacular coincidences were observed in the Iron Gate reservoir, at Budapest, and downstream of the Arges (demonstrating the impact of Bucharest).

The coincidence of benzo[a]pyrene and lead shows a combination of compounds characteristic of fossil fuel combustion and using leaded fuels. The coincidence factor here shows peaks coinciding with industrial activities in Germany, along the Slovak-Hungarian Danube, and the accumulation in the Iron Gate reservoir and downstream of the Arges river introducing waste discharges from Bucharest.

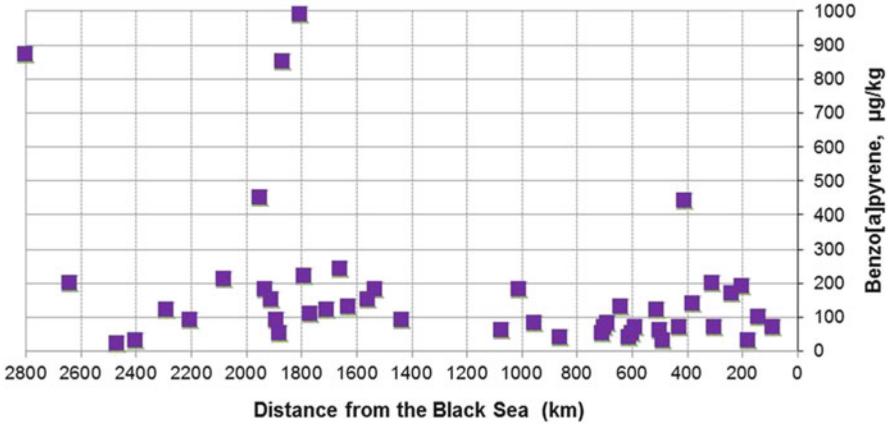

Fig. 4 Distribution of benzo[a]pyrene in Danube sediments

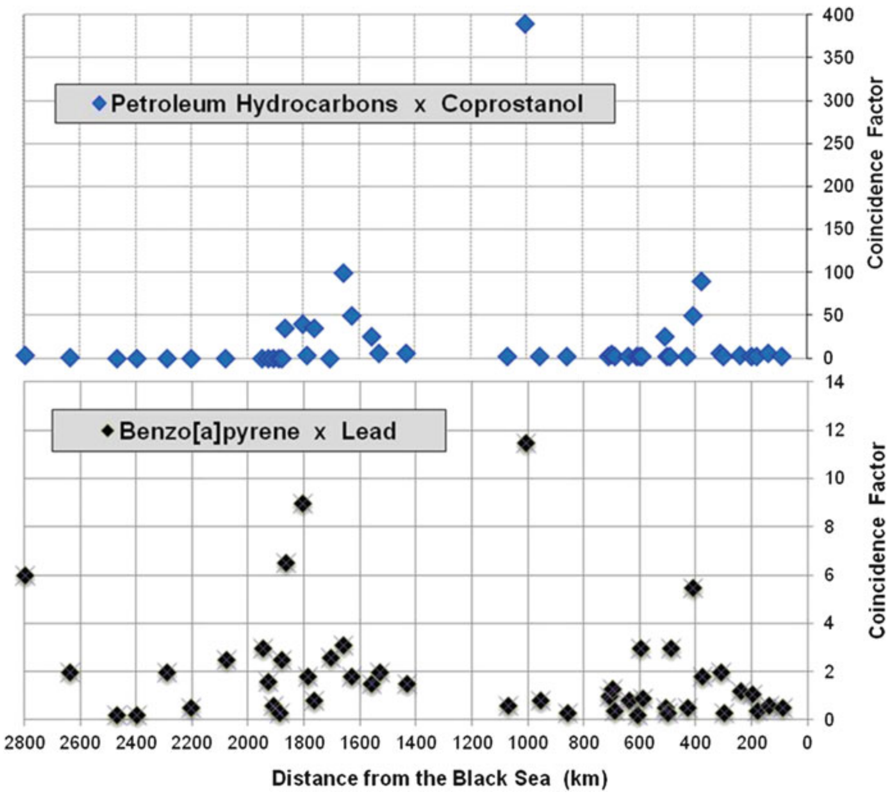

Fig. 5 Hot spot identification with coincidence factors

4.2 *The Joint Danube Surveys*

As continuation of the "along the Danube survey" by the Cousteau team, the ICPDR initiated Danube surveys with joint participation of the riparian Danube country institutions. The first Joint Danube Survey (JDS) was conducted in 2001, planned on the basis of the lessons learned from the previous surveys and also considering the requirements of the EU Water Framework Directive [4].

Among the chemical characteristics, petroleum hydrocarbons and PAHs were analyzed in water, SPM, bottom sediment and biota (mussels) samples. The first joint survey (JDS1) was followed by JDS2 (2007) and JDS3 (2013). The petroleum hydrocarbons were determined with different analytical methods during the JDS1. Based on the first results, the method based on measurement of fluorescence (fluorescence fingerprinting as detailed in Sect. 3.1) was agreed to be used during the following surveys.

Figure 6 demonstrates visual comparison between the different samples collected from representative sampling sites along the Danube.

The fingerprints in Fig. 6 show the results of the analysis of the cyclohexane extracts of the water samples. They demonstrate that the most water-soluble mono-aromatic (BTEX) compounds are dominating in samples from rkm 2,204 and rkm 532, likely originating from pollution with gasoline. In the case of the Morava river, the fingerprint indicated that the water was polluted with gasoline, diesel, and even with some crude oil residues.

The fingerprints of the Danube suspended solids and bottom sediment extracts demonstrate the presence of higher ring-number aromatic compounds, a mixture of diesel and crude oils, as well as weathered petroleum residues. It is interesting to note that these fingerprints look similar at different sampling sites; however, considering the contour intervals, the contamination of SPM and bottom sediment in the Morava river was about 10 times higher compared to the upstream Danube site (rkm 2,204). The oil pollution inputs discharged into the Danube between Vienna and Bratislava significantly increased the petroleum contamination in both the SPM and bottom sediment between Bratislava and the end of the Slovak-Hungarian Danube reach (1,707 rkm).

4.2.1 Petroleum Hydrocarbons in Water, SPM, and Bottom Sediment

Petroleum Hydrocarbons in Water

The calculation of the correlation between the concatenated spectra of the cyclohexane extracts of the water samples and the arbitrary standards resulted in highest correlation with gasoline in 16, with diesel oil in 44, and with crude oil in eight water samples. The petroleum hydrocarbon concentration in each water sample was calculated from the calibration with the relevant standard. The results are shown in Fig. 7.

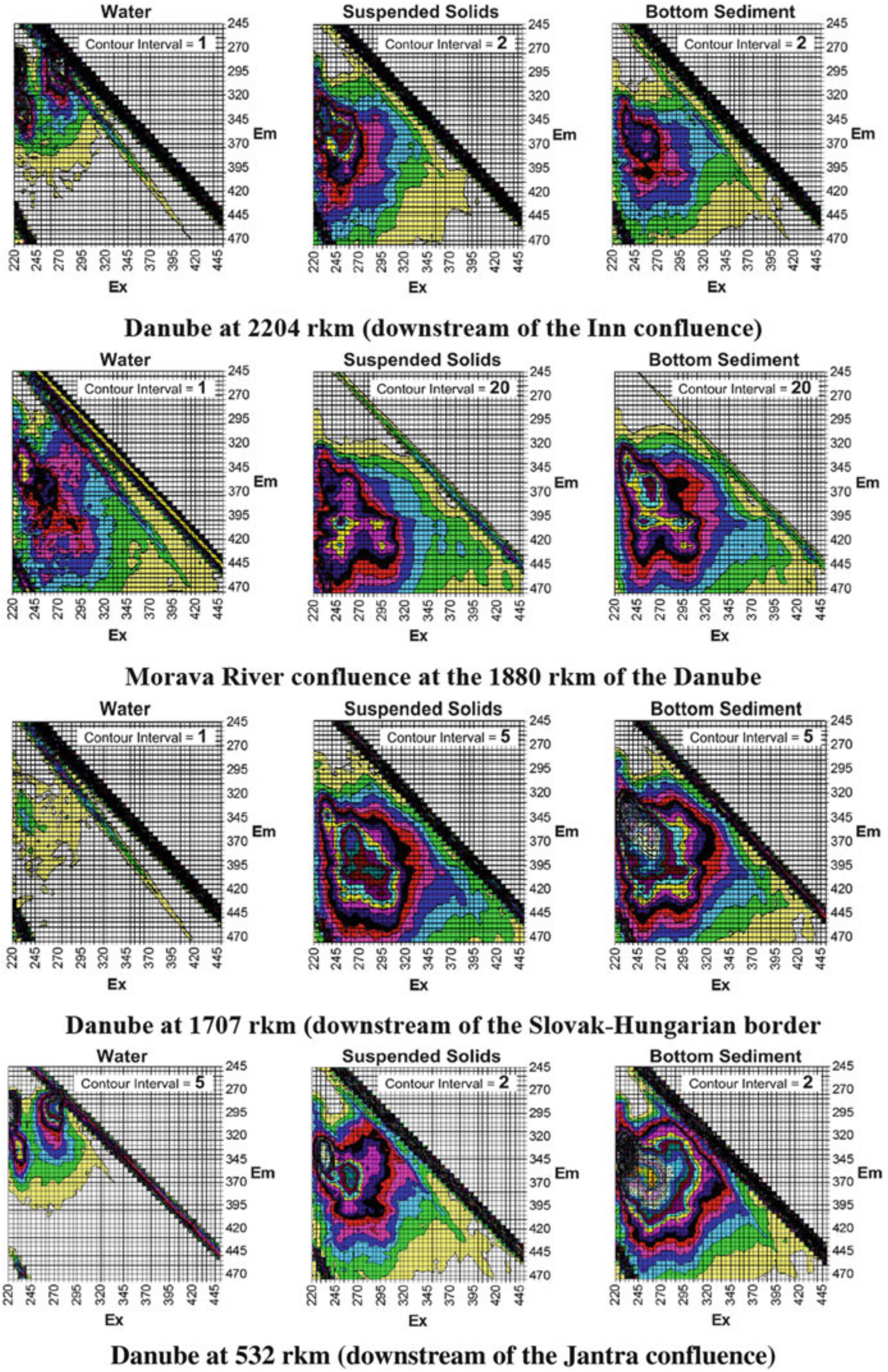

Fig. 6 Fluorescence fingerprints of water, SPM, and bottom sediment samples collected at selected sampling sites during JDS1

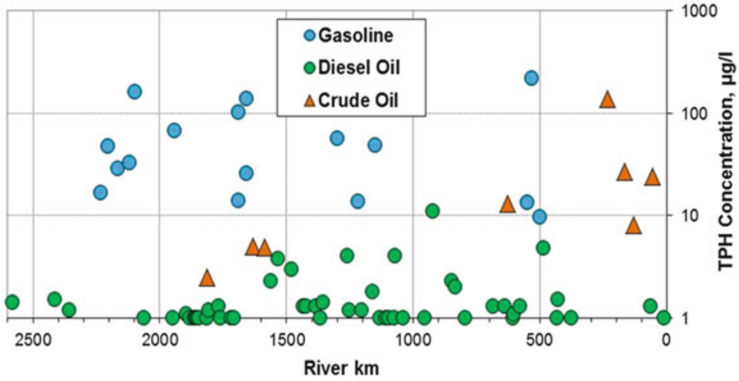

Fig. 7 Petroleum hydrocarbon contamination in the Danube water during JDS1

The concentration of the petroleum hydrocarbons was high in the samples with gasoline-type contamination likely due to the higher solubility of the monoaromatic hydrocarbons. The relatively high crude oil type contamination in the lower Danube reach was likely from the oil industrial discharges.

The usefulness of the one-time analysis of oil contamination in the water has been questioned after JDS1; therefore, this type of petroleum hydrocarbon analysis was discontinued. Instead, determination of PAHs in water was carried out as required by the EU WFD.

Petroleum Hydrocarbons in SPM

In both SPM and bottom sediment samples and during all three surveys, the highest correlation was observed with the crude oil standard, and the petroleum hydrocarbon contamination was calculated and expressed in crude oil equivalent.

Figure 8 shows the variation in the petroleum hydrocarbon contamination in the SPM along the Danube during the JDS1, JDS2, and JDS3 surveys.

The survey results distinguished three characteristic sections along the Danube: (1) upstream of the Gabčikovo reservoir, (2) section between the Gabčikovo and the Iron Gate dams, and (3) downstream of the Iron Gate reservoir, similar to the observation during the bottom sediment survey by the Cousteau team. The most significant variation in contamination levels was observed along the middle section.

At most of the sampling sites, the highest concentrations of petroleum hydrocarbons were observed during JDS2, the lowest during JDS1, while during JDS3, the contamination level was between the results of JDS1 and JDS2, with few exemptions when the highest contamination level was found during JDS3. This was particularly significant downstream of the Arges confluence (at rkm 432).

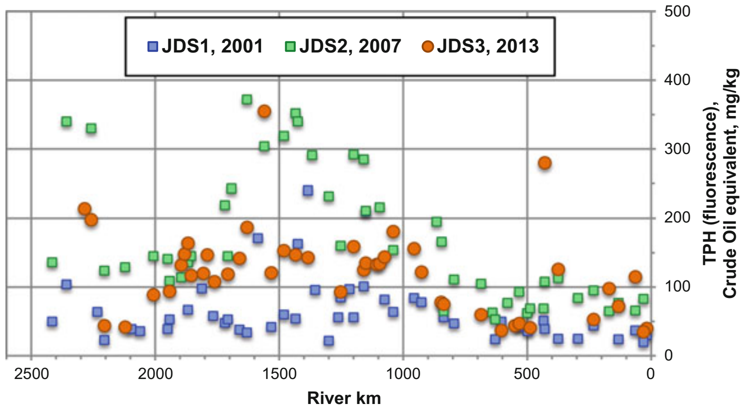

Fig. 8 Variation in petroleum hydrocarbon contamination in the SPM along the Danube River during Joint Danube Surveys

Petroleum Hydrocarbons in the Bottom Sediment

Figure 9 shows the variation in the petroleum hydrocarbon contamination in the bottom sediment along the Danube during the JDS1, JDS2, and JDS3 surveys.

The three characteristic Danube sections can be distinguished also by the results obtained for the bottom sediment samples. The highest variation was observed along the middle section of the Danube. It is likely that the highly contaminated SPM (observed in the period of JDS2) mainly settled to the bottom which resulted in an increase in the oil contamination of the bottom sediment from JDS1 through JDS2 to JDS3. The high concentration of oil pollution in the upper Danube (in Germany) as well as upstream of the Iron Gate reservoir can also be due to sedimentation of the contaminated SPM.

The significant difference between the correlation with the crude oil and the other two standards showed that: (a) gasoline-type discharges evaporate relatively fast; BTEX compounds are more soluble in the water (this was demonstrated during JDS1, showing the highest correlation with the gasoline in the water samples) and show limited adsorption to the particulate matter and (b) decreasing correlation with crude oil and increasing correlation with the diesel oil from the Iron gate reservoir to the Danube Delta indicate higher inputs from refined petroleum products (mainly diesel oil) and limited weathering of the hydrocarbon pollutants.

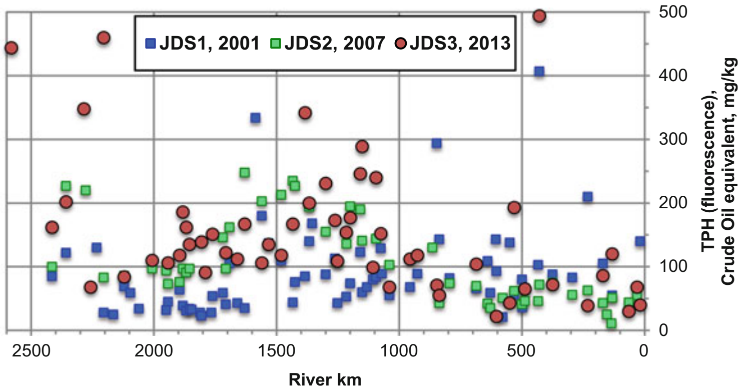

Fig. 9 Variation in petroleum hydrocarbon contamination in the bottom sediment along the Danube River during Joint Danube Surveys

4.2.2 Polyaromatic Hydrocarbons (PAHs) in Water, SPM, Bottom Sediment, and Biota

PAHs in the Water Samples

Table 3 shows the maximum concentration of individual PAH substances listed among the priority or priority hazardous substances in Directive 2013/39/EU in water samples collected during JDS3.

With the exception of benzo[g,h,i]perylene, the maximum concentration of the other PAH substances on the list was significantly below the relevant maximum allowable concentration, the MAC-EQS. It is also important to note that the detection limit of benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene was exceeded in three, five, and one water samples, respectively. Furthermore, in case of a one-time sampling and analysis of water, only the comparison to the MAC-EQS is appropriate.

PAHs in the SPM Samples

Table 4 shows the maximum concentrations of individual PAH substances in the SPM samples during JDS3.

The maximum concentration of most of the PAH substances was found at the most upstream site (at Böfinger Halde). Only the maximum concentration of benzo [a]pyrene and benzo[k]fluoranthene exceeded limit concentration indicated in the EU Priority Substances data sheet. However, even the maximum concentration of benzo[a]pyrene was far below the PELs = 763 μg/kg (see Table 2).

Table 3 Concentration of PAHs in water samples during JDS3

Substance	MAC (μg/L)	LOQ (μg/L)	Number of samples > LOQ	Maximum concentration (μg/L)
Anthracene	0.1	0.002	67	0.0401
Fluoranthene	0.12	0.002	17	0.0098
Naphthalene	130	0.002	59	0.0204
Benzo[a]pyrene	0.27	0.002	3	0.0024
Benzo[b] fluoranthene	0.017	0.002	5	0.0027
Benzo[k] fluoranthene	0.017	0.002	1	0.0022
Benzo[g,h,i] perylene	0.0082	0.0005	66	0.029

Table 4 Concentration of PAHs in SPM samples during JDS3

Substance	EU Priority data sheet (μg/kg)	LOQ (μg/kg)	Number of samples > LOQ	Maximum concentration (μg/kg)
Anthracene	24	20	2	21
Fluoranthene	2,000	20	48	191
Benzo[a] pyrene	91.5	20	35	110
Benzo[b] fluoranthene	70.7	20	39	122
Benzo[k] fluoranthene	67.5	20	25	55
Benzo[g,h,i] perylene	42	20	33	75

PAHs in the Bottom Sediment Samples

Table 5 shows the maximum concentration of individual PAH substances in the bottom sediment.

With the exception of the fluoranthene, the maximum concentrations of the other PAH substances on the list exceeded the limit concentration indicated in the EU priority substances data sheet. However, in the case of anthracene and benzo[a] pyrene, even the maximum concentration was far below the PELs = 245 and 763 μg/kg, respectively, in the Canadian Sediment Quality Guidelines (see Table 2).

Polyaromatic hydrocarbons are major contributors to the fluorescence in the cyclohexane extracts of environmental samples. The cyclohexane extract of some selected bottom sediment samples used for fluorescence fingerprinting was analyzed for PAHs. The particular reason was to compare the concentration of selected PAHs to the results of the fluorescence fingerprints. Table 6 shows the results for comparison.

Table 5 Concentration of PAHs in the bottom sediment samples during JDS3

Substance	EU Priority data sheet (μg/kg)	LOQ (μg/kg)	Number of samples > LOQ	Maximum concentration (μg/kg)
Anthracene	24	20	3	57
Fluoranthene	2,000	20	55	690
Benzo[a] pyrene	91.5	20	41	370
Benzo[b] fluoranthene	70.7	20	49	489
Benzo[k] fluoranthene	67.5	20	16	259
Benzo[g,h,i] perylene	42	20	33	328

Table 6 Concentration of selected PAHs in selected bottom sediments during JDS3

Substance	Unit	High TPH samples	Low TPH samples	Min–max during JDS2
Fluoranthene	μg/kg	215–265	21–45	15 and 853
Benzo[a]pyrene	μg/kg	104–114	41–52	10 and 115
Benzo[a]anthracene	μg/kg	66–71	26–32	
Benzo[b] fluoranthene	μg/kg	183–214	35–56	
TPH (fluorescence)	mg/kg	444–550	56–90	11 and 248

The results in Table 6 demonstrate that the higher TPH concentrations correspond to higher concentration of the PAHs. Unfortunately, the recent Directive 2013/39/EU shows EQS for water and biota only. However, considering the Canadian Sediment Quality Guidelines (CCME, 2001), even the maximum concentration of the selected PAHs is far below the PELs (probable effect limits), being 2,355, 782, and 385 μg/kg for fluoranthene, benzo[a]pyrene, and benzo[a]anthracene, respectively.

PAHs in Biota (Mussel) Samples

Mussel samples were analyzed for PAHs during JDS1. Figure 10 shows the sum of the individual PAH substances in biota.

The mussel samples contained PAHs at similar levels as during earlier surveys [1, 2]. A slight increasing trend can be observed downwards along the Danube to the Delta. The highest accumulation was measured in mussels collected from tributaries in the middle Danube reach where petroleum hydrocarbon contamination was the highest in other matrices.

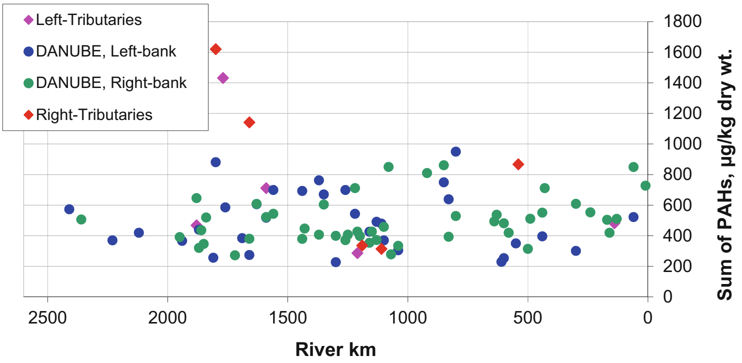

Fig. 10 Variation in the concentration of PAHs in the mussel samples collected from the Danube and its tributaries during JDS1

5 Conclusions

There are several analytical methods to measure petroleum hydrocarbons in the environment. Each method can provide information about the characteristics of the contamination. Comparison and interpretation of the data (usually called as "TPH") obtained with different analytical methods require specific treatment and considerations.

The fluorescence spectroscopy for characterizing fluorescing compounds being mostly persistent hydrocarbons (i.e., pollutants with aromatic rings, usually causing adverse effects to the environment) provided a sensitive, moderately selective, and cost-effective analytical tool for monitoring and assessment of oil pollution. The polluting aromatic hydrocarbons can be characterized in terms of fluorescence patterns of the fluorescence fingerprints; the concentration of the petroleum hydrocarbons can be calculated from the fluorescence intensity at specified excitation/emission wavelengths.

Interpretation of the fluorescence fingerprint of cyclohexane extracts of water, SPM, and bottom sediment samples, collected during the Joint Danube Surveys (in 2001, 2007, and 2013), provided information on the characteristics and level of the petroleum hydrocarbon contamination, concluding as follows:

- Petroleum hydrocarbons contamination in water was mainly characterized with the fluorescence of gasoline. The concentrations varied in the range of 2–300 µg/L, in gasoline equivalent.
- The level of oil contamination was similar in the SPM and the bottom sediment, characterized with the fluorescence of crude oil. The concentrations varied between 5 and 500 mg/kg, in crude oil equivalent.
- The petroleum hydrocarbon contamination in the bottom sediment showed slowly increasing trends during the three surveys, characterized with the highest contamination in 2013, likely caused by settling of the contaminated SPM, which showed the highest TPH concentration in 2007.

- Characteristics of the petroleum hydrocarbon contamination divided the Danube into three sections: (1) upstream of the Gabčikovo reservoir, (2) section between the Gabčikovo and the Iron Gate dams, and (3) downstream of the Iron Gate reservoir. High contamination was detected in the upper Danube reach, and significant variation in the contamination levels was observed along the middle section.
- The PAH compounds determined in water, SPM, bottom sediment, and biota (mussels) showed similar trends in contamination as observed in the case of petroleum hydrocarbons. However, even the highest concentrations in the different matrices were usually below the EQS according to EU Directive 2013/39/EU or the PELs in the Canadian Sediment Quality Guidelines.

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Pollution of Groundwater in the Danube River Basin by Hazardous Substances

Andreas Scheidleder

Abstract The implementation of the EU Water Framework Directive and the EU Groundwater Directive and the reporting there under give a very good overview of those hazardous substances which are of considerable concern in the Danube River Basin. Thirty-two hazardous substances could be identified of definitely causing considerable pollution of groundwater in the Danube River Basin as they are causing poor chemical status of at least one groundwater body.

The establishment of groundwater threshold values for 72 hazardous substances also indicates that these substances are either already causing significant pollution or are reasonably suspicious of bearing potential to significant pollution. As threshold values are established on a risk-based approach at national, river basin or groundwater body level, considerable variations are evident within the Danube River Basin District. Additionally, national legislations identifying those substances which have to be prevented from entering groundwater according to Article 6 of the EU Groundwater Directive give strong indication of further hazardous substances being relevant.

Keywords Groundwater directive, Groundwater quality, Hazardous substances, Pollution, Water framework directive

Contents

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Abbreviations

DRB	Danube River Basin
DRBD	Danube River Basin District
DRPC	Danube River Protection Convention
GWD	EU Groundwater Directive (2006/118/EC)
ICPDR	International Commission for the Protection of the Danube River
RBD	River Basin District
RBM Plan	River Basin Management Plan
TV	Threshold value
WFD	EU Water Framework Directive (2000/60/EC)
WISE	Water Information System for Europe

1 Introduction

Groundwater is of extraordinary importance in the Danube River Basin (DRB) as it is the major source of drinking water, supplying at least 59 Mio inhabitants. About 72% of the drinking water in the DRB is produced from groundwater and 28% is abstracted from surface waters. Due to the heterogenic situation in the DRB (e.g. different hydrogeological, topographic, climatic, pressure and pollution conditions), the share of groundwater used for drinking water purposes varies considerably and ranges from 30% (Bulgaria) to 100% (Austria). The individual shares, illustrated in Fig. 1, do not refer to the countries as a whole but only to the areas of the DRB within these countries.

Apart from the drinking water aspect, groundwater is also an important resource for industry (cooling purposes, food, etc.), agriculture (e.g. irrigation) and thermal water supply (balneology, heating purposes). Furthermore, it plays an essential role in the hydrological cycle, being critical for the maintenance of wetlands and feeding river flows. It acts as an important buffer during dry periods, and it provides base flow to many surface water systems [2].

Groundwater is exposed to a broad variety of human pressures ranging from different land use practices, industrial and other activities, accidents, intrusions from connected surface and marine waters and effects induced due to climate change. A considerable amount of the groundwater in the DRB is located in karstic aquifers which are highly vulnerable to contamination due to their high permeability. The percolation time for contaminants is very short, and therefore natural

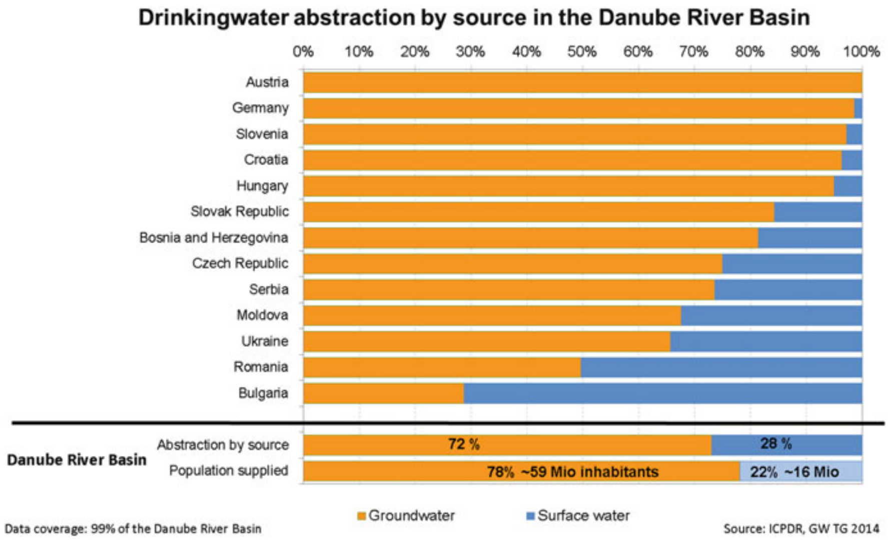

Fig. 1 Drinking water abstraction by source in the Danube River Basin. *Source*: ICPDR Groundwater Task Group, ICPDR 2014. *Note*: The statistics given for the countries do not refer to the countries as a whole but only to the areas of the Danube River Basin District within these countries. The overview focuses on the contracting parties of the ICPDR (14 countries sharing over 2,000 km^2 of the DRB). In contrary to the definition in the Joint Questionnaire OECD/Eurostat [1], bank-filtered water was agreed by the Groundwater Task Group to be considered as groundwater. Reference year of the data: 2006 (Serbia), 2007 (BiH-Federation of Bosnia and Herzegovina, Bulgaria, Germany, Romania and Ukraine), 2008 (Hungary and Slovak Republic), 2009 (Austria), 2010 (Czech Republic and Slovenia), 2011 (BiH-Republic of Srpska and Moldova)

purification processes are very limited; contaminations may reach the receptors (humans, ecosystems) within hours. Porous aquifers are usually much better protected, and contaminations may reach the groundwater decades or even later after the polluting activity took place. But this also means that once polluted, remediation measures will take very long and are extremely costly or not effective at all. Hence, the precautionary principle and measures to prevent and limit inputs into groundwater are the keys for (cost) effective groundwater management.

2 Groundwater Governance Under the ICPDR

When the EU Water Framework Directive (WFD, 2000/60/EC) was adopted in October 2000 in the European Community, all countries cooperating under the Danube River Protection Convention (DRPC) decided to make all efforts to implement the WFD throughout the whole basin and to prepare a common River Basin Management Plan (RBM Plan). This decision is fully in line with WFD Article 13 which requires that “Member States shall endeavour to produce a single river basin management plan”. With the implementation of the WFD, the ‘groundwater body’ was introduced as the main management unit in achieving the environmental objectives.

About 80% of the Danube River Basin District (DRBD) is part of the European Union, and nine national (and even more detailed) RBM Plans in the area of the DRBD were reported to the European Commission. In total 722 national groundwater bodies were identified in the area of the DRBD.

For the remaining part (20%) of the DRBD, the identification of groundwater bodies by non-EU Member States is in progress and only partly completed, as the non-EU Member States are basically not obliged to stick to the very tight implementation schedule of the WFD and its legal requirements. Therefore, the status and level of implementation and the level of available information are diverse, and the identification of pressures and risks and the systematic monitoring of relevant hazardous substances in groundwater are partly not so far developed in the non-EU Member States.

As the ICPDR decided to focus its efforts and activities and also the common Danube RBM Plan on aspects of basin-wide importance, it was agreed to identify and put focus on bilaterally agreed transboundary groundwater bodies of basin-wide importance. Importance of such groundwater bodies was defined either by size (larger than 4,000 km^2) or by various criteria, e.g. socio-economic importance, uses, impacts, pressures and interaction with aquatic ecosystem.

Finally, 11 groundwater bodies or groups of groundwater bodies of basin-wide importance were identified by the contracting parties of the ICPDR and bilaterally agreed. These 11 groundwater bodies are formed by in total 59 individual national groundwater bodies.

To coordinate the cooperation on groundwater within the DRB, the ICPDR contracting parties established the Groundwater Task Group in 2004 to deal with groundwater-related issues of basin-wide concern. All the principles and decisions within the GW TG are summarized in a guidance document [2] which is regularly reviewed and updated.

3 Hazardous Substances in Groundwater

3.1 Relevant Information Sources

The most relevant information source for assessing which hazardous substances are supposed to cause significant groundwater pollution on a basin-wide level is the reporting under the WFD and the legislation established in response to the requirements of the EU Groundwater Directive (2006/118/EC, GWD), at least in that part of the Danube Basin which falls into the territory of the European Union (~80%). In contrary to specific case study investigations which are usually focused on very narrow locations and specific point sources and substances of pollution, both directives focus on the aspects endangering the good chemical status of groundwater bodies as a whole. Therefore, substances reported under the WFD are usually of high relevance when assessing basin-wide concerns.

Within the River Basin Management Plans (RBM Plans), EU Member States have to systematically and periodically investigate and report on the anthropogenic pressures and the effects of human activity on groundwater status considering the functions of groundwater in relation to the health of associated aquatic and dependent terrestrial ecosystems and all legitimate uses of groundwater. If Member States identify that there might be a risk that the good groundwater status cannot be achieved, Member States have to implement sufficient monitoring, establish groundwater threshold values and implement all measures necessary to achieving good status. Measures might also be needed to keep the good status and to avoid any deterioration in the status.

In addition to achieving the environmental objectives of the WFD, Article 6 of the GWD obliges Member States to implement all measures necessary to prevent inputs of hazardous substances into groundwater and to identify those substances which are relevant.

Hence, the following three reporting elements within the implementation of the WFD and the GWD give reliable indications about the most relevant hazardous substances endangering groundwater in the DRB:

1. All substances which were reported to cause poor chemical status in groundwater bodies
2. All substances for which groundwater threshold values were established
3. All hazardous substances (groups of substances) where inputs into groundwater have to be prevented (Article 6 of the GWD)

As not all countries in the DRB are EU Member States and obliged to WFD reporting, the groundwater experts from the non-Member States in the ICPDR Groundwater Task Group helped to complement the basin-wide overview as far as relevant information was available.

3.2 Hazardous Substances Causing Poor Groundwater Chemical Status

The substances causing poor chemical status of groundwater bodies according to the WFD are definitely of major relevance. Reporting of the RBM Plans showed that although the dominant substances causing pollution of groundwater bodies in the DRBD as well as in Europe are nitrates from agricultural activities, also hazardous substances are endangering groundwater chemical status to a certain degree. The sources of pollution by hazardous substances are mainly agricultural

Table 1 Chemical status of groundwater bodies in the national shares of EU Member States in the Danube River Basin District

Member State	Total GWBs	Good chemical status	Unknown status	Poor chemical status	Poor chemical status due to hazardous substances
Austria	128	125	0	3	0
Bulgaria	50	32	0	18	2
Czech Republic	54	10	0	44	29
Germany	46	32	0	14	7
Hungary	185	147	0	38	8
Poland	2	2	0	0	0
Romania	142	123	0	19	0
Slovak Republic	97	58	26	13	6
Slovenia	18	14		4	2
DRBD	**722**	**543**	**26**	**153**	**54**
% in terms of numbers	–	75%	4%	21%	7%
% in terms of area	–	79%	2%	19%	–

Bold values indicate that this is the sum of all countries above. The figures for the whole Danube River Basin District

Note: A groundwater body can be in poor status due to more than one substance which is the reason why the numbers of groundwater bodies do not necessarily coincide with the figures in Table 2. No groundwater bodies of poor status for hazardous substances in Austria, Poland, Romania and Serbia

Source: WISE 2013 and RBM Plans 2009

activities with the application of pesticide products but also industrial activities and point sources of pollution like contaminated sites and waste disposal sites.

In total, 722 groundwater bodies are identified in the nine Member States national shares of the DRBD. Five hundred and forty-three groundwater bodies (75% in terms of numbers and 79% in terms of area) are of good chemical status, 26 are of unknown status (in Slovak Republic) and 153 groundwater bodies are failing to meet good chemical status (21% in terms of numbers and 19% in terms of area).

About one third (54 of 153) of the groundwater bodies of poor chemical status (in six Member States) are failing good status due to pollution by hazardous substances (see Table 1); Table 2 lists all related 32 hazardous substances (six naturally occurring and 26 synthetic substances) and the number of groundwater bodies affected. Hence, these substances are definitely causing considerable pollution of groundwater in the DRB.

Table 2 Hazardous substances causing poor chemical status and number of groundwater bodies concerned in the national parts of EU Member States in the Danube River Basin District

Substances	Number of groundwater bodies affected (total)	BG	CZ	DE	HU	SI	SK
Natural occurring substance							
Cadmium	10	–	9	–	–	–	1
Lead	10	–	10	–	–	–	–
Arsenic	5	–	2	–	–	–	3
Mercury	5	–	5	–	–	–	–
Aluminium	3	–	3	–	–	–	–
Chromium	2	2	–	–	–	–	–
Synthetic substance							
Atrazine	23	–	6	5	6	2	4
Tetrachloroethylene	20	–	18	–	1	–	1
Benzo(a)pyrene	13	–	13	–	–	–	–
Benzene	12	–	12	–	–	–	–
Desethylatrazine	11	–	5	7	4	–	–
Benzo(k)fluoranthene	10	–	10	–	–	–	–
Benzo(g,h,i)perylene	9	–	9	–	–	–	–
Indeno(1,2,3-cd) pyrene	9	–	9	–	–	–	–
Benzo(b)fluoranthene	8	–	8	–	–	–	–
Fluoranthene	8	–	8	–	–	–	–
Naphthalene	7	–	7	–	–	–	–
Simazine	7	–	–	2	3	–	2
Trichloroethylene	5	–	2	–	2	–	1
Propazin	3	–	–	1	2	–	–
Terbuthylazine	3	–	–	1	2	–	–
2,4-D	2	–	–	–	2	–	–
Desethylsimazin	2	–	–	2	–	–	–
Dieldrin	2	–	2	–	–	–	–
Metribuzin	2	–	–	–	2	–	–
Triazine, total	2	–	–	–	2	–	–
4,4-DDT	1	–	1	–	–	–	–
Bromacil	1	–	–	1	–	–	–
Desethylsebuthylazin	1	–	–	1	–	–	–
Desethylterbuthylazine	1	–	–	1	–	–	–
Diuron	1	–	–	1	–	–	–
Metazachlor	1	–	–	1	–	–	–

Note: No groundwater bodies at risk/poor status for hazardous substances in Austria, Poland, Romania and Serbia
Source: WISE 2013, RBM Plans 2009 and ICPDR Groundwater Task Group

3.3 Hazardous Substances for Which Groundwater Threshold Values Were Established

A further indication whether a substance is considered relevant and is suspected of posing threat to groundwater is when groundwater threshold values (TVs) were established. TVs have to be established by Member States for each pollutant that characterizes a groundwater body at risk of not achieving the good chemical status objectives, and they act as national groundwater quality standards. TVs need to be established in accordance with the provisions given in the GWD and reported within the RBM Plans. They play a key role in the assessment of groundwater chemical status and trends as – beside other aspects – the monitoring data are compared with the threshold values.

In the establishment of TVs, Member States need to consider the interactions between groundwater and associated aquatic and dependent terrestrial ecosystems, the interference with actual or potential legitimate uses or functions of groundwater and the natural levels of substances in groundwater. TVs are established at the most appropriate scale, either at the national level, at the level of River Basin Districts (RBDs) or national parts of international RBDs or at the level of groundwater bodies or groups of groundwater bodies.

In total, for 88 different substances/indicators, groundwater TVs were reported in the national RBM Plans of nine Member States in DRBD and of Serbia. Looking into more detail, 72 of the reported substances can be referred to as more or less hazardous. Out of these 18 substances are naturally occurring and belong to the group of metals and 54 are (individual) pesticide substances or metabolites, hydrocarbons or other synthetic substances. The reported hazardous substances are listed in Table 3 (naturally occurring substances) and Table 4 (synthetic substances), indicating the Member States where respective TVs have been established at their national shares of the DRBD.

The establishment of a TV gives evidence that the corresponding substance is relevant in the respective Member State or RBD, either causing already significant pollution or reasonably suspicious of bearing potential to significant pollution. It is not fully clear on the basis of the information provided in WISE, which of the listed substances/indicators pose actual risk to groundwater bodies of not meeting good chemical status in 2015 because a number of TVs were primarily established to enable risk and status assessment for the preparation of the RBM Plan of 2009 and not as a result of an actual risk. But nevertheless, a substance for which a TV has been established is considered as relevant.

3.3.1 Variations of Groundwater Threshold Values in the DRB

Due to the risk-based approach applied by each individual Member State, groundwater threshold values cannot be uniform throughout Europe and not even within an RBD. This was also illustrated by a report from the European Commission to the

Table 3 Naturally occurring hazardous substances for which groundwater TVs were established in the national shares of EU Member States in the Danube River Basin District

	Total	AT	CZ	DE	HU	PL	RO	SK
Cadmium	7	x	x	x	x	x	x	x
Lead	7	x	x	x	x	x	x	x
Arsenic	6	x	x	x	–	x	x	x
Mercury	6	x	–	x	x	x	x	x
Chromium	4	x	–	x	–	x	–	x
Copper	4	x	–	x	–	x	–	x
Nickel	3	x	–	x	–	x	–	–
Aluminium	2	–	x	–	–	x	–	–
Antimony	2	–	–	x	–	x	–	–
Barium	2	–	–	x	–	x	–	–
Cobalt	2	–	–	x	–	x	–	–
Molybdenum	2	–	–	x	–	x	–	–
Selenium	2	–	–	x	–	x	–	–
Vanadium	2	–	–	x	–	x	–	–
Zinc	2	–	–	x	–	x	–	–
Silver	1	–	–	–	–	x	–	–
Thallium	1	–	–	x	–	–	–	–
Titanium	1	–	–	–	–	x	–	–

Note: No groundwater threshold values for naturally occurring hazardous substances established/reported in Bulgaria, Slovenia and Serbia
Source: WISE, RBM Plans 2009 and ICPDR Groundwater Task Group
x stands for a tick mark

Council and the European Parliament on groundwater TVs [3] which showed significant differences across the European Union. Subsequently, an in-depth assessment was conducted to further explore the reasons behind these variations using the example of selected substances by considering the information published in the first RBM Plans and further details collected from Member States [4]. The analysis also depicts how the flexible elements in the WFD and GWD ensure or hinder a comparable level of implementation and thereby a comparable level of groundwater status results within the EU.

Looking at the substances for which TVs were established most often in the DRBD, considerable differences appear for some of the substances. Table 5 lists all substances for which TVs were established by at least three Member States in the DRBD. Table 6 compares for selected substances the individual groundwater TVs which were established in the individual national RBDs belonging to the overall DRBD.

Due to the complex compliance regime established by the WFD and the GWD, only a very detailed look into the underlying factors considered may allow drawing conclusions whether the established and reported TVs provide a comparable level of groundwater status assessment in the national shares of the DRBD or not.

Table 4 Synthetic hazardous substances for which groundwater TVs were established in the national shares of EU Member States in the Danube River Basin District

Synthetic substances	Total	AT	CZ	DE	HU	SI	SK
Tetrachloroethylene	6	x	x	x	x	x	x
Trichloroethylene	6	x	x	x	x	x	x
1,2-Dichloroethane	4	x	–	x	–	x	x
Benzene	4	x	x	x	–	–	x
Aldrin	3	x	x	–	–	x	–
Benzo(a)pyrene	3	–	x	x	–	–	x
Dieldrin	3	x	x	–	–	x	–
Atrazine	2	–	x	–	–	–	x
Benzo(b)fluoranthene	2	–	x	x	–	–	–
Benzo(g,h,i)perylene	2	–	x	x	–	–	–
Benzo(k)fluoranthene	2	–	x	x	–	–	–
Fluoranthene	2	–	x	x	–	–	–
Heptachlor	2	x	–	–	–	x	–
Heptachlorepoxide	2	x	–	–	–	x	–
Hexachlorobenzene	2	–	x	x	–	–	–
Hydrocarbons	2	x	–	x	–	–	–
Indeno(1,2,3-c,d)pyrene	2	–	x	x	–	–	–
Nonylphenol	2	–	–	x	–	–	–
PAH, Sum	2	x	–	x	–	–	–
Simazine	2	–	x	–	–	–	x
1,1-Dichloroethane	1	–	–	–	–	x	–
4,4′-DDT	1	–	x	–	–	–	–
Alachlor	1	–	x	–	–	–	–
Alkylated benzols, total	1	–	–	x	–	–	–
Anthracene	1	–	–	x	–	–	–
Carbon tetrachloride	1	–	–	–	–	–	x
CHC, total	1	–	–	x	–	–	–
Chlorobenzene	1	–	–	x	–	–	–
Chlorophenol, total	1	–	–	x	–	–	–
Chlorpyrifos	1	–	x	–	–	–	–
Cyanides	1	–	–	x	–	–	–
Desethylatrazine	1	–	x	–	–	–	–
Dibenz(a,h)anthracene	1	–	–	x	–	–	–
Dichloromethane	1	–	–	–	–	x	–
Endrin	1	–	x	–	–	–	–
Epichlorohydrin	1	–	–	x	–	–	–
Hydrogen cyanide	1	–	x	–	–	–	–
Isodrin	1	–	x	–	–	–	–
Isoproturon	1	–	x	–	–	–	–
MTBE	1	–	–	x	–	–	–
Naphthalene	1	–	x	–	–	–	–

(continued)

Table 4 (continued)

Synthetic substances	Total	AT	CZ	DE	HU	SI	SK
Naphthalene + methylnaphthalene	1	–	–	x	–	–	–
PCB, total	1	–	–	x	–	–	–
Pentachlorobenzene	1	–	x	–	–	–	–
Phenol	1	–	–	x	–	–	–
Styrene	1	–	–	–	–	–	x
Tetrachloromethane	1	–	–	–	–	x	–
Toluene	1	–	–	–	–	–	x
TOX	1	–	–	–	x	–	–
Trifluralin	1	–	x	–	–	–	–
Trihalomethane, sum	1	x	–	–	–	–	–
Vinyl chloride	1	–	–	x	–	–	–
Volatile aliphatic halogenated hydrocarbons, total	1	–	–	–	–	x	–
Xylene	1	–	–	–	–	–	x

Note: No groundwater threshold values for synthetic hazardous substances established/reported in Bulgaria, Romania, Poland and Serbia
Source: WISE, RBM Plans 2009 and ICPDR Groundwater Task Group
x stands for a tick mark

Table 5 Ranges of groundwater threshold values for substances most commonly reported in the Danube River Basin District

Substance	Member States	Lowest TV µg/l	Highest TV µg/l	TV ranges (x times)
Cadmium	7	0.5 (DE)	27 (RO)	54
Lead	7	5 (CZ)	320 (RO)	64
Arsenic	6	5.25 (SK)	40(RO)	7.6
Mercury	6	0.2 (DE)	1	5
Tetrachloroethylene	6	2 (SI)	10	5
Trichloroethylene	6	2 (SI)	10	5
1,2-Dichloroethane	4	1.65 (SK)	3 (SI)	1.8
Benzene	4	0.75 (SK)	1 (CZ, DE)	1.3
Chromium	4	7 (DE)	50 (PL)	7
Copper	4	14 (DE)	1800 (AT)	129
Nickel	3	14 (DE)	20 (PL)	1.4
Aldrin	3	0.03		1
Dieldrin	3	0.03		1
Benzo(a)pyrene	3	0.006 (SK)	0.01(CZ, DE)	1.7

Source: WISE, RBM Plans 2009 and ICPDR Groundwater Task Group

In establishing TVs the GWD follows a risk-based approach and requests Member States to take regard of the relevant receptors of the groundwater as well as the risks and functions, the characteristics and behaviour of the pollutants and the hydrogeological characteristics represented by the natural background levels.

Table 6 Comparison of selected TVs (in μg/l) in the different national RBDs which belong to the Danube River Basin District

RBD code	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
AT1000	9	4.5	45	1,800	9	0.9	18	–
BG1000	–	–	–	–	–	–	–	–
CZ_1000	10	1	–	–	5	–	–	–
DE1000	10	0.5	7	14	7	0.2	14	58
HU1000	–	5	–	–	10	1	–	–
PL1000	20	5	50	200	100	1	20	1,000
RO1000	10–40	5–27	–	–	10–320	1	–	–
SI_RBD_1	–	–	–	–	–	–	–	–
SK40000	5.25–10	1.525–2.5	25–27	500.2–504	5.25–10	1	–	–
Total	**5.25–40**	**0.5–27**	**7–50**	**14–1800**	**5–320**	**0.2–1**	**14–20**	**58–1000**

Source: [4], WISE, RBM Plans 2009 and ICPDR Groundwater Task Group
Bold values indicate that this is the total range within the Danube River Basin District

The individual consideration of the relevant elements, potentially adapted to each individual groundwater body, leads to the various approaches followed by the Member States [3].

In the DRB, the highest ranges of groundwater TVs are evident with naturally occurring substances as the natural background levels vary quite considerably due to the complex geological setting within the basin. The differences between the lowest and the highest TVs for these substances range between 1.4 and 129 times.

For synthetic substances the differences are rather small as the TVs are either derived from drinking water standards as far as the groundwater is used for drinking water purposes and/or environmental quality standards as far as aquatic or terrestrial ecosystems are connected or dependent to the groundwater.

3.4 Compilation: Hazardous Substances with Threshold Values and Substances Causing Poor Groundwater Chemical Status

The following Tables 7 and 8 provide a compilation of all hazardous substances (distinguished between naturally occurring and synthetic substances) which cause poor groundwater chemical status within the DRBD and for which groundwater threshold values were established and reported to WISE.

Table 7 Naturally occurring hazardous substances – poor status and threshold values

Substance	Poor status	TVs	Substance	Poor status	TVs	Substance	Poor status	TVs
Aluminium	x	x	Antimony	–	x	Selenium	–	x
Arsenic	x	x	Barium	–	x	Silver	–	x
Cadmium	x	x	Cobalt	–	x	Thallium	–	x
Chromium	x	x	Copper	–	x	Titanium	–	x
Lead	x	x	Molybdenum	–	x	Vanadium	–	x
Mercury	x	x	Nickel	–	x	Zinc	–	x

x stands for a tick mark

3.5 Substances Where Input to Groundwater Has to Be Prevented

Further strong indication of whether a hazardous substance is considered relevant regarding the pollution of groundwater in the DRB gives the national lists of identified hazardous substances which have to be prevented from entering groundwater.

According to Article 6(1)(a) of the GWD, Member States shall implement all measures necessary to prevent the input of any hazardous substances into groundwater. For any other substance which is not considered hazardous, the input into groundwater has to be limited so as to ensure that such inputs do not cause deterioration or significant and sustained upward trends in the concentrations of pollutants in groundwater.

According to this Article, Member States are obliged to identify those substances that they consider hazardous on the basis of their intrinsic properties. Therein Member States take account of hazardous substances belonging to the families or groups of pollutants referred to in Annex VIII to the WFD. Hazardous substances effectively replace the previous List 1 substances under the old Groundwater Directive (80/68/EEC) [5].

Table 9 gives an overview of the hazardous substances which were quoted in the respective national legislations whose input into groundwater is to be prevented. An entry to such a national list does not mean that there is groundwater pollution evident in the Member State but, e.g. the explicit nomination of a certain metal can give evidence that significant specific pressures exist that could cause considerable pollution by that substance if not specifically tackled by a regulation.

Table 8 Synthetic hazardous substances – poor status and threshold values

Substance	Poor status	TVs	Substance	Poor status	TVs	Substance	Poor status	TVs
4,4'-DDT	x	x	Metribuzin	x		Hexachlorobenzene		x
Atrazine	x	x	Propazin	x		Hydrocarbons		x
Benzene	x	x	Terbuthylazine	x		Hydrogen cyanide		x
Benzo(a)pyrene	x	x	Triazine, total	x		Isodrin		x
Benzo(b)fluoranthene	x	x	1,1-Dichloroethane		x	Isoproturon		x
Benzo(g,h,i)perylene	x	x	1,2-Dichloroethane		x	MTBE		x
Benzo(k)fluoranthene	x	x	Alachlor		x	Naphthalene + methylnaphthalene		x
Desethylatrazine	x	x	Aldrin		x	Nonylphenol		x
Dieldrin	x	x	Alkylated benzols, total		x	PAH, total		x
Fluoranthene	x	x	Anthracene		x	PCB, total		x
Indeno(1,2,3-c,d) pyrene	x	x	Carbon tetrachloride		x	Pentachlorobenzene		x
Naphthalene	x	x	CHC, total		x	Phenol		x
Simazine	x	x	Chlorobenzene		x	Styrene		x
Tetrachloroethylene	x	x	Chlorophenol, total		x	Tetrachloromethane		x
Trichloroethylene	x	x	Chlorpyrifos		x	Toluene		x
2,4-D	x		Cyanides		x	TOX		x
Bromacil	x		Dibenz(a,h) anthracene		x	Trifluralin		x
Desethylsebuthylazin	x		Dichloromethane		x	Trihalomethane, sum		x
Desethylsimazin	x		Endrin		x	Vinyl chloride		x
Desethylterbuthylazine	x		Epichlorohydrin		x	Volatile aliphatic halogenated hydrocarbons, total		x
Diuron	x		Heptachlor		x	Xylene		x
Metazachlor	x		Heptachlorepoxide					

x stands for a tick mark

Table 9 Hazardous substances to be prevented from input into groundwater

Substances	AT	BG	DE	HU	PL	RS	SI	SK
Organohalogen compounds and substances which may form such compounds in the aquatic environment	x	x	x	x	x	x	x	x
Organophosphorus compounds	x	x	x	x	x	x	x[a]	x
Organotin compounds	x	x	x	x	x	x	x	x
Substances and preparations, or the breakdown products of such, which have been proved to possess carcinogenic or mutagenic properties or properties which may affect steroidogenic, thyroid, reproduction or other endocrine-related functions in or via the aquatic environment	x	x	x	x	x	x	x	x
Persistent hydrocarbons and persistent and bioaccumulable organic toxic substances			x	x	x		x	x
Mineral oils and other hydrocarbons	x	x		x	x	x	x	x
Cyanides	x	x	x	x		x	x	x
Cadmium and its compounds	x	x	x	x	x	x	x	x
Lead and its compounds			x				x	
Mercury and its compounds	x	x	x	x	x	x	x	x
Nickel and its compounds			x				x	
Thallium			x					
Arsenic and its compounds			x				x	
Biocides and plant protection products							x	

[a]It concerns Chlorfenvinphos, Chlorpyrifos and Glyphosate
Source: ICPDR Groundwater Task Group
x stands for a tick mark

4 Conclusions

The reporting of the EU Member States under the WFD in the year 2013 allows for compiling a comprehensive overview of hazardous substances posing a threat to groundwater. In total, 32 hazardous substances are causing poor chemical status of at least one groundwater body in the DRB, and for another 51 substances/indicators, groundwater threshold values have been established, which indicates risk of failing the objectives of the WFD. A further strong indication of the relevance of hazardous substances in the DRB gives national legislations identifying those substances or groups of substances which have to be prevented from entering groundwater according to Article 6 of the EU Groundwater Directive. According to the WFD the EU Member States have now to respond by an appropriate programme of measures in order to remediate, enhance and protect Europe's groundwater bodies.

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Hazardous and Emerging Substances in Drinking Water Resources in the Danube River Basin

Florian R. Storck, Frank Sacher, and Heinz-Jürgen Brauch

Abstract This article gives an overview on hazardous and emerging substances in several European streams and compares and discusses actual findings from the Danube. Concentrations of priority pollutants, pesticides, pharmaceuticals, industrial chemicals, and artificial sweeteners are mostly lower in the Danube and its tributaries than in the Rhine River. However, tributaries with low discharge and a high portion of wastewater or industrial emissions may strongly contribute to the overall pollution of the Danube and finally the Black Sea. Direct use of surface water without advanced treatment or indirect use of bank-filtrated water with short retention times during subsurface passage is common in parts of the Danube catchment to prepare drinking water. However, due to the comparatively low concentrations of pollutants, drinking water production at the Danube is currently not endangered.

Keywords Acesulfame, Bank filtration, Diatrizoate, Emerging substances, Pharmaceuticals, Danube River, Surface water, Water quality

Contents

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Abbreviations

4Me-BTZ	4-Methyl-1*H*-benzotriazole
5Me-BTZ	5-Methyl-1*H*-benzotriazole
AA-EQS	Annual average environmental quality standard
AGR	Artificial groundwater recharge
AOS	Adsorbable organic sulfur compounds
AOX	Adsorbable organic halogen compounds
BTZ	1*H*-Benzotriazole
CH	Switzerland
DDT	Dichlorodiphenyltrichloroethane
DE	Germany
DOC	Dissolved organic carbon
EQS	Environmental quality standards
HRO	Croatia
HU	Hungary
HVOC	Highly volatile organic compounds
IAWD	International Association of Water Supply Companies in the Danube River Catchment Area
ICPDR	International Commission for the Protection of the Danube River
LOQ	Limit of quantification
NDS	Naphthalenedisulfonate
NL	The Netherlands
NS	Naphthalenesulfonate
PAH	Polycyclic aromatic hydrocarbons
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonate
PFCs	Perfluorinated compounds
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
POP	Persistent organic pollutant
RS	Serbia
TOC	Total organic carbon
X-RCM	Iodinated X-ray contrast media

1 Introduction

At present, management of real or perceived contamination of water resources (freshwater or seawater) with thousands of industrial and natural chemical compounds is one of the key questions, concerning the preventive protection of natural water resources as well as the safety of drinking water production. The term hazardous compounds is clearly defined in the literature: hazardous substances are regarded as well-known contaminants such as heavy metals, pesticides, chlorinated or halogenated compounds, as well as polycyclic aromatic hydrocarbons for which standard or guideline values have been set. Regarding drinking water quality, most of these substances have been regulated for decades due to potential health effects. Depending on their biological and chemical properties like toxicity, persistency, and bioaccumulation, potential hazardous substances may also have an impact on aquatic organisms and water quality in general. According to legal regulations in Europe (Water Framework Directive), monitoring and management strategies have already been set in order to protect freshwater resources and to enable a sustainable use of natural water systems in the future. In this context, the list of priority substances (at present, 45 individual compounds or classes of compounds) with their respective environmental quality standards (EQS) is a tool for the EU policy to manage water resources and to improve water quality in general.

Besides the well-known hazardous compounds, a huge number of anthropogenic chemicals have been found in water resources in low concentrations (low μg/L–ng/L range). These so-called emerging contaminants are potentially hazardous substances as information on possible toxic effects for aquatic organisms and humans is often missing. Today, emerging contaminants are regularly defined as substances for which health-based or ecology-based standard or guideline values have not been set so far. Furthermore, emerging substances are currently not included in routine monitoring programs in major river basins. Emerging contaminants comprise, e.g., pharmaceuticals, hormones, perfluorinated compounds (PFCs), flame retardants, benzotriazoles, artificial sweeteners, siloxanes, musks, algal toxins, perchlorate, or pesticide transformation products. For a general compilation of current research, regulation, and analytical methods on emerging contaminants, see the review by Richardson and Ternes [1].

2 Drinking Water Production in the Danube River Basin

The Danube is the second largest river in Europe and about 80 million people are dependent on freshwater resources in the Danube basin for their drinking water supply. In general, various drinking water sources are regularly used: in the upper regions of the Danube basin (e.g., Germany and Austria), springwater from the Alpine regions and/or groundwater are the main resources. Additionally, riverbank-filtrated water contributes to a low percentage to the drinking water supply in some major cities like Ulm and Regensburg in Germany or Linz in Austria.

Riverbank-filtrated water – this means groundwater which interacts with river water dependent on water flow regime – has a good quality and is well suited for drinking water preparation. Riverbank filtration is regarded as a natural water treatment process which generally improves water quality and may reduce water treatment costs. Overall benefits of riverbank filtration and subsurface treatment (artificial groundwater recharge – AGR) are the removal of particles, microorganisms, pathogens, natural organic matter, organic and inorganic chemicals, as well as hazardous substances [2–14]. In general, river water undergoes a diversity of natural attenuation processes, significantly improving water quality, without the need for adding process chemicals, and resulting in a high-quality natural water.

In Slovakia, Hungary, and Serbia, the capitals Bratislava, Budapest, and Belgrade use 50–100% of riverbank-filtrated groundwater for their drinking water supply. In the lower part of the Danube River Basin (especially the lowlands of Romania and Bulgaria), bank filtration sites are rare due to inappropriate geological conditions or low flow velocities and – for several tributaries – due to temporarily low discharges. In those areas, the population has to rely mainly on surface water which is regularly treated by chemicals (chlorine). Similar to the Danube River, water supply along the major tributaries like the rivers Drava, Sava, and Tisza is based on the spring water or groundwater in the upper regions which does not directly interact with river water. Bank filtration (and partly AGR) is applied in the middle and lower catchment sections, if conditions are appropriate.

Drinking water production in the Rhine River Basin is different from the situation in the Danube catchment. In Switzerland, as well as in the southern regions of Germany, spring- or well water is used and lakes (lake of Zurich, lake of Constance, etc.) serve as large drinking water reservoirs. Artificial groundwater recharge is used to produce drinking water, e.g., for the agglomeration of Basel (Switzerland) and large parts of the Ruhr metropolitan region (Germany). In the upper Rhine valley, downstream of the City of Basel, drinking water supply is generally based on groundwater which does not interact with the river. In the middle and lower sections of the catchment, drinking water supply of the major cities of Mainz, Wiesbaden, Koblenz, Köln, Düsseldorf, Duisburg, etc., is based on riverbank filtration (and partly AGR) since more than 100 years. In the Netherlands, dune infiltration for improving water quality is frequently applied after direct abstraction of river water. Additionally, raw water and groundwater are generally treated by appropriate modern and efficient technical processes.

3 Monitoring Programs in the Danube River Basin

Since 1994, the International Association of Water Supply Companies in the Danube River Catchment Area (IAWD) has been conducting a yearly monitoring program with the general goal to determine physical, chemical, and microbiological parameters which are important for drinking water production. This monitoring program covers most of the parameters which are relevant for drinking water surveillance under the EU Drinking Water Directive. In detail, basic parameters (temperature, conductivity, pH value, oxygen, turbidity), nitrogen and phosphorus species (nutrients), toxic elements, and heavy metals as well as organic surrogate parameters like TOC (total organic carbon), DOC (dissolved organic carbon), and AOX (adsorbable organic halogen compounds) are regularly measured. In addition, the microbiological parameters *E. coli*/coliform bacteria, *Enterococci*, and *Clostridium perfringens* are determined due to their general relevance for drinking water production.

The International Commission for the Protection of the Danube River (ICPDR) also conducts annually a routine and comprehensive monitoring program with up to 116 sampling sites across the entire Danube basin. This program mainly reflects the requirements of the EU Water Framework Directive and takes less account of the demands of drinking water production. The data and results of both monitoring programs however provide a good overview on the water quality of the Danube River and its major tributaries.

In addition, ICPDR carried out the very comprehensive Joint Danube Surveys in 2001 and 2007 as well as in 2013 in order to get more detailed information on the chemical, biological, ecological, and hydromorphological status of the Danube River and the major tributaries analyzing water, sediments, and suspended solids as well as biota. The results of the ongoing monitoring programs can be downloaded from the respective websites (www.iawd.at, www.icpdr.org). Data on the occurrence of hazardous compounds (priority pollutants) and emerging substances in the Danube River Basin are generally less available as the determination of a major list of organic substances requires well-equipped and experienced laboratories and is more expensive than the determination of basic or inorganic parameters. Therefore, in most cases, results of short-term sampling campaigns are available and will be presented in the following sections.

4 Hazardous and Emerging Substances of Concern

4.1 Priority Substances

In the past, hazardous compounds such as highly volatile organic compounds (HVOC), polycyclic aromatic hydrocarbons (PAH), as well as pesticides and highly chlorinated insecticides were analyzed more frequently as limit and guideline values have been set for surface water, groundwater, and drinking water. Some of these individual substances are listed as priority substances under the EU Water Framework Directive [15]. This means that priority pollutants have to be regularly monitored in European countries and data are also available for the Danube River Basin. Although a ban on the production, marketing, or use of many of those listed priority substances had been placed already several years ago, their emission into water bodies is still possible either from sewage treatment plants or from diffuse runoff. In general the concentrations currently found in the Danube River and its tributaries are mostly marginal and often below the limit of quantification (LOQ) of the analytical methods used. This is mainly due to the very low solubility of these compounds in water as well as to their marked tendency to adsorb onto solids. This also results in a near-total or total removal by means of natural riverbank filtration (infiltration) and technical treatment processes.

The following table lists average and maximum values for priority pollutants in Danube River water (Table 1) (location Regensburg, km 2,354). In addition, the LOQs and respective environmental quality standards (EQS) are given.

For most of the analyzed substances, the average concentrations found were far below the environmental quality standards. For fluoranthene (No. 15) and benzo(a) pyrene (No. 28), it is not possible to give a definite statement regarding compliance with the EQS, as the LOQ is significantly higher than the respective EQS. Only the pesticide isoproturon was found occasionally in samples above the LOQ. As a conclusion, it can be pointed out that findings of priority substances in the Danube River near Regensburg are rather seldom, and in most cases, concentrations found are far below the environmental quality standards. Thus, regarding the compounds discussed here, a negative impact for drinking water production cannot be recognized.

4.2 Perfluorinated Compounds (PFCs)

Some years ago, perfluorinated compounds (PFCs) have been assessed as hazardous substances in water due to their toxicity, bioaccumulation potential, and persistency. The most relevant compounds have been perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) which were found in surface water, groundwater, and drinking water from the low ng/L to the low μg/L range. Although restricted regulation on the use of PFOS has been expected by the European Parliament and a voluntary initiative was launched to reduce emissions of PFOA in 2006, contamination of aquatic systems has not stopped at all because of the manifold use and application of PFC. PFOS has been classified as a persistent organic pollutant (POP) by the Stockholm convention [17] and has recently been listed in Annex I as a priority substance under the EU Water Framework Directive [15]. An overview on PFC is given in a recent monograph edited by T. P. Knepper and F. T. Lange, including information on the presence of PFC in European waters [18].

Although first investigations of PFC in the Danube River Basin showed that concentrations found were relatively low (ng/L range), some hot spots of PFC emissions into smaller tributaries have been identified. In eastern Bavaria, Germany, very high concentrations of PFOA (7.5 μg/L) were found in the small Alz River [19]. Groundwater was also contaminated (up to 7.4 μg/L) and drinking water supply was severely affected in that area [20]. The reason for the

Table 1 Concentrations of priority substances in Danube River water (location Regensburg, km 2,354) in μg/L

No.[a]	Name of substances	AA-EQS	LOQ	2011		2012	
				Mean	Max	Mean	Max
2	Anthracene	0.3	0.01	<0.01	<0.01	<0.01	<0.01
3	Atrazine	0.6	0.02	<0.02	<0.02	<0.02	<0.02
9b	DDT total	0.025	0.005	<0.005	<0.005	<0.005	<0.005
11	Dichloromethane	20	10	<10	<10	<10	<10
13	Diuron	0.2	0.02	<0.02	<0.02	<0.02	<0.02
14	Endosulfan	0.005	0.002	<0.002	<0.002	<0.002	<0.002
15	Fluoranthene	0.0063	0.01	<0.01	<0.01	<0.01	<0.01
16	Hexachlorobenzene	–	0.001	<0.001	<0.001	<0.001	<0.001
18	Hexachlorocyclohexane	0.02	0.005	<0.005	<0.005	<0.005	<0.005
19	Isoproturon	0.3	0.02	<0.02	0.074	<0.02	0.037
22	Naphthalene	2	0.01	<0.01	<0.01	<0.01	<0.01
28	Benzo(a)pyrene	1.7×10^{-4}	0.01	<0.01	<0.01	<0.01	<0.01
29	Simazine	1	0.02	<0.02	<0.02	<0.02	<0.02
32	Trichloromethane	2.5	0.1	<0.1	<0.1	<0.1	<0.1

Data source: [16]

[a]Alphabetic sequence referred to Annex II, Directive 2013/39/EU [15]

AA-EQS annual average environmental quality standards for inland surface water, *LOQ* limit of quantification, *DDT* Dichlorodiphenyltrichloroethane contamination was the emission of PFOA used as an emulsifier in the production of fluoropolymers at an industrial site. Elevated concentrations propagated downstream and were also found in the Inn River, in the Danube at the location Jochenstein (German/Austrian border [19, 21]), and further downstream in the Austrian and Hungarian section of the Danube River [22].

In Table 2, ranges of concentrations of PFOA and PFOS as well as of other PFCs in major European rivers are listed. As a conclusion, concentrations of PFOA and PFOS in the Rhine River as well as in the Danube River are quite low (low ng/L range). However, local hot spots have to be kept in mind if already known. For instance, the natural drainage of contaminated groundwater from the Alz aquifer to receiving water is supposed to continue for 30 years [20]. The presence of PFOA and PFOS can be evaluated as an indicator for man-made pollution.

4.3 Pharmaceuticals

Within the last 10–15 years, a lot of studies have been conducted concerning the occurrence and fate of pharmaceuticals in European waters, particularly in surface water. The major rivers like Rhine, Meuse, Elbe, and Danube as well as smaller tributaries were investigated for more than 100 individual compounds. A very important conclusion of those findings and results was the fact that the concentrations found are strongly dependent on the portion of wastewater in the respective river basin. This means that smaller rivers with low discharges have shown generally higher concentrations of pharmaceuticals. Out of more than 100 individual substances, only a small set of pharmaceuticals like carbamazepine, diclofenac, sulfamethoxazole, and metoprolol as well as some iodinated X-ray contrast media (X-RCM) have been found in all European rivers so far. This is due to the fact that those compounds are very persistent and more polar and therefore are hardly removed in conventional wastewater treatment plants. Furthermore, these properties hamper the elimination in natural and technical water treatment processes so that residual concentrations can even be found in drinking water. Although hazards and risks for human health cannot be recognized via drinking water, consumers are worried and concerned about those findings. Tables 3 and 4 give an overview on the concentrations found in European rivers for the most relevant compounds. Continuous information on pollution of the Danube River with certain pharmaceutical residues is practically lacking, particularly for the parts of the catchment downstream of Austria.

4.4 Artificial Sweeteners and Benzotriazoles

The artificial sweeteners acesulfame and sucralose have recently been recognized as interesting compounds which are monitored by waterworks in the Rhine

Table 2 Range of perfluorinated compound concentrations in European rivers in ng/L

River/lake	Location	Year	PFBA	PFHxA	PFOA	PFBS	PFHxS	PFOS
Rhine (CH)	Basel[a]	2012	<1–2	<1	1–3	<1–16	<2	3–7
Rhine (DE)	Karlsruhe[a]	2012	<1–3	1–2	<1–6	<1	<2	4–9
Rhine (DE)	Mainz[b]	2012	<1–10	1–2	<1–3	<1–26	<1–4	4–15
Rhine (DE)	Köln[b]	2012	<1–5	<1–2	1–5	<1–3	<1–6	<1–14
Rhine (DE)	Düsseldorf[b]	2012	<1–24	<1–2	2–3	4–23	<1–2	4–10
Rhine (NL)	Lobith[c]	2012	<1–8	1–3	<1–6	2–42	<10	<1–2
Main (DE)	Frankfurt[b]	2012	<1–6	<1–2	1–3	<1–11	<1–1	3–9
Ruhr (DE)	Whole catchment[d]	2008–2012	<10–59	<10–40	<10–93	<10–250	<10	<10–91
Ruhr (DE)	Mülheim[e]	2013	<10–14	<10–13	<10–15	<10–29	<10[f]	<10–22
Danube (DE)	Leipheim[g]	2006	No data	<1–1.7	<1–1.7	<1–3.2	<1–2.2	1.6–7.8
Danube	JDS2[h]	2007	No data	No data	>1–46	No data	No data	>1–19
Danube tributaries	JDS2[h]	2007	No data	No data	<1–60	No data	No data	<1–101

[a] *Data source*: [23], $n = 12$ (Basel) and $n = 13$ (Karlsruhe)
[b] *Data source*: [24], $n = 13$
[c] *Data source*: [25], $n = 13$
[d] *Data source*: [26], decreasing concentrations for PFOA, $n = 161$
[e] *Data source*: [27], $n = 12$
[f] PFHxS once >10 ng/L
[g] *Data source*: [28], $n = 12$
[h] *Data source*: [22], Joint Danube Survey 2, whole catchment, $n = 52$, tributaries $n = 50$

PFBA perfluorobutanoic acid, *PFHxA* perfluorohexanoic acid, *PFOA* perfluorooctanoic acid, *PFBS* perfluorobutane sulfonate, *PFHxS* perfluorohexane sulfonate, *PFOS* perfluorooctane sulfonate

Table 3 Concentration ranges of pharmaceuticals in European rivers in ng/L

River/lake	Location	Year	Carbamazepine	Diclofenac	Sulfamethoxazole	Metoprolol
Rhine (CH)	Basel[a]	2012	<10–45	<10–33	<10–20	<10–16
Rhine (DE)	Karlsruhe[a]	2012	16–31	<10–34	11–19	<10–21
Rhine (DE)	Mainz[b]	2012	20–47	<10–46	16–29	16–43
Rhine (DE)	Köln[b]	2012	26–80	17–85	20–46	27–73
Rhine (DE)	Düsseldorf[b]	2012	32–68	12–97	27–47	33–100
Rhine (NL)	Lobith[c]	2012	Up to 110	14–110	No data	33–120
Main (DE)	Frankfurt[b]	2012	26–170	27–160	23–91	32–200
Ruhr (DE)	Whole catchment[d]	2008–2012	<25–190	<25–180	<25–140	<25–340
Ruhr (DE)	Mülheim[e]	2013	<50–280	46–160	<25–130	61–330
Danube (DE)	Bavaria[f]	2010	<2–90	<2–130	<2–50	<2–370
Danube	JDS2[g]	2007	18–66	<1–7	9–28	No data
Danube tributaries	JDS2[g]	2007	<1–945	<1–52	<1–204	No data

[a] *Data source*: [23], $n = 12$ (Basel) and $n = 13$ (Karlsruhe)
[b] *Data source*: [24], $n = 13$
[c] *Data source*: [25], $n = 13$
[d] *Data source*: [26], $n = 161$
[e] *Data source*: [27], $n = 10$–12, carbamazepine $n = 194$
[f] *Data source*: [29], Danube (3 sites) and tributaries (18 sites), $n = 2$ for each site
[g] *Data source*: [22], Joint Danube Survey 2, whole catchment, $n = 52$, tributaries $n = 50$

Table 4 Concentration ranges of iodinated X-ray contrast media in European rivers in ng/L

River/lake	Location	Year	Amidotrizoic acid	Iohexol	Iomeprol	Iopamidol	Iopromide
Rhine (CH)	Basel[a]	2012	<10–47	10–52	23–480	52–660	27–230
Rhine (DE)	Karlsruhe[a]	2012	17–110	14–69	68–320	76–1,100	61–170
Rhine (DE)	Mainz[b]	2012	52–190	12–75	140–520	130–690	73–150
Rhine (DE)	Köln[b]	2012	90–320	29–160	190–630	90–410	55–190
Rhine (DE)	Düsseldorf[b]	2012	85–330	39–190	220–710	120–410	77–220
Rhine (NL)	Lobith[c]	2012	61–520	43–220	200–1,000	50–620	71–280
Main (DE)	Frankfurt[b]	2012	270–1,000	86–180	250–730	97–590	160–550
Ruhr (DE)	Whole catchment[d]	2008–2012	<50–780	<50–1,400	<50–1,700	<50–2,000	<50–350
Ruhr (DE)	Mülheim[e]	2013	99–810	No data	260–1,800	110–1,400	<50–610
Danube (DE)	Bavaria[f]	2010	<50–1,000	No data	No data	No data	No data

[a] *Data source*: [23], $n = 12$ (Basel) and $n = 13$ (Karlsruhe)
[b] *Data source*: [24], $n = 13$
[c] *Data source*: [25], $n = 13$
[d] *Data source*: [26], $n = 161$
[e] *Data source*: [27], $n = 10$–12
[f] *Data source*: [29], Danube (3 sites) and tributaries (18 sites), $n = 2$ for each site

Table 5 Concentration ranges of artificial sweeteners and 1*H*-benzotriazole in European rivers in ng/L

River/lake	Location	Year	Acesulfame	Sucralose	1*H*-Benzotriazole
Rhine (CH)	Basel[a]	2012	450–890	60–90	110–250
Rhine (DE)	Karlsruhe[a]	2012	400–940	60–120	150–300
Rhine (DE)	Mainz[b]	2012	610–1,300	60–140	200–460
Rhine (DE)	Köln[b]	2012	820–2,100	70–220	260–1,000
Rhine (DE)	Düsseldorf[b]	2012	850–1,800	90–260	270–660
Rhine (NL)	Lobith[c]	2012	670–2,400	50–240	290–1,200
Main (DE)	Frankfurt[b]	2012	970–3,800	60–360	400–1,300
Ruhr (DE)	Mülheim[d]	2013	No data	No data	300–1,500
Danube	JDS2[e]	2007			Up to 380

[a]*Data source*: [23], $n = 12$ (Basel) and $n = 13$ (Karlsruhe)
[b]*Data source*: [24], $n = 13$
[c]*Data source*: [25], $n = 13$
[d]*Data source*: [27], $n = 10$–12
[e]*Data source*: [22], Joint Danube Survey 2, whole catchment, $n = 10$

catchment. This is mainly due to their persistence during natural water treatment processes like riverbank filtration [30] and their comparatively high concentrations detected in surface water: acesulfame concentrations up to 3.8 μg/L were reported in the year 2012, whereas sucralose concentrations were generally one order of magnitude lower (Table 5). Benzotriazoles, mainly 1*H*-benzotriazole, 4-methyl-1*H*-benzotriazole, and 5-methy-1*H*-benzotriazole, which are used as corrosion inhibitors, in dishwashing agents and in deicing/anti-icing fluids, are another compound class under investigation. Surface water concentrations of 1*H*-benzotriazole regularly exceeded 100 ng/L in rivers Rhine, Main, and Ruhr, and maximum concentrations up to 1,500 ng/L were reported close to the mouth of the Ruhr River (Table 5). During the Joint Danube Survey 2, a few samples were taken in the Danube catchment and concentrations did not exceed 380 ng/L. However, at present, there is no comprehensive data available on the occurrence of sweeteners and benzotriazoles in the Danube catchment.

5 Sampling Campaign in the Year 2011

5.1 Motivation and Analyzed Parameters

The overall goal of this sampling campaign was to obtain more information on the occurrence of emerging substances in the Danube basin. Besides basic and inorganic parameters (nutrients, toxic elements, heavy metals, etc.), organic surrogate parameters (TOC, DOC, AOX, AOS – adsorbable organic sulfur compounds), and mainly emerging substances like pesticides, pharmaceuticals, iodinated X-ray contrast media, naphthalenesulfonates, benzotriazoles, synthetic chelating agents,

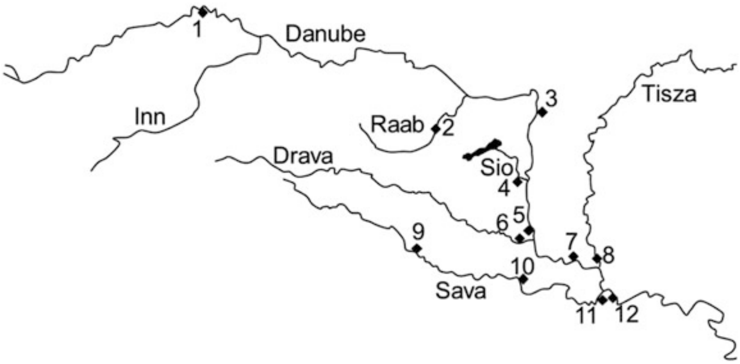

Fig. 1 Sampling sites. Numbered locations are listed in Table 6

and artificial sweeteners were analyzed. For a description of the analytical methods applied, see [12, 31].

5.2 Sampling Sites

Grab samples of river water were taken in September 2011 few meters from the riverbank, in the middle course of the Danube River as well as at several locations situated at the tributaries Sava, Drava, Raab, and Tisza. Sampled sites are shown in Fig. 1; for locations and exact sampling dates, see Table 6. Samples were cooled right after sampling until analysis.

5.3 Naphthalenesulfonates

Figure 2 shows the results of naphthalenesulfonates found at different locations. The highest concentration of 1,5-naphthalenedisulfonate (1,5-NDS) was determined in the Raab River at Sarvar. The sources for the elevated concentrations are for many years well-known emissions into the Raab River from an industrial site. For 1,5-NDS, the emissions from the Raab River have been estimated to contribute 75% to the load of 1,5-NDS in the Danube River near Budapest [32].

5.4 Benzotriazoles

Another class of industrial chemicals that was investigated during the monitoring in September 2011 were the benzotriazoles. 1*H*-Benzotriazole (BTZ) is only partly

Table 6 Locations sampled in September 2011 in the Danube catchment. Tisza was sampled on both sides of the river

Item	River	Location	Date
1	Danube	Geisling, DE	09.09.11
2	Raab	Sarvar, HU	05.09.11
3	Danube	Budapest, HU	06.09.11
4	Sio	Szekszard, HU	06.09.11
5	Danube	Batina, HRO	06.09.11
6	Drava	Osijek, HRO	06.09.11
7	Danube	Novi Sad, RS	11.09.11
8a	Tisza (l)	Titel, RS	11.09.11
8b	Tisza (r)	Titel, RS	11.09.11
9	Sava	Jasenovac, HRO	11.09.11
10	Sava	Zupanja, HRO	11.09.11
11	Sava	Makis, RS	09.09.11
12	Danube	Vinca, RS	09.09.11

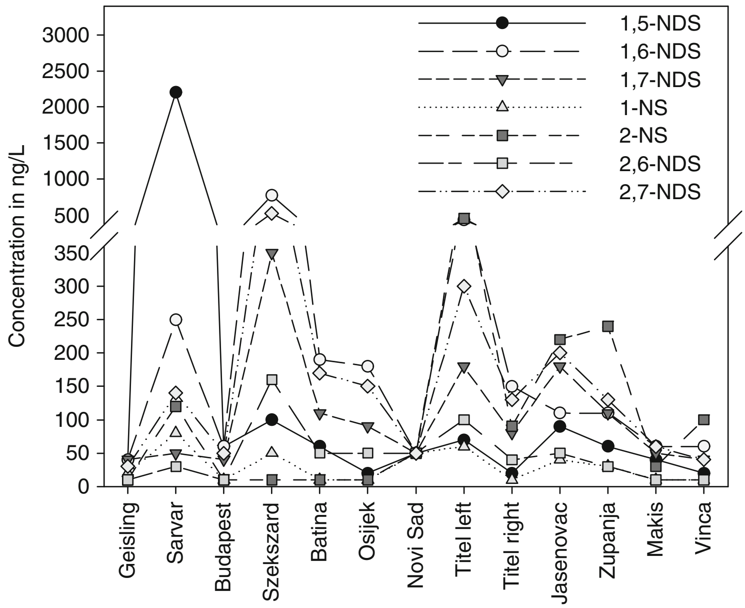

Fig. 2 Concentrations of naphthalenesulfonates in the Danube River and in several of its tributaries. Note *y*-axis break. LOQ: 20 ng/L, at Novi Sad for all parameters 100 ng/L. *NDS* naphthalenedisulfonate, *NS* naphthalenesulfonate. Values < LOQ are displayed as 0.5*LOQ

degraded during wastewater treatment, whereas 4-methyl-*1H*-benzotriazole (4Me-BTZ) and 5-methyl-*1H*-benzotriazole (5Me-BTZ) seem to be even more persistent [33]. Concentrations of BTZ and 4Me-BTZ were mostly above 100 ng/L. For both substances, a slight tendency toward lower concentrations was observed following the Danube downstream (Fig. 3). A similar tendency was

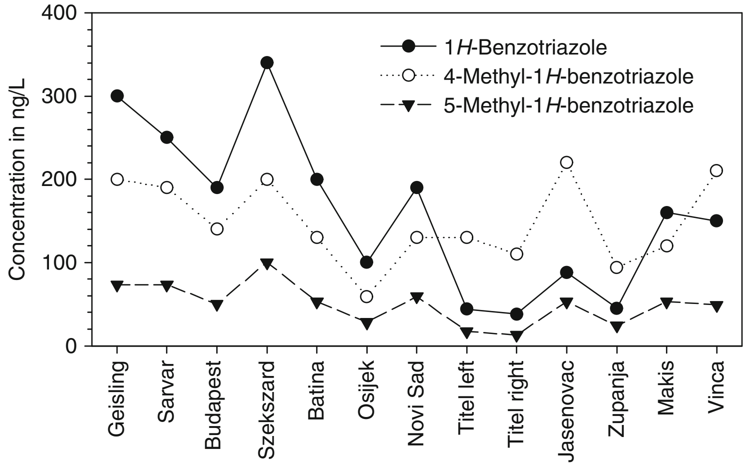

Fig. 3 1*H*-Benzotriazole, 5-methyl-1*H*-benzotriazole, and 4-methyl-1*H*-benzotriazole concentrations in the Danube River and in several of its tributaries. LOQ: 10 ng/L

observed for 5Me-BTZ, but the concentration level did not exceed 100 ng/L. In contrast to the Danube River, concentrations of BTZ in the Rhine River increase downriver, and the concentration levels both in the Rhine and in its tributaries Ruhr and Main are generally higher than in the Danube catchment (compare Table 5).

5.5 Iodinated X-Ray Contrast Media and Pharmaceuticals

Concentrations of five iodinated X-ray contrast media are displayed in Fig. 4. The highest concentrations were found in the Sio River, a small tributary of the Danube in Hungary. Sio River serves as a receiving water system for treated wastewater from the Balaton area. Concentrations of amidotrizoic acid (diatrizoate), iomeprol, iopamidol, iopromide, and iohexol ranged up to several 100 ng/L. In contrast, the concentrations found in other tributaries and in the Danube were much lower and mostly below 100 ng/L. The same holds true for carbamazepine and the metamizol metabolites *N*-acetyl-4-aminoantipyrine and *N*-formyl-4-aminoantipyrine, for which the highest concentrations were detected in the Sio River near Szekszard (Fig. 5). Concentrations of diclofenac, ibuprofen, and several beta-blockers were mostly <10 ng/L and thus smaller than in the Rhine catchment (compare Tables 3 and 4). Diclofenac concentrations in Geisling, Sarvar, Budapest, and Szekszard ranged from 12 to 32 ng/L. Atenolol was only detected downstream of Belgrade (11 ng/L) and metoprolol occurred in Geisling, Sarvar, and Szekszard in concentrations up to 27 ng/L.

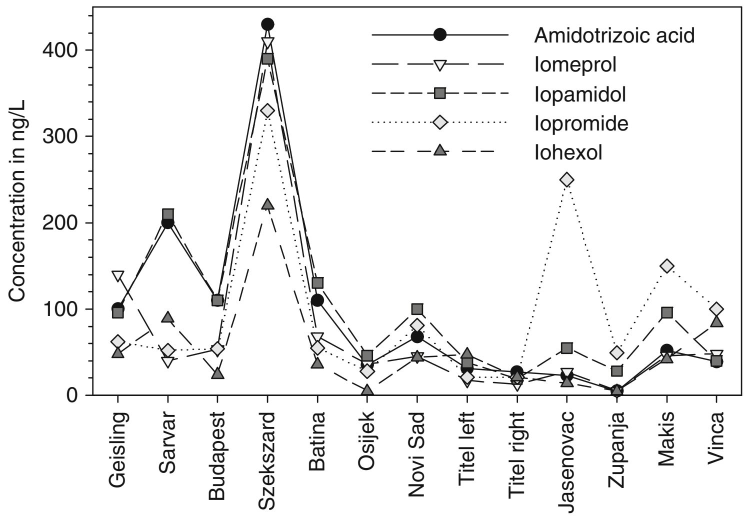

Fig. 4 Concentrations of iodinated X-ray contrast media in the Danube River and in several of its tributaries. LOQ: 10 ng/L. Values < LOQ are displayed as 0.5*LOQ (locations 6 and 10)

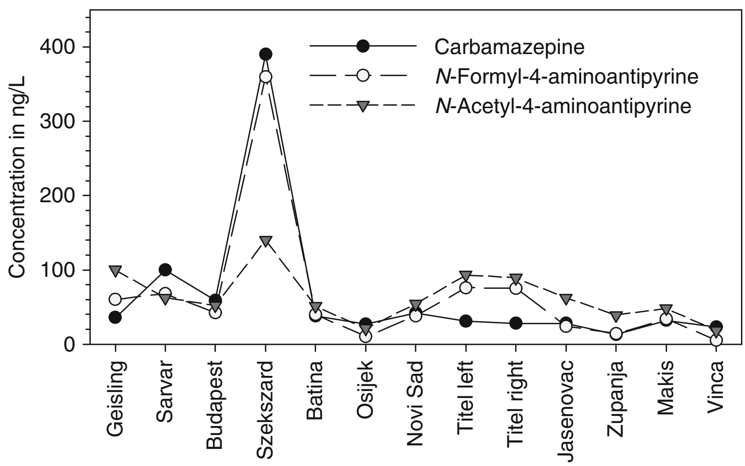

Fig. 5 Concentrations of *N*-acetyl-4-aminoantipyrine, *N*-formyl-4-aminoantipyrine, and carbamazepine in the Danube River and in several of its tributaries. LOQ: 10 ng/L. *N*-Formyl-4-aminoantipyrine values < LOQ are displayed as 0.5*LOQ (location 12 – Vinca)

5.6 Artificial Sweeteners

Among the artificial sweeteners (Fig. 6), acesulfame was the compound with the highest concentrations found in this campaign (up to 8 μg/L in the Sio River at the location Szekszard). The main reasons for the elevated concentrations of acesulfame in river water are its high persistency and good solubility in water in

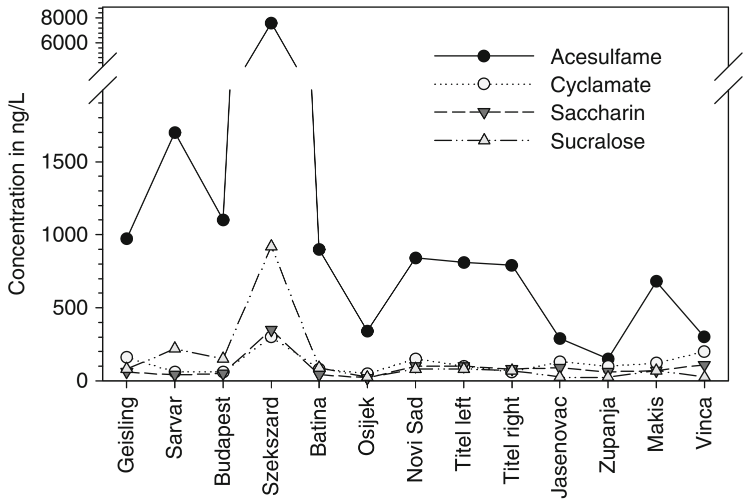

Fig. 6 Concentrations of artificial sweeteners in the Danube River and in several of its tributaries. LOQ: 10 ng/L, sucralose: 50 ng/L. Sucralose values < LOQ are displayed as 0.5*LOQ (locations 6, 9, 10, 12 – Osijek, Jasenovac, Zupanja, Vinca)

comparison to the substances cyclamate and saccharin which are readily biodegradable in wastewater treatment plants [34, 35]. Sucralose is classified as not readily biodegradable [34, 35], but the amounts used in food and beverages are comparably lower at present. In general, acesulfame and sucralose concentrations are lower in the Danube River Basin than in the Rhine catchment, especially in the middle and lower stretches of the river. While concentrations of both compounds increase from source to the mouth of the Rhine (compare Table 5), the opposite seems to hold true for the Danube. This tendency is most probably related to the higher discharge of the Danube and the higher consumption of dietary food in the Rhine catchment, as both acesulfame and sucralose are quite persistent in surface water [30]. The ratio of acesulfame to sucralose observed in this campaign ranged from 7.7 to 12.1 and is thus lower than reported by Scheurer et al. [31] for the Rhine River.

6 Summary

Emerging substances are found in river waters throughout the Danube catchment. The concentrations of individual substances are generally dependent on river water flow and the extent of wastewater inflow and its composition. This means that smaller rivers with minor discharges exhibit mostly higher concentrations of emerging substances, if the input occurs via wastewater effluents. In some cases, however, high emissions of individual compounds (see Fig. 2) have been discovered which can be traced several hundred kilometers downstream. It seems to be appropriate to stop such an emission of hazardous or emerging substances into the river in order to protect the aquatic environment as well as the drinking water resources. The importance of preventive action and early identification of hot spots and contamination (even indirect and retarded as due to leaching and drainage from contaminated aquifers) gets clear from the example of PFC.

Concentrations found in the Danube catchment area are mostly lower than in the Rhine River as well as in smaller Rhine tributaries. This can be explained by higher amounts of pharmaceuticals, industrial chemicals, and other emerging substances used in Western Europe and higher wastewater portions in comparison to the middle and lower Danube River Basin. The latter holds true even for the campaign conducted in 2011, when all sampled rivers had low discharge and the portion of wastewater can be regarded as relatively high.

Due to the importance of the Danube River as a direct or indirect source of drinking water, substances can be evaluated and assessed by means of the "Memorandum regarding the protection of European rivers and watercourses in order to protect the provision of drinking water" [36]. According to this memorandum, tolerable surface water concentrations of substances with effects on biological systems like pesticides and their metabolites, endocrine active compounds, pharmaceuticals, and perfluorinated or halogenated compounds are 0.1 μg/L. The same threshold value is valid for substances which have not been evaluated yet or with non-evaluated transformation products and metabolites which cannot sufficiently be removed by natural steps of drinking water treatment (e.g., bank filtration or AGR). Substances which were toxicologically evaluated and classified as not harmful to human beings and persistent compounds were set to maximum target concentrations of 1 μg/L.

In this perspective, emissions of X-RCM into the Danube River and some of its tributaries should be lowered, as concentrations exceed 0.1 μg/L when dilution is low (upper catchment of the Danube, tributaries). For pharmaceuticals, the situation is better, with exception of small rivers with a high portion of wastewater. The artificial sweeteners acesulfame and sucralose are rather persistent, but currently, concentrations in the Danube rarely exceed 1 and 0.1 μg/L, respectively. Industrial chemicals like benzotriazoles, naphthalenesulfonates, and PFCs are not a problem at most locations, but emissions from hot spots should be reduced to protect small rivers and to avoid unneeded pollution of the Danube River and an accumulation of persistent substances in the Black Sea. However, as many waterworks in the Danube catchment use surface water without advanced treatment or with short retention times during bank filtration and AGR, the state of pollution must be surveyed on a regular basis to recognize potentially problematic trends early.

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Radioactivity in the Danube

Franz Josef Maringer, Claudia Seidel, Andreas Baumgartner, and Michael Stietka

Abstract In this chapter, a general review of radioecological research and exemplarily results of radioactivity measurements carried out in the Danube freshwater ecosystem in the last 30 years are presented. Sample collection techniques and sample preparation and radiometric measurement methods, developed and applied in radioecological studies of the Danube River, are shown comprehensively. Results of radiometric analysis of bottom sediment samples, collected continuously by sediment traps and additionally by grab sampling during Danube research cruises, are given and discussed. The main goal of the radioecological research studies is the protection of the environment to manage sustainable use and conservation of the Danube freshwater resource against harmful radioactive exposure.

Keywords ^{137}Cs, Natural radionuclides, Radiation protection, Radioactive contamination, Radioactive pollution, Radioactivity, Radioecology

Contents

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1 Introduction

In the recent decades, anthropogenic radioactive burden appeared increasingly in all freshwater ecosystems around the world. This is due to a progressive impact of nuclear industry, NORM[1] industry, nuclear applications in medicine and proliferation of nuclear weapons. Globally effective bad examples of this development are the catastrophic nuclear accidents of Chernobyl in 1986 and Fukushima in 2011. Besides these two fatal experiences, many nuclear accidents and incidents happened with implications to the freshwater environment since the processing and application of nuclear material. Elevated levels of artificial and natural radionuclides in the hydrosphere lead to increased health risk of the population consuming contaminated drinking water or fish. Additionally, the use of contaminated fresh water for irrigation of agricultural areas could increase the health risk by consumption of the products. Therefore, the continuous radioecological investigation and monitoring of river ecosystems is of high relevance to assess the impact of radioactive contamination nuclides on the public health. The results of these investigations serve as basis for environmental management and population protective countermeasures.

After the global radioactive contamination following the atmospheric nuclear weapons tests in the 1950s and 1960s, intensive radioecological research including the continental freshwater resources had been started worldwide. In the second half of the twentieth century, the radioecological monitoring of the Danube had been started by the Austrian Federal Institute for Water Quality [1] and continued by the Federal Institute for Testing and Research Arsenal [2] and the International Atomic Energy Agency (IAEA) [3]. At this time, some other European countries started radioactive monitoring of the Danube in the frame of the International Association for Danube Research (IAD). This scientific association was founded in 1956 and is the longest existing international scientific network in the Danube Region with the goal of promoting and coordinating activities in the fields of limnology, water management, water protection and sustainable development in the Danube River basin (http://www.iad.gs/).

After the Chernobyl accident in May 1986, several researchers had monitored a big increase of artificial radionuclides in the Danube catchment area and the Danube water and sediments [4,5]. Within the Danube Field Excursion, carried out in 1988 by the IAD, the radioactive contamination of 28 bottom sediment samples taken from river km 1,819 to 16 had been analysed radiometrically

[1] Naturally Occurring Radioactive Material

[6]. Radioecological monitoring, though, did not only emphasise on nuclear fission radionuclides, e.g. ^{137}Cs ($T_{1/2}$ ~ 30 y), ^{90}Sr ($T_{1/2}$ ~ 29 y) and ^{239}Pu ($T_{1/2}$ ~ 24·10^{3} y), but also took into account naturally occurring radionuclides originating from natural geochemical background and NORM industry activities, e.g. ^{232}Th, ^{238}U, ^{228}Ra, ^{226}Ra, ^{210}Po and ^{210}Pb [7].

Major naturally occurring radioactive constituents of the earth's crust are the isotopes ^{238}U ($T_{1/2}$ ~ 4.5·10^{9} y) and ^{232}Th ($T_{1/2}$ ~ 14.1·10^{9} y). Being created during the cosmic formation of the elements in a supernova and then building the earth's matter, these radioisotopes, due to their extremely long physical half-life, now can be found in almost every sort of rock and its weathering product, the soil. The ubiquity of these radioisotopes also implies the presence of its decay products in rocks and soil like ^{226}Ra ($T_{1/2}$ ~ 1.6·10^{3} y) and ^{228}Ra ($T_{1/2}$ ~ 5.7 y), respectively.

^{226}Ra and ^{228}Ra analysis of sediments allows identifying the geochemical background and the influence of the mining industry as well as changing sediment sources. The influence of the main tributaries, e.g. Drava, Tisa, Sava and Velika Morava, on the radionuclide activity concentrations has been also evaluated. Sediment cores taken in the Iron Gate reservoir are used to analyse sedimentation sequences.

The levels of natural (^{40}K, ^{226}Ra, ^{228}Ra, ^{232}Th) and artificial (^{137}Cs) radionuclide concentrations of 72 sediment samples collected during the joint survey cruise in the Danube and the main tributaries have been radiometrically analysed [8].

The results of the radioecological studies have been established as basic data for radiation protection of the public and additionally for environmental research and scientific applications such as sediment genesis and dating, effects of climate change and assessment of soil erosion [9,10].

2 Sample Collection

In the 1960s, collection of water and sediment grab sample had been started for hydrobiological and chemical investigations of the Danube. Triggered by the large-scale radioactive contamination caused by the atmospheric weapons tests in the early 1960s, specific sample collection campaigns had been carried out in many Danube countries to investigate the radioactive contamination of the hydrosphere and especially of the Danube. In the 1980s, coordinated and well-structured research work on the radioecology of the Danube and routine environmental radionuclide monitoring programmes had been established successfully. After some specific short-term radioecological investigations in the Austrian part of the Danube, a long-term continuous radioecological research and monitoring programme has been started immediately after the Chernobyl contamination. To obtain a comprehensive data set for this research, continuously collected water and sediment samples have been taken in Austria at four different locations along the Danube at river km 2,146.7 (Ottensheim/Wilhering), river km 2,094.5 km

(Wallsee/Mitterkirchen), river km 1,949.2 (Greifenstein) and river km 1,933.2 (Vienna/Nussdorf) on a monthly cycle since 1987. Three sediment traps had been installed in the cooling water circuit of Danube hydropower plants and one at the right shore of the Danube in Vienna (Nussdorf). After inflow of the Danube water into the traps, the flow velocity is reduced by deflection and the suspended sediment particles settle down into sedimentation recipients. The sediment samples have been taken out of the sedimentation recipients every month. At the same locations, suspended matter was collected by centrifugation of continuously collected water samples (40–60 l each month). The applied sediment and suspended matter sampling methods are described in detail in Maringer [11].

Additionally to the routine continuous sample collection, water and sediment samples had been collected during Danube research cruises. These cruises had been carried out in 1988 by the International Working Group for Danube Research (IAD), in 2004 in the integrated research project AquaTerra (in the frame of the European Commission FTD Program 6) and in 2007 in the Joint Danube Survey 2 (JDS2) of the International Commission for the Protection of the Danube River (ICPDR). The sampling locations have been chosen to be upstream as well as downstream of major cities (e.g. Vienna, Bratislava, Budapest, Novi Sad, Belgrade/Pancevo), main tributaries (e.g. Drava, Tisa, Sava, Velika Morava) and at specific locations of interest, e.g. reservoirs (e.g. Gabcikovo). The selection of the sampling positions was based on the interest on potential influence of the cities, tributaries and reservoirs on the radionuclide distribution in the river ecosystem.

3 Sample Preparation and Radiometric Analytics

In the radioecological research work of the Danube carried out in Austria, the collected bottom sediment samples have been air-dried and homogenised. Partially, grain-size fractions <20 μm and <63 μm had been radiometrically analysed, and grain-size distributions of bottom sediment samples had been analysed by sieving and optical methods (SediGraph). To separate the suspended matter and the solved radionuclide phase, the collected water samples had been flow-through centrifuged. The remaining water samples after centrifugation had been evaporated in large-volume vacuum rotation flasks to obtain the solved particles including the radionuclides for radiometric analytics. The applied sampling and preparation methods are described in detail in Maringer [11] and Tschurlovits and Maringer [12].

The radiometric analyses had been carried out in the Low-Level Counting Laboratory Arsenal, Vienna [13]. The measuring room of this laboratory is surrounded with a 4π-shield heavy mineral concrete (thickness 1.6 m), low-level lead (30 mm) and low-level steel (6 mm).

Five low-level Ge(HP) detectors were used for gamma spectrometry: two p-type coaxial, one p-type coaxial with active anti-compton NaJ(Tl) shielding, one n-type planar and one n-type low-energy Ge(HP) detector. The laboratory runs an ISO/IEC 17025 [14] quality management system. Energy and efficiency calibration and routine quality check of all detectors were periodically done by standard reference materials of the Federal Office of Metrology and Surveying (BEV, Vienna), the Physikalisch-Technische Bundesanstalt (PTB, Germany) and the US National Bureau of Standards (NIST).

The sample activity detection limits (measuring time 24 h) are between 10 and 60 mBq for ^{137}Cs, ^{226}Ra and ^{228}Ra according to the Austrian Standard Procedure ON S 5250-2 [15]. The relative extended uncertainty ($k = 2$) of the measured radionuclide activity concentrations of sediment samples are between 10% and 40%. At low activity concentrations, the main contribution to the total analytical uncertainty is due to counting statistics. At mean and higher activity concentrations, the uncertainties of the efficiency calibration and the coincidence summing correction are dominant [16].

4 Results

The average mineralogical composition of the sediment samples collected in the Austrian part of the Danube is 44% clay and mica minerals, 30% quartz and feldspar, 22% carbonates and 4% organic matter; the mean grain-size distribution is 15% clay fraction (<2 μm), 70% silt fraction (2–63 μm) and 15% fine sand fraction (63–250 μm) [11].

In Fig. 1, the relative distribution of ^{137}Cs in relation to the particle grain size of Austrian Danube sediment samples is shown [11]. This important result means that

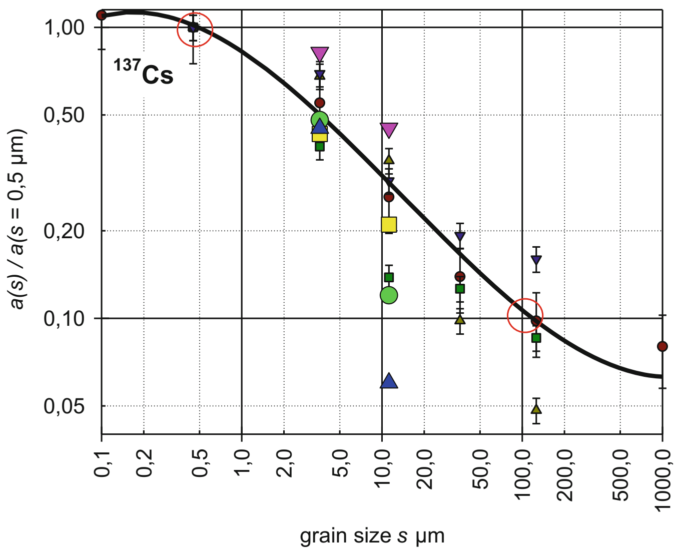

Fig. 1 Fitted function of relative ^{137}Cs activity concentration of Danube sediment relating to sediment particle grain size $s = 0.5$ μm [11]

the binding capacity of sediment particles decreased with increasing grain size. This effect is caused by the high binding capacity of clay and mica to ^{137}Cs ions. This specific behaviour of ^{137}Cs has an impact on the activity concentration of sediment samples with different grain-size distribution: the more fine-grained particle of sediments, the higher the ^{137}Cs activity concentration. This effect had been also detected for other (but not for all) radionuclides (e.g. ^{210}Pb, ^{226}Ra; not for ^{40}K).

A useful radioecological indicator for the radionuclide's activity partitioning between water and matter particles is the K_d factor, defined as equilibrium ratio of the radionuclide's activity concentration of suspended matter particles (Bq/kg) to activity concentration of this radionuclide solved in the water phase (Bq/m^3). In this research work, K_d factor median values of 120 m^3/kg for ^{137}Cs, 30 m^3/kg for ^{226}Ra and 9 m^3/kg had been analysed in the Danube [12].

The regional and chronological development of the radioactive contamination of the Danube is shown in Fig. 2. A good indicator for the radioactive contamination is ^{137}Cs. In Fig. 2, the ^{137}Cs activity concentration of prepared bottom sediment samples with grain-size fraction <20 μm, collected at three Danube research cruises in the years 1988, 2004 and 2007 [6,8,17], is given. The ^{137}Cs activity concentration decreases from the upper part to the lower part of the Danube. This is due to the relatively high environmental radioactive contamination after the Chernobyl nuclear accident in 1986. The chronological decrease of the ^{137}Cs contamination of the Danube sediment between 1988 and 2007 by a factor of about 10 had been detected (Fig. 2). This means a total ecological half-life of ^{137}Cs in Danube

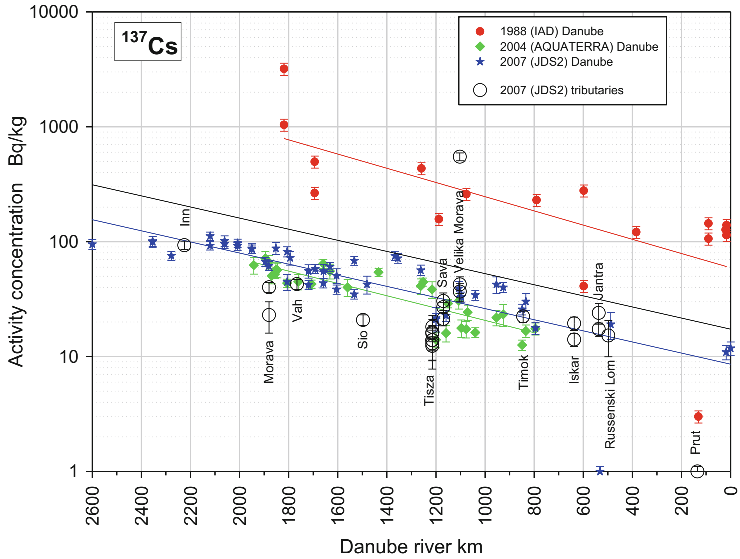

Fig. 2 ^{137}Cs activity concentration of sediment samples, grain-size fraction <20 μm, collected at three Danube research cruises in the years 1988, 2004 and 2007 [6,8,17]

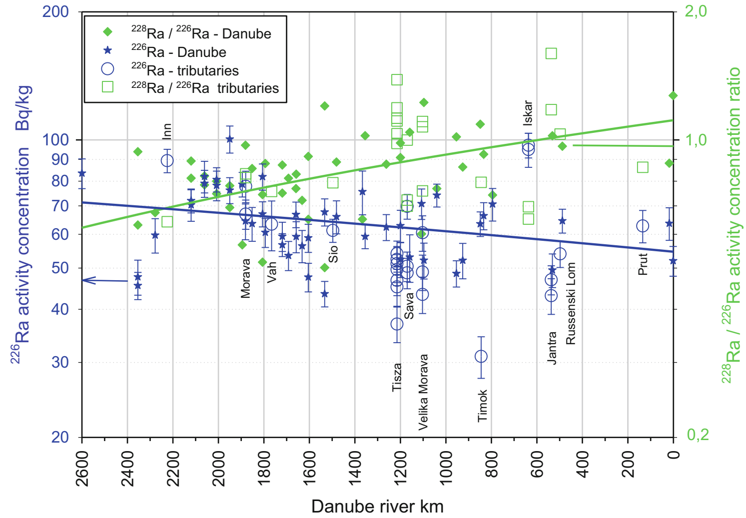

Fig. 3 ^{226}Ra activity concentration of sediment samples (grain-size fraction <63 μm) and $^{228}Ra/^{226}Ra$ activity concentration ratio – Danube and tributaries [8]

sediments of about 5 years in contrast to the physical half-life of ^{137}Cs of about 30 years.

As an example for the radioecological behaviour of natural radionuclides, the results of the analysed activity concentrations of $^{228}Ra/^{226}Ra$ and their ratio of sediment samples collected during JDS2 are shown in Fig. 3. The activity concentration ratio of $^{228}Ra/^{226}Ra$ ration can be used as indicator of the geochemical source of the sediment particle, as ^{228}Ra is derived from the ^{232}Th decay chain and ^{226}Ra is formed by the decay of ^{238}U. Ratios of approximately 0.7–0.8 had been found as far as upstream Budapest (km 1,659), gradually increasing downstream, where ratio in the range between 0.8 and 1.1 could be observed (Fig. 3). 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 region. Further downstream the Alpine influence is apparently reduced in favour of more regional influence. In this investigation, upstream Timok (km 849), a very low ratio of 0.4 was found (Fig. 3), coinciding with the extremely high concentration of the ^{226}Ra decay product ^{210}Pb at the same location. This can be explained by the presence of the well-established copper extraction industry upstream, along the Timok River, mining copper and gold from predominantly volcanic and hydrothermal deposits [8].

The long-term development of the radioactive contamination of the Danube caused by the release of fission radionuclides during Chernobyl accident is given in Fig. 4. In the Austrian part of the Danube – which is one of the most contaminated region in Europe after the Chernobyl accident – the ^{137}Cs activity concentration of Danube sediments started with values above 1,000 Bq/kg in 1987 to about 30 Bq/kg in 2011 (Fig. 4). The subsequent decrease of ^{137}Cs activity concentration of Danube

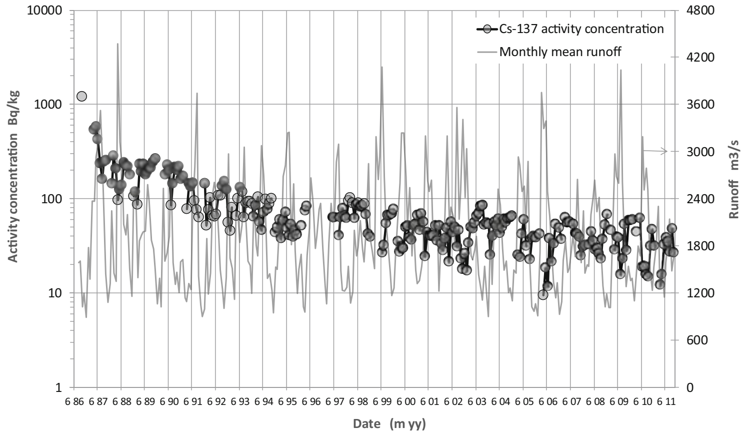

Fig. 4 ^{137}Cs activity concentration of monthly continuously collected Danube sediment samples, river km 1,933.2 (right bank), Vienna/Nussdorf, years 1987 until 2011

sediment can be approximated by exponential decline. With this result, the ecological half-life of ^{137}Cs in Danube sediment of about 5 years could be derived (describing the time the radionuclide remains in the hydrological sediment compartment). This is in good agreement with the results obtained from the three Danube research cruises (Fig. 2).

After the Chernobyl contamination, a rather rapid decay is characterised by an ecological half-life of about 4 months. Beginning in 1988, the decline of ^{137}Cs activity concentration describes a much more flat, though still exponential, decrease, showing an ecological half-life of around 4 years (Fig. 5). Since 1998, a slower average decrease of ^{137}Cs in Danube sediments has been observed so far. The slightly higher ecological half-life since 1998 could be explained by mixing of higher ^{137}Cs contaminated 'old' remobilised Danube reservoir bottom sediments with recently soil-eroded (lower ^{137}Cs activity concentration) sediment particles.

The comprehensive data set of this long-term research and monitoring is given by boxplot frequency distributions of various natural and artificial radionuclides' activity concentrations (Fig. 6) and natural radionuclides' mass concentrations (Fig. 7) of Danube bottom sediments and suspended matter.

An illustrative example of the impact of hydrological situation on the natural radionuclides' activity concentration of Danube bottom sediment is given for ^{210}Pb in Fig. 8. With acceptable statistical significance ($r^2 = 0.56$), the dependency of the ^{210}Pb activity concentration (Bq/kg) on the runoff (m^3/s) is $a(^{210}\mathrm{Pb}) = 6.43 \cdot 10^{3} \cdot Q^{-0.579}$.

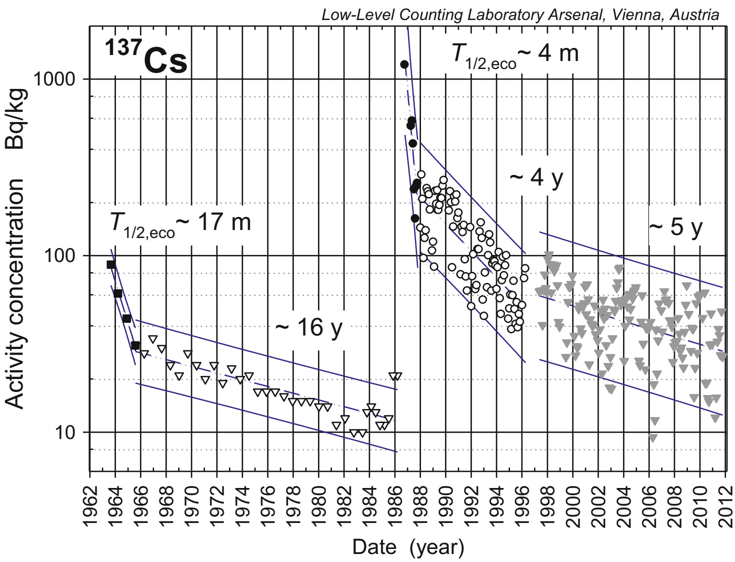

Fig. 5 ^{137}Cs activity concentration of bottom sediment samples, Danube upper part, river km 2,163.5 (year 1965–1986) and river km 1,933.2 (year 1987–2011)

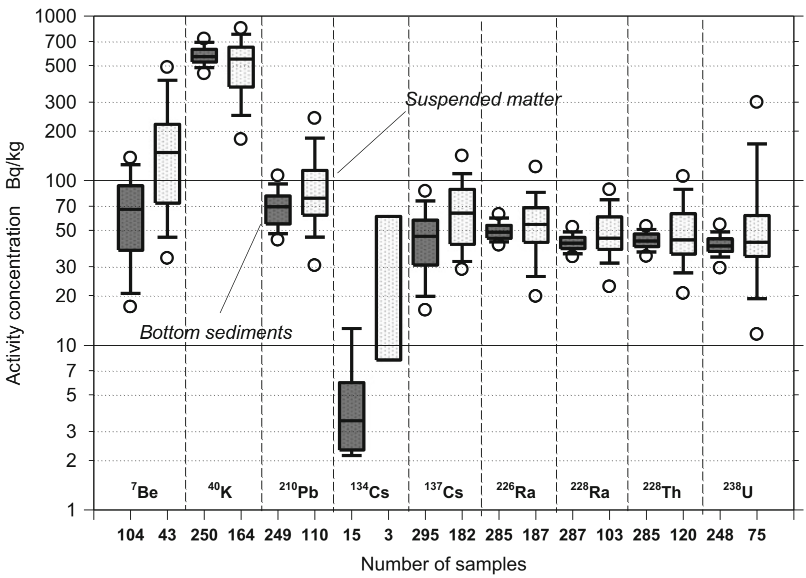

Fig. 6 Artificial and natural radionuclides' activity concentrations of bottom sediment and suspended matter samples, Danube river km 2,150 to km 1,933, years 1989 until 2011 (sum frequency boxplots quantiles: 5, 10, 25% and 75, 90, 95% median)

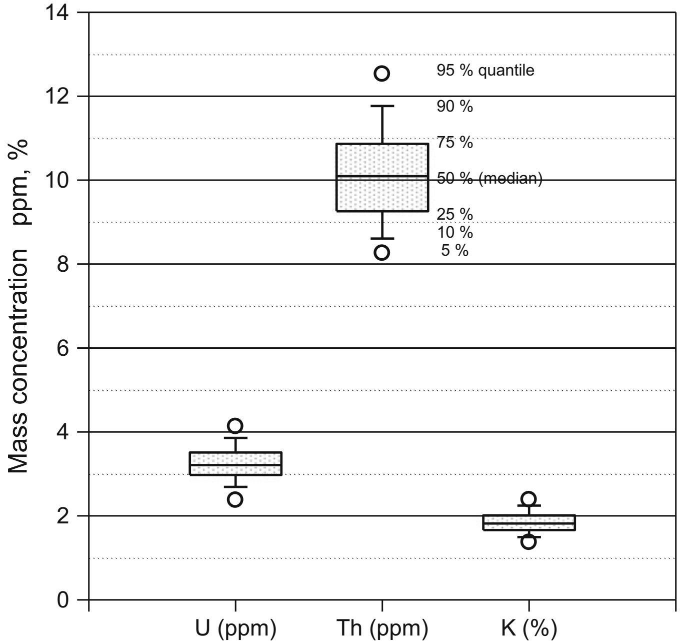

Fig. 7 Sum frequency distributions of mass concentration of uranium, thorium and potassium in bottom sediment samples, upper part of Danube, river km 2,150 to km 1,933, years 1989 until 2011

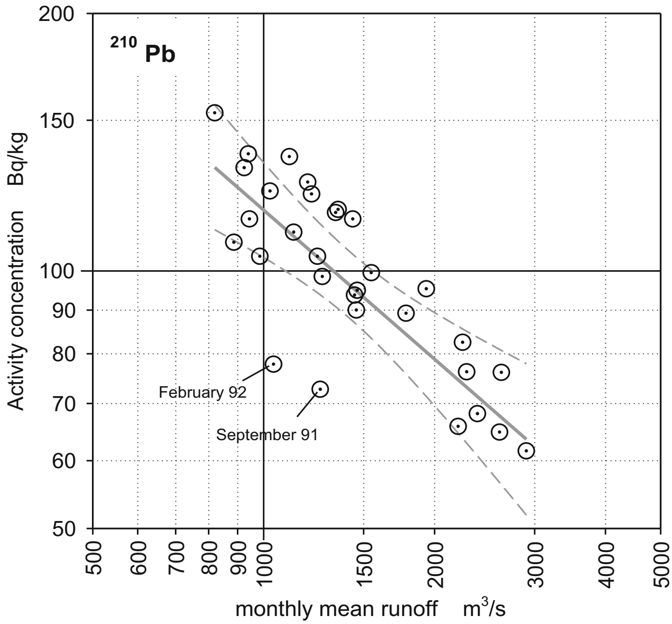

Fig. 8 Dependency of ^{210}Pb activity concentration on runoff situation – monthly continuously collected bottom sediment samples, Danube upper part (Austria), years 1989–1992 [18]

5 Discussion and Conclusions

Whereas ^{137}Cs, ^{210}Pb and ^{226}Ra activity is transported primarily in the Danube by suspended matter soil particles, ^{40}K, ^{90}Sr and ^{238}U run mainly solved in the water. The particle-bound relative partition of the radionuclides transported in the upper Danube river water is in average 50% for ^{40}K, 70% for ^{226}Ra and 90% for ^{137}Cs. In the 1990s, the annual transported radionuclide activities in the Danube's upper part water are approximately 6 TBq ^{40}K, 0.3 TBq ^{226}Ra and 1 TBq ^{137}Cs. The largest part of the annual radioactivity burden is transported during flood events only during some few days in a year.

Clearly, a strong particle grain-size influence on ^{137}Cs and ^{210}Pb activity concentration of sediments has been observed. The observed ecological half-life of ^{137}Cs in the Danube bottom sediment was about 5 years until 2009. Currently, there is a stagnation of ^{137}Cs activity concentration of bottom sediments around about 30 Bq/kg.

The extensive radioecological research and radiometric monitoring of the Danube in Austria since 30 years together with radiometric results of three Danube research cruises allow reliable environmental assessment of the river ecosystem. The long monitoring period allows long-term assessment of the activity of natural and artificial radionuclides in the Danube river compartments.

The generation and evaluation of radiometric data for environmental and public radiation protection applications need radiometric low-level measurement methods to support sufficient detection levels and applicable measurement uncertainties. A reliable data set is a necessity when using them as input in radioecological models and radiation dose calculations.

Since the 1986 Chernobyl nuclear accident, derived radionuclides are the major source of artificial radioactivity in the Danube basin [19]. 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) has been observed since 1988. Since 2004, a generally constant ^{137}Cs activity level was detected along the Danube, except for the upper section where a slight increase of radioactivity was observed. This effect could be explained by the downstream transport of remobilised sediment and by locally increased ^{137}Cs input to the freshwater ecosystem of the Danube by soil erosion in the catchment area. The researched radioecological behaviour of the Danube since 30 years is a good illustration of the impacts of climatic change causing seasonal and regional changes in contaminated soil erosion in the Danube catchment. There are clear relations between ^{137}Cs activity concentration in Danube sediment and hydrological conditions in the Danube Basin. For example, in spring and summer seasons, the ^{137}Cs activity concentration of Danube bottom sediment samples generally decreases due to increasing mean runoff caused by snow melting and summer flood events which leads to increasing grain size of soil erosion in the catchment and sediment particles bed down in the river (Figs. 1 and 4).

The levels of natural (e.g. ^{228}Ra, ^{226}Ra) and artificial (e.g. ^{137}Cs) radionuclide activity concentrations in the Danube water, suspended matter and bottom sediment have been analysed in Austria continuously since 1987. Between Vienna and the Danube delta, bottom sediment grab samples were collected during research cruises in the years 1988, 2004 and 2007. In comparison to the activity concentration data set of the 1988 cruise, clearly reduced ^{137}Cs activity concentrations were observed in 2004 and 2007. The ^{137}Cs activity concentration of bottom sediment is gradually decreasing downstream Vienna. This fact has been observed during all surveys carried out so far (Fig. 2). The influence of tributaries and reservoirs on the radionuclide concentrations of sediments is shown: lower ^{137}Cs activity concentration of Morava, Sio and Tisa sediments and higher ^{137}Cs activity concentration of Velika Morava sediment (Fig. 2). ^{228}Ra and ^{226}Ra analysis allows identifying the influence of geological/geochemical changing sediment sources: higher ^{226}Ra activity concentration of Iskar sediment and higher ^{228}Ra/^{226}Ra activity concentration ratio of Tisa and Jantra sediments (Fig. 3). The results indicate relations between sediment activity concentrations, grain-size distribution, basin contamination, geochemical background and NORM industrial activities.

Due to gradually decreasing artificial radioactivity levels in the Danube River, currently no health risk for the population could be derived. The current radiological risk for the population due to consumption of purified Danube water and fish and irrigation of agricultural areas with Danube water is small. The evaluated total dose due to ingestion of contaminated water and foodstuffs is less than 1% of the natural radiation background.

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 has been in a good radioecological status since 1988. However, locally elevated concentrations, especially in the tributaries (e.g. Inn, Velika Morava; Fig. 3), caused either by contaminated soil erosion (Chernobyl accident) or by emissions from NORM industrial sites (mining activities increasing the natural radioactivity), must be mentioned as well.

The obtained research results on artificial and natural radionuclides occuring in the Danube freshwater compartments serve as certain input data for freshwater radioecological long-term modelling for public exposure assessment. Additional to radiation protection applications, the provided low-level radioactivity measurement results are applicable for environmental research because of their sufficient low detection levels and reasonable measurement uncertainties. Eventually, the results serve as complete and sound basis for current and future environmental research and application in radiation protection, e.g. river and sediment management, flood investigation, soil erosion, sediment particles' geochemical 'fingerprinting' and public exposure assessments. The future most challenging application of radiometric data of the Danube would be the scientific field of climate and global change research.

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Part II
Biology and Hydromorphology

Short Overview on the Benthic Macroinvertebrate Fauna of the Danube River

Wolfram Graf, Patrick Leitner, and Florian Pletterbauer

Abstract This article gives a rough overview on the occurrence and distribution of selected benthic invertebrates along the Danube River. The description of the benthic community within typological units of the Danube is based on the results from the Joint Danube Surveys. Species richness and abundance illustrate the structure and dominant groups of the benthic community. Furthermore the role of environmental impacts like hydromorphological changes, pollution, navigation as well as neozoa is shortly addressed and highlighted. In this context a conceptual framework of the multi-stressor complex of large rivers is introduced and discussed. Finally the biodiversity losses of selected species are reflected on a European scale.

Keywords Biodiversity, Danube, Distribution, Environmental impacts, Joint Danube Survey, Macroinvertebrates

Contents

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1 Introduction

About the macroinvertebrate fauna of the Danube, who has the knowledge and the overview? Due to the overwhelming diversity, most approaches, which aim to give a comprehensive picture, were bound to fail due to the ever-changing nature of large rivers and either naturally or anthropogenically induced faunal shifts along the time axis.

The longitudinal, lateral and vertical dimensions of large rivers have been in the focus of limnologists since the last 50 years only, and we are just at the beginning to understand the principles of ecological processes and functions. Even during that short period, large rivers have changed their character dramatically due to exposure to multiple stresses induced by human uses. The first systematic documentations of large rivers in the 1960s give us a glimpse of the organisms present at that time. Profound baseline information and monitoring efforts started much later and were confined to some national stretches of interest. All we got from earlier times – revealing more pristine conditions – are some flashlight information from outstanding naturalists, scientists and specialists on specific groups, scattered in regional publications, which has to be evaluated according to the taxonomic resolution of the time of publication.

The macroinvertebrate fauna of the Danube is highly diverse consisting of numerous systematic groups including annelids, molluscs, crustaceans and insects and comprises an incredibly high diversity. Some of these animals have a high adaptive potential to changing environmental conditions; some have been documented only once and are thought to be extinct since their discovery 250 years ago, and other Danubian elements may have never been recorded at all. Their documentation is extremely dependent on the chosen methods and seasonal aspects which is the reason why some of us still are curious and search for those legendary and long-lost organisms of large rivers which may be still out somewhere in the dark. Some have been rediscovered in tributaries, and some few have recolonised the Danubian river bottom from unknown refugia indicating a recovery of specific habitats and the overall ecological integrity.

One major basis for the evaluation of the biological inventory of the Danube is provided by the two large expeditions within the Joint Danube Survey, JDS1 and JDS2, as these include recent and methodologically reproducible results. Other sources are local information and historic records which are included in a rather subjective way. Summarising, this article tries to sketch a rough picture of the author's subjective knowledge on general distribution patterns, occurrence of typical species and faunal losses and major changes of ecological processes in the past including examples from other large rivers of Central Europe.

2 Typological Aspects and Longitudinal Zonation Patterns of the Fauna

Sources on information on macroinvertebrates can be categorised as follows: (1) - species-specific data published by specialists scattered in time and space, starting from the middle of the eighteenth century, (2) ecologically oriented academic or applied studies from the 1950s up to now, (3) data focusing on biodiversity conservation issues and (4) data from systematic documentation of benthic assemblages which was initiated by the beginning of water resources management approaches and by especially saprobiological surveys (mainly the middle of the twentieth century) leading to huge datasets focusing on abundance and dominance of higher taxonomic units and species.

While (1) builds in general the basis for species-level information, (2) is improving our knowledge on the interactions of environmental variables on organisms mainly based on case studies; (3) provides data on selected and somehow unbalanced species groups, mainly FFH species comprising of few Odonata and Mollusca within the large and heterogeneous group of macroinvertebrates; and (4) initiated a high number of various national and international monitoring efforts. With the implementation of the Water Framework Directive (WFD) in 2000, a new dimension in the conservation of freshwater ecosystems was achieved, as the overall ecological status of surface water bodies has to be assigned within the EU member states, based on bioindicative organism groups, including macroinvertebrates. Within this reference-based assessment system, a sound typology is a prerequisite and various attempts have been performed to classify the Danube River. Frequently top-down approaches based on different eco-geographic units were applied [1, 2]. Moog et al. [3] included the macroinvertebrate fauna alongside geomorphological factors like river slope, hydrology, geology and dominating substrate type in their analyses and stressed the importance of the ecoregions according to Illies ([4]; the Central Highlands, the Hungarian Lowlands, the Pontic Province, the Carpathians and the Eastern Balkan) which resulted in ten distinct Danube River sections. Nesemann [5, 6] discussed the distribution patterns of molluscs and leeches and stressed palaeoclimatic factors to be responsible for the phenomena of disjunct species distributions and faunal inhomogeneity along the Danube course. The Upper Danube can be characterised by glacial and postglacial relicts according to Nesemann who highlights recent historic events as additional parameters shaping the fauna other than geomorphology.

Like in most European large rivers, the original aquatic fauna is under extreme pressure. Damming, pollution, navigation, habitat fragmentation and the invasion of neozoa are among the main stressors leading to an insensitive, cosmopolitan and less indicative benthic assemblage [7]. Many of section-type-specific species listed in Sommerhäuser et al. [8] have not been found for decades and have hopefully survived in discrete habitats; others are expanding their areas and are invading new sections. These range oscillations in combination with a nowadays more or less homogenised fauna along the entire Danubian stretch seriously hamper a biologically based typology as well as a sound ecological assessment system.

2.1 General Distribution Patterns

Dudich [9] compiled a first reliable and comprehensive species list of nearly all systematic groups from the entire Danube based on a literature review. He annotated national occurrences and even some ecological comments on the species. During the introduction he stated his concerns about the validity of his compilation regarding obsolete literature, the changing of environmental conditions of the Danube along timescales, nomenclatorial ambiguities and obscure locality records. Although the mentioned obstacles are obvious (and still do exist), he listed 1,623 species and gave the first overview summarising the contemporary knowledge from scattered publications. Huge new data have been collated in the last 50 years, but still the overall value of Dudich [9] lies in the documentation of distribution patterns especially of Ponto-Caspian species and rheophilous species of the Upper and Middle Danube, respectively, as massive migration and irreversible faunal changes started soon after. He characterised marine groups being restricted to the delta region or to the adjacent regions (especially Gastropoda and Bivalvia and Amphipoda, Mysidacea, Cumacea, respectively) and realised some insect orders like the Plecoptera and Ephemeroptera to have their main area in the Upper and Middle Danube. Additionally the enormous densities of the Amphipoda genus *Celicorophium* in Bulgaria have attracted attention (242,136 ind./m^2 according to Russev [10]) and were discussed as essential food resources for fishes.

Twenty-eight years after Dudich [9], Moog et al. [11] published 1,142 invertebrate species from the Austrian stretch of the Danube summarising literature data including records from the floodplains which contributed considerably to the overall diversity. Their data indicate a clear north-western shift of invasive amphipods compared to Dudich's compilation. Regarding diversity 74% of the total species inventory belonged to insects.

Although the distribution of benthic macroinvertebrates along the Danube River has been investigated in earlier studies [12–14], the most coherent data were provided by the Joint Danube Surveys 1 in 2001 [2] and 2 in 2007 [15], respectively. Macroinvertebrate data were collected with comparable and standardised methods along the Danube from Ulm to the Black Sea during a defined period (August to September). General characteristics of the fauna are given in Fig. 1 (taxa richness per group) and Fig. 2 (abundance per taxa group), respectively (data referring to JDS2, typology after Literáthy et al. [2]).

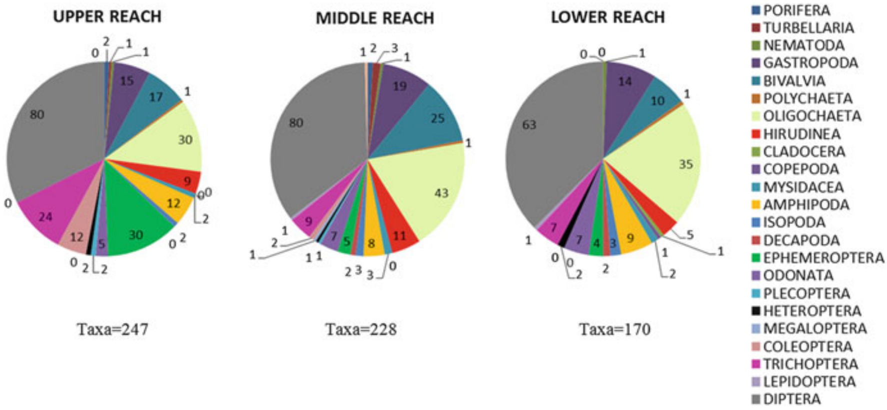


Fig. 1 Number of taxa per taxa group along the different reaches of the Danube

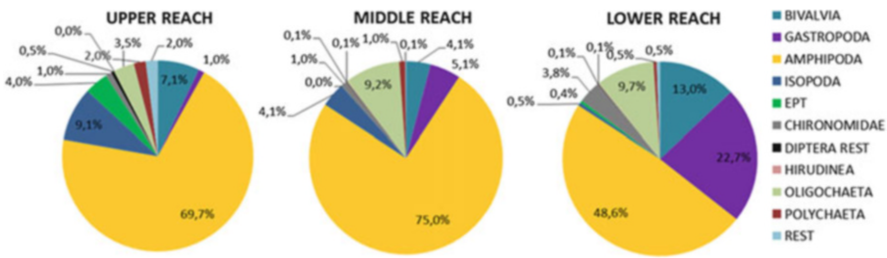


Fig. 2 Abundance of taxa per taxa group along the different reaches of the Danube

The most heterogeneous groups were Diptera (mainly Chironomidae, 174 taxa) and Oligochaeta (53 taxa) followed by Ephemeroptera (42 taxa), Trichoptera (35 taxa) and Mollusca (Bivalvia 26 taxa, Gastropoda 27 taxa, respectively). Coleoptera (17 taxa), Amphipoda (13 taxa) and Hirudinea (11 taxa) were as well noteworthy. This overall characteristic in diversity does not change along the three reaches of the Danube, although the number of insects, other than chironomids, decreases considerably downstream.

Regarding abundance (ind./m^2), Amphipoda were the dominant group in all Danube reaches and constitute up to 75%, while Isopoda (mainly *Iaera istri*) play an essential role in the upper reach and decrease downstream. Oligochaeta and Mollusca were found in increasing numbers in the lower reach.

In terms of biomass Mollusca were 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 (10–35%). Looking at the different reaches of the Danube, the increasing dominance of Mollusca from the upper to the lower reach becomes evident (Fig. 3). Although Crustacea are the most abundant group, they play only a minor role regarding biomass.

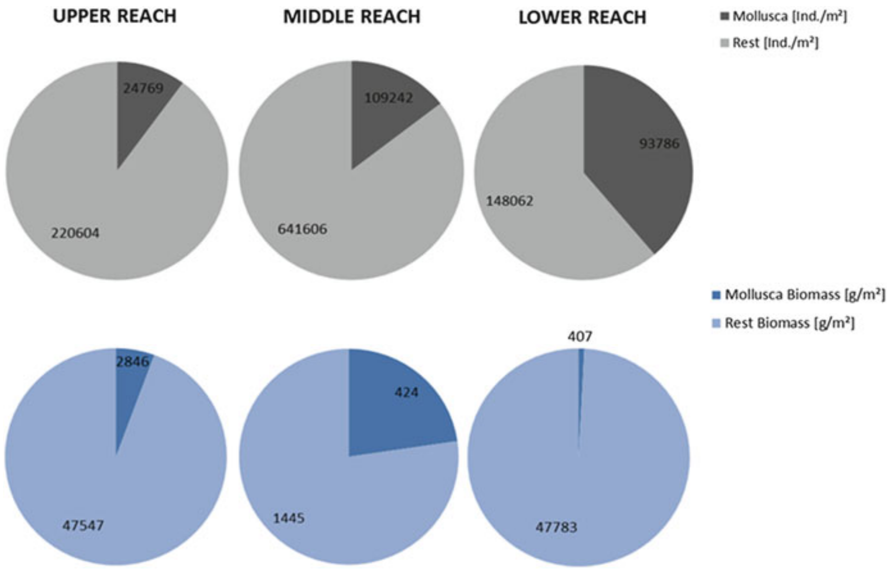


Fig. 3 Abundances and biomasses of Mollusca in comparison to the taxa rest (Airlift/Multicorer/MHS)

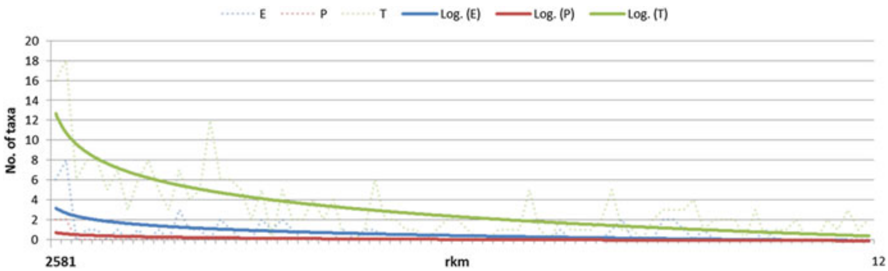


Fig. 4 Total numbers of EPT taxa recorded during JDS2 along the Danube

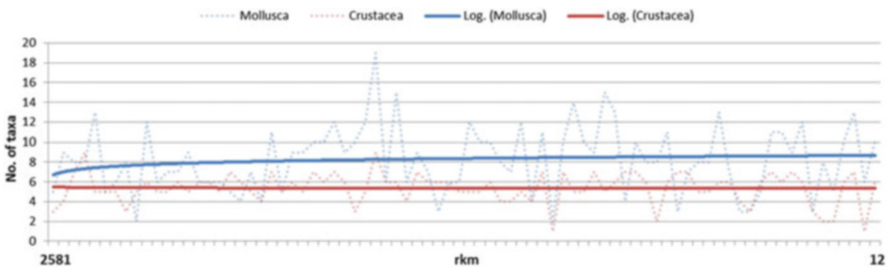


Fig. 5 Total numbers of Mollusca and Crustacea taxa recorded during JDS2 along the Danube

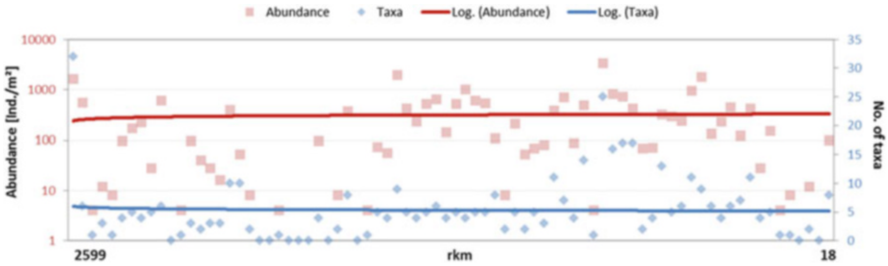


Fig. 6 Abundances and total taxa numbers of Chironomidae recorded during JDS2 along the Danube

Within insects EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) are rarely found – with the exception of the upper reach. Among Trichoptera the net spinning, filtering genus *Hydropsyche* covers in considerable densities the whole stretch. Figures 4, 5 and 6 give schematically the development of diversity within EPT taxa, Crustacea, Mollusca and Chironomidae along the river course based on the results of JDS2.

Within aquatic insects exclusively, Chironomidae play a major role both in diversity and abundance.

3 Wetland Faunas

During the last decades floodplains of large rivers came in the focus of applied and basic limnological science (e.g. Amoros and Roux [16], Junk et al. [17], Schiemer [18], Ward et al. [19] and Findlay et al. [20]). Floodplains are an essential part of the aquatic ecosystem depending entirely in their spatial and temporal dimension on the pulses obtained from the river; due to regulations and damming, these hot spots of biodiversity [21] are among the most threatened ecosystems worldwide [18, 22–27]. Up to 90% of all floodplains in Europe and Northern America are heavily impacted [21]; exemplarily for land-use developments in Central Europe, floodplain areas have been reduced by 85% in Austria [28]. Within the Danube catchment floodplains have been reduced by 80% from the early nineteenth century up to now [29]. Conservation and restauration of persisting floodplains are therefore of highest priority within modern effective and sustainable aquatic ecosystem management [30–40].

Floodplains are generally seen as biodiversity hot spots as they form an ecotone from aquatic to terrestrial habitats and provide linkages between biological processes at various temporal and spatial scales [16, 17, 22, 41, 42]. Hydrological conditions and connectivity have been increasingly considered to be key drivers in creating structural and habitat diversity (Fig. 7).

Based on the distribution of habitat types within the hypothetical framework of floodplains [43, 44], Waringer et al. [45] classified 256 benthic invertebrate species

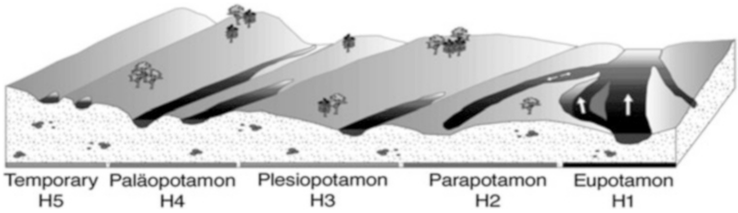


Fig. 7 Scheme of a hypothetical floodplain with habitat types according to Amoros et al. [43, 44]

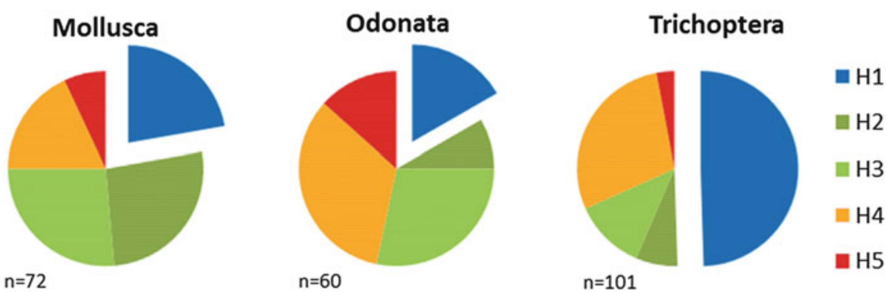


Fig. 8 Percentage of floodplain habitat type per taxa group (Mollusca, Odonata and Trichoptera); classifications according to Waringer et al. [45]

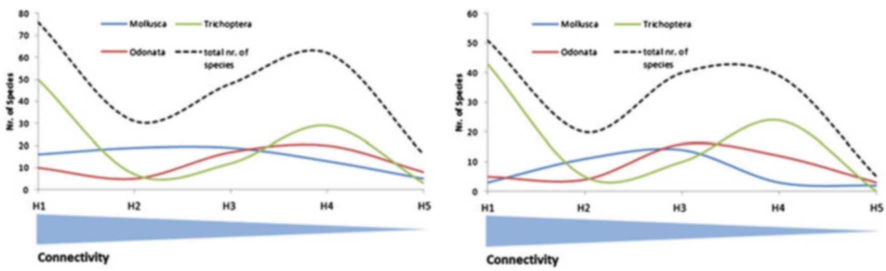


Fig. 9 *Left*: Theoretical diversity patterns of Mollusca, Trichoptera and Odonata along the connectivity gradient based on classifications taken from Waringer et al. [45]. *Right*: Species numbers of Mollusca, Trichoptera and Odonata (163 species) along the connectivity gradient documented at the floodplains near Vienna during 2001 and 2009

(Odonata, Trichoptera and Mollusca) occurring along the Austrian Danube according to their habitat-type preferences [43, 44]. Based on this data, Fig. 8 gives the percentage of species with specific habitat-type affinities which clearly indicates the dominance of floodplain species within the species pool of Mollusca, Odonata and Trichoptera along the Austrian Danube. Figure 9 left shows the potential species richness along the connectivity gradient within floodplains, peaking both at the Eupotamon and the Paläopotamon. This fits well with the conceptual biodiversity pattern along floodplains [46, 47], stressing the importance of wetlands in general. Studies on the floodplains in the vicinity of Vienna (Klosterneuburg, Lobau, Stopfenreuth, Altenwörth, Mühlwasser; investigation period 2000–2011) have confirmed these findings by high species numbers of typical floodplain organisms (in total 87 Trichoptera, 43 Odonata and 33 Mollusca species) representative for other macroinvertebrate groups (Fig. 9, right).

Under the holistic perception that floodplains are one essential part of large rivers, existing assessment systems are lacking this speciality, and new assessment approaches are currently under development to enlarge and complement WFD-compliant methods to evaluate the ecological status of large rivers and their floodplains based on macroinvertebrates [48, 49].

4 Environmental Impacts on Macroinvertebrates and Species Losses

Aquatic habitats of large rivers in Central Europe have been tremendously altered by diverse human impacts within the last centuries [50]. After river regulations for flood protection and navigation in the second half of nineteenth century and pollution due to industrialisation and human settlements, the building of hydropower plants and damming led to completely different stream characteristics regarding hydromorphological features like habitat dynamics, substrate and flow velocities. Decoupling the main river corridor from its floodplains and associated processes (like regular floods) changed nutrient cycles and influenced the characteristics of the faunal assemblages severely. Moreover large rivers are subject to invasions of nonindigenous species within the last decades which are supposed to have additional severe negative effects on the remaining native elements.

4.1 Hydromorphological Impacts

4.1.1 Channelisation

Large rivers and the connected floodplains are sensitive and complex ecosystems which are mainly determined by hydrological processes. Lateral connectivity and interactions between river and floodplain are most essential processes for ecosystem functioning [16–18, 20, 41, 42, 51–53]. During the centuries in man’s desire of land reclamation and security, the alterations initiated regarding large rivers tangle processes on catchment, reach as well as local scales. The most severe ecological impacts of river straightening led to scouring processes, thus decoupling the river from its floodplains, and a tremendous reduction of aquatic area in general, especially of lentic, riparian zones.

Demek et al. give a precise summary of the well-documented development at the Danube [54]. The first systematic large-scale channelisation schemes at the Upper

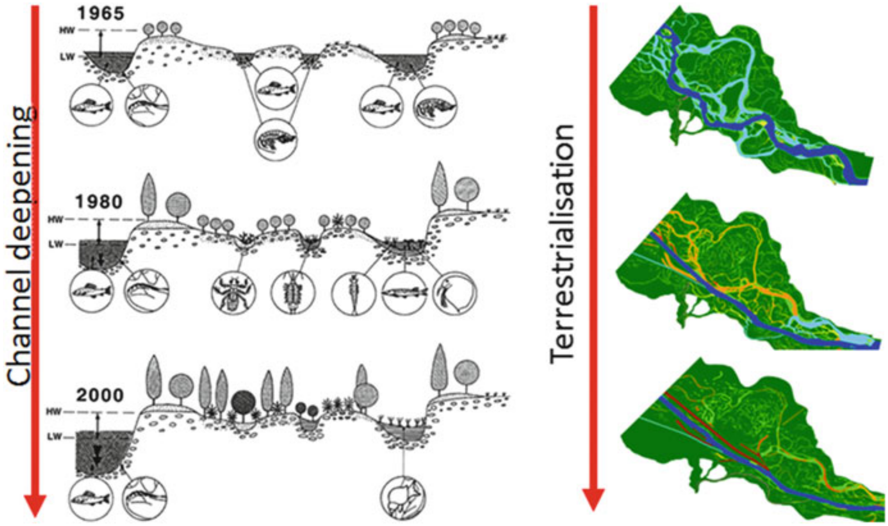


Fig. 10 Terrestrialisation processes due to River regulation and faunal reaction (*left*, Ward et al. [60]; *right*, Graf et al. [49], Danube River at Vienna)

Danube River and the Upper Rhine River were initiated as early as the end of the Napoleonic Wars (1805–1815) [55]. Hohensinner [56] and Hohensinner et al. [57–59] describe in detail the development of channelisation at the Austrian Danube since the early eighteenth century. In Fig. 10 (right) hydromorphological changes from 1715 up to now are illustrated. On the left-hand side the turnover of functional groups and the loss of biodiversity are schematically depicted.

4.1.2 Damming

In general damming leads to increasing sedimentation of fine particles due to the reduction of current velocity in longitudinal, lateral and vertical (clogging of the interstitial) dimensions [61, 62]. Faunal changes are well documented and have different extent from headrace to the weir [62–68]. In general a dramatic change of functional groups from rheophilous to stagnophilous organisms and from scraper/filter feeders to detritivorous, respectively, can be observed. Due to enhanced autotrophic production in dammed areas, the nutrient cycle is altered and filter-feeding assemblages increase below dams (e.g. Statzner [69] and Mauch [70]). Besides these local impacts damming influences the discharge regime and sediment transport considerably and changes the overall character of riverine systems (e.g. Habersack et al. [71]). The homogenised discharge dynamics and summation effects of dam chains lead to a loss of type-specific organisms which are replaced by pioneers and more opportunistic and insensitive faunal elements [72, 73] as documented by Usseglio-Polatera and Bournaud [74] and Fruget [75] at the Rhone River.

Fragmentation of habitats, especially like the succession of dams at the Upper Danube, may suppress genetic exchange of populations [76] and represent a major threat for biodiversity in general [77].

4.2 Pollution

An excellent description of various pollution pathways in Vienna during the Middle Ages is given by Kohl [78] which may be generally applied on most European cities and connected large rivers of that time. Liebmann and Reichenbach-Klinke [79] list pollution sources along the entire course of the Danube and provide a historical outline of organic pollution (e.g. the first biological water quality map of the Austrian Danube). As one example of large rivers, Tobias [80] gives an overview of the development of the oxygen and ammonium content from 1970 to 1994 at the river Main with highest pollution loads between 1972 and 1980 and a recovery afterwards which clearly correlates with the revival of the mayfly *Ephoron virgo*. Since that time water quality has substantially been enhanced during the last decades mainly because of raised environmental awareness based on continuous saprobiological surveys and subsequent improved purification processes.

Organic pollution has generally lost its primary role as stressor in aquatic systems of Central Europe and has been replaced nowadays by hydromorphological degradation. Anyhow, organic pollution had its negative effects in the past, and detailed monitoring campaigns have impressively initiated a reduction of organic pollution in the Danube (e.g. Jungwirth et al. [50], Fig. 33). In regard to water chemistry, hazardous and endocrine substances which impact biological quality elements are currently a main issue in water management. The effects of currently applied substances in agriculture as well as in industrial processes together with effluents of sewage treatment plants and their combined effects via the whole catchment areas are poorly understood (e.g. Van Der Geest et al. [81]).

4.3 Navigation

Vessel-induced waves lead to high shear stress at the river banks [82] and Liebmann and Reichenbach-Klinke already observed severe negative effects by navigation in 1967, especially caused by wave action. Juvenile fish were reported to be hurled at the riparian zone, fish were disturbed during spawning in general, and oil was polluting the substrate. Especially wave wash effects have impacts on juvenile fish as reported by Hirzinger et al. [83], Kucera-Hirzinger et al. [84] and Schludermann et al. [85]. Gabel et al. [86–88] investigated the reactions of selected macroinvertebrates and their interactions with fish under the influence of wave actions. Their findings underlie the magnitude of ecological impacts and stress, e.g. the fact that the neozoon *Dikerogammarus villosus* is more flexible than its congeners among the genus *Gammarus* thus suppressing it and other native species.

Negative effects on merolimnic organisms by mechanical damaging especially during moulting processes at the shoreline can be expected but have not been studied yet in detail; in fact the majority of insects still persisting nowadays in the Danube moult nearly exclusively at the water surface.

Furthermore ships are generally suggested to enhance the spreading of neozoa as vectors through ballast water and vessel hulls as suitable colonising substrate. The role of navigation in the process of globalisation of the fauna – the so-called McDonaldisation [89] – is hardly investigated comprehensively in all its aspects, therefore poorly understood and remains still underestimated.

4.4 Neozoa

Nonindigenous species will be discussed in detail by Paunović et al. [90] giving comprehensive and clear definitions. As neozoa are decisive and dominant elements within the benthic community of the Danube for decades, some aspects are shortly addressed here additionally.

Neozoa are per definition species which colonised a given area after the year 1492. Reliable studies on macroinvertebrates started with Linnaeus back at the end of the eighteenth century which makes the designation of certain species difficult due to lack of detailed distributional information. Zoogeographical patterns are the result of mainly climatic conditions and various either recent or historic shifts have been documented. For example, *Dreissena polymorpha* is documented from Tertiarian times in Central Europe, survived glaciation in southern areas and returned during the eighteenth century [91]. Species ranges have been and will be oscillating, but anthropogenically induced pressures like climate change and others speed up these processes and enhanced the awareness of this specific environmental problem [92].

The increasing massive occurrence of invasive alien species in connection with the increasingly documented loss of indigenous faunas of large rivers is observed on a European-wide scale (e.g. Moog et al. [93], Arbačiauskas et al. [94], Graf et al. [95], Panov et al. [96] and Füreder and Pöckl [97]). Besides biodiversity issues this phenomenon is intensively discussed in the context of ecological assessment systems and the closely linked management actions (e.g. Schöll and Haybach [98, 99], Arbačiauskas et al. [94], Panov et al. [96], Olenin et al. [100], Cardoso and Free [101] and Orendt et al. [102]).

The Danube River is – besides a northern corridor via the Volga to the Baltic Sea and a central pathway via the Dnieper to the Elbe and the Rhine – the main southern migration route of aquatic Ponto-Caspian elements [103], and the majority of neozoa in the Danube therefore clearly belong to Crustacea and Mollusca from this region, while only few others like *Atyaephyra desmaresti*, *Eriocheir sinensis* and *Corbicula fluminea* and *Sinanodonta woodiana* and *Potamopyrgus*

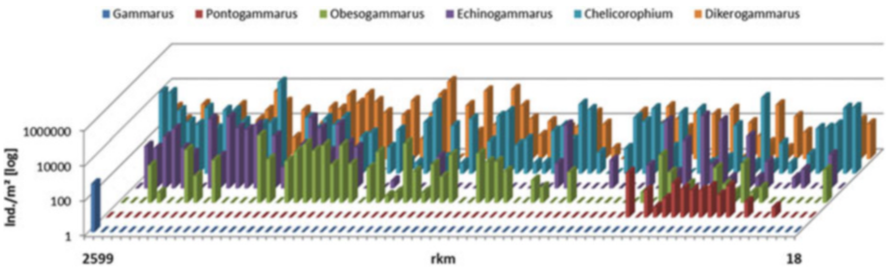


Fig. 11 Distribution of Amphipoda genera with densities along the Danube based on JDS2 data

antipodarum, respectively, are of other origins (the Mediterranean, East Asia and New Zealand; [93]). Figure 11 gives the distribution of the genera Amphipoda with densities along the Danube. Only the genus *Gammarus* is considered to be native in the Upper and Middle Danube.

Direct negative influences of invasive alien species on the original fauna have been hardly testified, but Schöll [104] found clear correlations between increasing densities of the amphipod *Dikerogammarus villosus* and the population decrease of the caddisfly genus *Hydropsyche* in the Rhine River. Moog et al. [105] describe similar interactions between *D. villosus* and *Gammarus fossarum* and *G. roeselii*, respectively, in the river Traun. According to Pöckl [106] the predator *D. villosus* shows higher fertility than the resident *G. fossarum* and *G. roeselii* and is successfully competing with them. Bącela et al. [107] also stated significant changes among the benthic associations after the new colonisation of *D. villosus* in Rhine, Oder, Danube and Meuse. Nowak [108] investigated the effect of *Dreissena bugensis* on other benthic invertebrates, but in general processes behind are still poorly understood.

The seriousness of this problem may be illustrated exemplarily by the recently documented structure of benthic assemblages of the Danube during the JDS2 expedition: Among the ten most frequent macroinvertebrate species sampled, nine are assigned as neozoa [95], above all occurring in very high densities and frequency (see Fig. 12).

In terms of abundance neozoa dominate clearly the benthic communities and reach up to 50% of all documented taxa in the Upper and Middle Danube (Fig. 13).

Neozoa are characterised by Statzner et al. [109] as ecologically flexible, as having high fertility rates, and as nonsensitive thereby being more robust which enables them to colonise disturbed environments. In fact, large river ecosystems are multiply stressed and among the most threatened ecosystems worldwide. Invasive elements may just fill up empty niches after the loss of indigenous elements. Analysing the enhanced invasions in Austria since the 1980s, Moog and Wieser [110] and Korte and Sommerhäuser [111] mention the increasing water temperatures as one essential trigger, which was also mentioned earlier by Rahel and Olden [112].

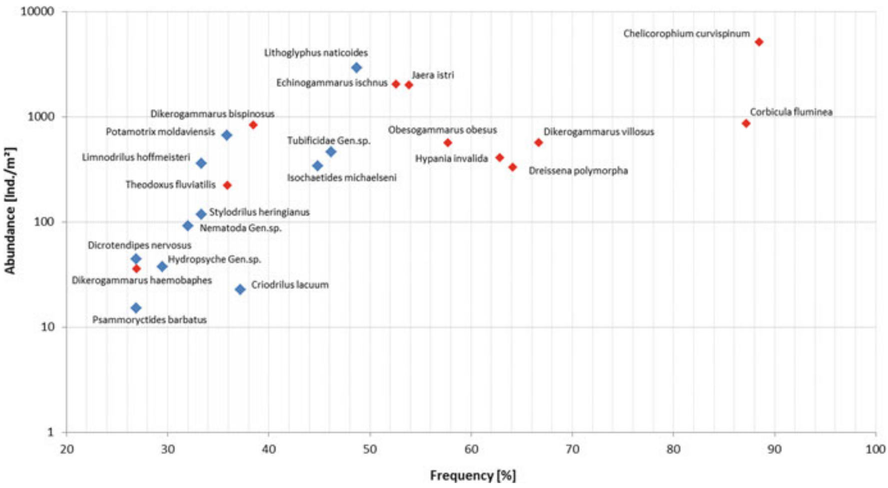


Fig. 12 Most dominant taxa (frequency >25%) and their average abundances (when present) in the Danube during JDS2; neozoa marked red

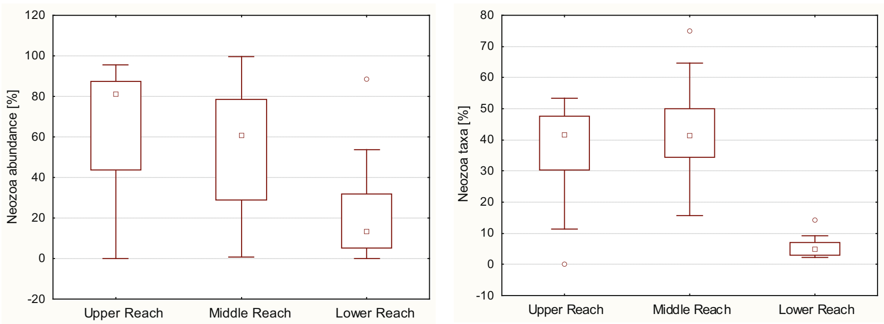


Fig. 13 Box-and-whisker plots of neozoa abundance and neozoa taxa numbers

From an ecological point, the most dominant neozoa have severe impacts on the entire functioning of aquatic ecosystems as they (1) reach high densities (e.g. 500,000 ind./m^2 of *Chelicocorophium curvispinum* in the Morava [113] dominate the benthic community and colonise niches of indigenous faunas), (2) act partly as bioengineers changing the habitat characteristics entirely (*Chelicocorophium* spp. alter the microhabitat structures by building tubes; *Corbicula* spp. provide a specific habitat for other species, respectively, as the diameter of adult shells resembles microlithal conditions; [114]) and (3) intervene significantly in the nutrient cycle, e.g. *Corbicula* spp. This Asian clam – an active filter feeder – shows mass occurrence and can reach a biomass of more than 7 kg/m^2 ([93]; Danube at Linz, Austria); Rey [115] stated even a biomass of 30.8 kg/m^2 in Lake Constance.

Nakano and Strayer [116] recently gave a worldwide overview on biology, impacts and ecosystem engineering of biofouling animals. They stress the fact that biofoulers are economically important and estimate a yearly global cost of 277 million US$ to be caused by them. Documentation of faunal changes (e.g. Paunovic et al. [117, 118], Borza [119, 120], Borza and Boda [121] and Borza et al. [122]) is therefore essential as it seems that shifts and range oscillations have not ended yet (e.g. Fischer [123] and Fischer et al. [124]). Large datasets as compiled by the Joint Danube Surveys are extremely useful and necessary in monitoring of the ecosystem functioning and potential changes in ecosystem services. Restoration of hydromorphological conditions hopefully will contribute to achieve improvements in ecological integrity, but as stated by Füreder and Pöckl [97], a substantial recovery is probably impossible.

5 Large River Species and Losses

Large rivers in Europe have undergone many anthropogenic modifications and have lost a high share of their indigenous fauna, especially sensitive insects like Ephemeroptera, Plecoptera and Trichoptera (EPT taxa). Other than in commercially important species like fish, we have few indications of the occurrence of macroinvertebrates on species level of large rivers during the centuries. Many of these species once covered a large area in Europe (summarised exemplarily for Plecoptera by Zwick [77]); nowadays nearly all of them are listed in Red Data books of most countries as threatened or even extinct. Den Hartog et al. [125] documented a disappearance of 85% of these species in the Lower Rhine, Mey [126] describes a similar phenomenon regarding Trichoptera, and Fittkau and Reiss [7] highlighted this fact in general.

The Danube River seems to be no exception. Among Trichoptera only the river-type specific *Hydropsyche contubernalis* and *H. bulgaromanorum* were found along all reaches accompanied by local populations of *Setodes punctatus* during JDS2. Other and more frequently documented species of that group are known to be more or less insensitive and typical for slow current velocity. Ephemeroptera were mainly represented by few species of the genus *Caenis* and *Heptagenia* only which occurred sporadically. Plecoptera could not be found downstream of the site Oberloiben, while Raušer [127] reported a rich indigenous stonefly community for the Danube and listed the following well-documented species according to literature: *Brachyptera trifasciata*, *B. braueri*, *Oemopteryx loewii*, *Taeniopteryx araneoides*, *T. nebulosa*, *Perlodes dispar*, *Isogenus nubecula*, *Isoperla obscura*, *Isoperla difformis*, *Marthamea vitripennis*, *Xanthoperla apicalis* and *Isoptena serricornis*.

The few historical information indicates that these species once indeed occurred in very large numbers. Calderini [128] described the disturbance of local people by masses of *Brachyptera trifasciata* in Italy, and Ausserer [129] mentioned this species to be "specialmente in primavera molto comune in tutta la fauna".

Kühtreiber [130] remarked “all silts and sand banks are teeming with them”, giving us possibly a hint on the substrate type preferred by this species. Bridges in Prague were so crowded with the nowadays nearly vanished *Brachyptera braueri* that the public called it the “Prague fly”. *Isogenus nubecula* was described in Brauer and Löw [131] as “very common” in the vicinity of Vienna. Mass emergence of the species *Oemopteryx loewii* was reported as early as 1775 by Schäffer [132] from Regensburg, of which nowadays only few females are left in museums. The last reliable finding is reported by Russev [133] from the Bulgarian Danube in 1955. Although cumulative effects of multiple stressor interactions are responsible for this losses, the last records of conspicuous species are well coinciding with the period of dam building at the Upper Danube.

Most of those potamal species had wide distributions in Europe once. Zwick [77] cites records of *Isogenus nubecula* from England, France (Paris), the Netherlands, the Danube at Ulm and Vienna, Dresden and Bulgaria, and similar large areas have been covered once by *Marthamea vitripennis* [134] and *Xanthoperla apicalis* [135].

Today’s populations are isolated and persisted exclusively in small and severely fragmented refuges as in the case of *Isogenus nubecula* in the river system Lafnitz/Raba in Austria/Hungary and the Tisza in Hungary [136, 137]. Other examples which demonstrate similar fates of large river species are given in, for example, Fittkau and Reiss [7], Zwick [77, 134] and Fochetti and Tierno de Figueroa [138]. A few of these species seem to have survived in discrete refuges and have been rediscovered only recently. *X. apicalis* of which some vouchers from 1884 (Danube at Vienna) exist in the Museum of Natural History in Vienna was recently collected in the middle of the 16th district of Vienna [139]. This long-lost species is apparently recolonising some large rivers in Central Europe (e.g. Braasch [140]).

Among Ephemeroptera *Ephoron virgo* is another example of a potamotypic species with mass emergence which was so conspicuous that Schäffer [141] reported it already in 1757. After some decades of disappearance, its revival was reported yearly by local newspapers along the Upper Danube as its numerous corpses can lead to operations of snowplough trucks to prevent accidents. Production of these potamotypic mayflies was incredibly high, and Tobias [80] cites old reports, whereas locals attracted specimens with fire and lamps and gathered them at the shore. At the river Saône in France, 100 tons of corpses were yearly collected and used as food for swines, fishes or birds and as fertilisers or even sold to pharmaceutical industries (Lampert [142], in Tobias [80]).

Another Ephemeroptera, the large species *Palingenia longicauda* (4 cm in body length), was formerly found from the Netherlands to Ukraine [143]. Nowadays *P. longicauda* covers 2% of its former range [144] which led to listing it as one of the few aquatic insects in Appendix II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). It is doubted to have colonised the Upper Danube [145] but was regularly recorded from the Bulgarian stretch and some tributaries. Incredibly high densities reached between up to 3,350 specimens/m^2 and biomasses up to 660 g/m^2 [146], contributing essential to food resources for, for example, fishes. According to Russev [143, 146] *P. longicauda* is a habitat specialist which burrows tubes in clayey substrates, the argillal. Since

1974 records from the Danube River are missing maybe caused by reduced habitat availability among other stressors as stated by Russev [147] in 1992. Recently some specimens were found by G. Chiriac at Braila in 2011 (personal communication) which doubtlessly confirmed its return or persistence in refuges of the Lower Danube stretch. Soldán et al. [148] report one population at the Danube Delta, and another well-known and famous site is the River Tisza where spectacular mass emergences can still be observed [149].

Both mentioned Ephemeroptera species are burrowers living in U-shaped tubes and are therefore eco-engineering their environment. They filter out fine organic particles; thus, their reduced occurrence influences the nutrient turnover of the ecosystem. Stief et al. [150] found that microbial communities of burrows are different to that of the sediment and conclude that the presence of *E. virgo* contributes significantly to the ecological connection between the water column and the sediment and to the biogeochemical processing of organic matter in the riverbed. This specific food niche now is occupied nearly entirely by the invasive filter feeders *Chelicocorophium* (Crustacea) and *Corbicula* (Mollusca), besides the trichopteran genus *Hydropsyche*. Additionally to their effects on the aquatic ecosystem – e.g. Gheracopol et al. [151] stated that the diet of a starlet consisted 69% of *P. longicauda* – their mass emergence transferred a huge biomass to the terrestrial, nourishing a long list of organisms like spiders, birds, bats, etc. This stresses the importance of macroinvertebrates as available resource for consumers in general and in 1967 Russev [152] stated a yearly production of 19.235 tons of benthic biomass in the Danube Delta.

Like the mentioned species above, some stenoecious trichopteran species of large rivers as *Platyphylax frauenfeldi* belong to the most endangered aquatic species on a European scale with only one known vital population at the River Drava in Hungary [153, 154]. Another species, *Parasetodes respersellus*, has undergone dramatic population losses since the 1960s in Central Europe. Recently it was rediscovered in the Tisza River [155]. It once inhabited the Lower Danube in Romania where it was found prior to 1962 for the last time [156]. These species may nowadays act as umbrella species for an intact community and their occurrence may indicate vital processes and essential river-specific abiotic-biotic interactions. However, in Trichoptera only one case of extinction has been documented (*Hydropsyche tobiasi*, [154]) though human-induced considerable regressions or even extinctions in several national states are regularly reported (e.g. Botosaneanu [157]).

In fact many typical and nowadays extinct or endangered species of large rivers show mass emergences and short but synchronic flight periods (*Ephoron virgo*, *Palingenia longicauda*, *Xanthoperla apicalis*, *Isoperla obscura*). This phenomenon seems to be essential for mating and reproduction success; as minimum population size is not known, slight reductions of swarming stages may lead to severe bottlenecks leading to abrupt species losses within the whole catchment.

As pointed out earlier, the benthic assemblages are nowadays clearly dominated by nonindigenous, invasive or cosmopolitan elements which probably have strong negative effects and misbalance the ecological functioning of the whole system.

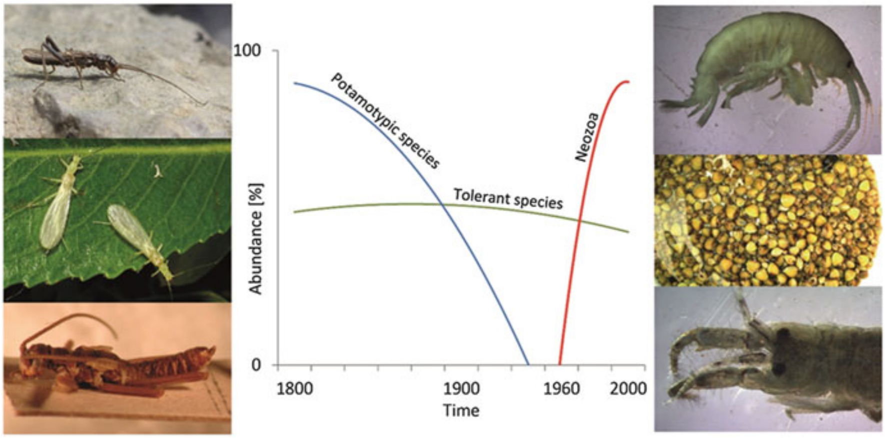


Fig. 14 Conceptual development of the fauna of large rivers in Central Europe from 1800 to 2000. Photos: *left*, indigenous species of the Danube, *Brachyptera trifasciata*, *Xanthoperla apicalis*, *Taeniopteryx araneoides* (pinned specimen, Museum Budapest, Photo: D. Murányi); *right*, invasive species, *Corbicula fluminea*, *Dikerogammarus villosus*, *Chelicocorophium curvispinum* (Graf and Pletterbauer, unpublished)

Figure 14 illustrates the above-mentioned processes documented in large rivers in Central Europe conceptually.

Molluscs are another typical and prominent element of large rivers and still colonise the Danube with many species. Two species, namely, *Unio pictorum* and *U. tumidus*, are the most common large mussels of the Danube which form the highest biomasses of benthic invertebrates in the main channel. The third species, *U. crassus*, which can only be rarely found in the Danube has undergone a strong decline throughout Europe in the recent decades; e.g. in Germany this species receded by about 90% of its former distribution area [158]. Consequently *U. crassus* is an endangered species which is mentioned in Annex II and IV of the European Fauna-Flora-Habitat Directive (e.g. Csar and Gumpinger [159]). Following Nesemann [5, 6] *U. crassus* occurs with several subspecies in the Danube basin (tributaries and Mosoni-Duna); only one living specimen from the main channel was recorded for the Austrian stretch [160]. Csányi et al. [161] report on the first record of *U. crassus* in the Lower Romanian Danube between Calarasi and Braila. *Anodonta anatina* is present in the Middle Danube, while the Asian species *Sinanodonta woodiana* increases steadily in density from the Middle Danube to the Delta but has invaded successfully backwaters all over the Danube floodplains. The Asian clam *Corbicula fluminea* covers the whole river stretch in high densities, while *C. fluminalis* is still rare and is present at few sites only. The zebra mussel *Dreissena polymorpha* is abundant on the Upper and Middle Danube; the newly invader *D. bugensis* has already spread to Vienna and above [123, 124].

Regarding snails, two *Viviparus* species (*Viviparus acerosus* and *V. viviparus*) are still common along the banks. Within Neritidae, *Theodoxus fluviatilis* has the widest distribution along the Danube; it is considered to be a neozoon. The Danube

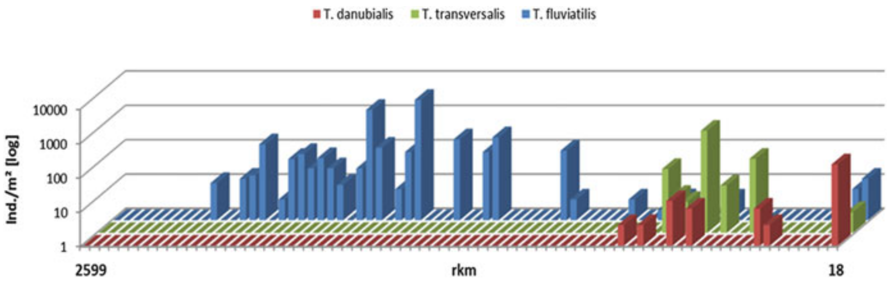


Fig. 15 Distribution of three species of the genus *Theodoxus* along the Danube recorded during JDS2

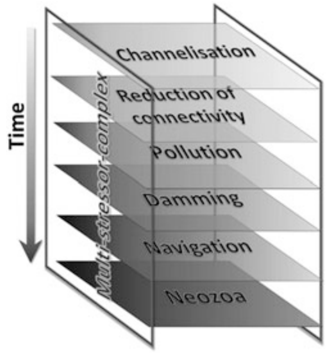
basin-specific *T. danubialis* is mainly restricted to the Lower Danube, while the formerly widespread *T. transversalis* is living now in a very restricted section at the Lower Danube (Fig. 15).

6 Conclusion

Large rivers have been altered for centuries (e.g. Tockner et al. [162, 163]), and Hering et al. [164] summarise the multiple interactions between various stressors of aquatic ecosystems worldwide. The Danube is regrettably no exception, but drivers and pressures fit well in a Pan-European scale. Rates of habitat modification of large rivers are currently so high that virtually all natural habitats and protected areas are destined to become ecological "islands" in surrounding "oceans" of altered habitats. This process of fragmentation and isolation in landscapes under human influence – main concepts in the island biogeography theory – is predicted to lead directly and indirectly to accelerated species extinctions at both the local and the global scales, thus reducing the world's biodiversity at all levels [165, 166]. In the context of the so-called McDonaldisation of the biosphere [89], the dispersal of many species is inhibited, while others – mostly more flexible species in ecological terms – become common and overtake the niches of indigenous species. Replacement of vulnerable taxa by rapidly spreading taxa that thrive in human-altered environments will ultimately produce a spatially more homogenised biosphere with much lower diversity. Regarding aquatic ecosystems and in particular large rivers, similar processes have already been observed by Fittkau and Reiss [7], Zwick [77, 134] and Fochetti and Tierno de Figueroa [138]. The multi-stressor complex appealing on large rivers, especially in Central Europe, is conceptually given in Fig. 16.

Potamal communities at the edge of their ecological capability might collapse when temperature increases due to climate change that adds to the deadly anthropogenic cocktail [167]. But with few exceptions there is no evidence of an actual decrease in species richness of rather flexible riverine and wetland assemblages in

Fig. 16 Conceptual framework of a multi-stressor complex of large rivers (Graf and Pletterbauer, unpublished)



lowlands of Central Europe, simply because most of these communities have been already dramatically shaped by anthropogenic pressures of various kinds; those surviving organisms are tolerant cosmopolitans which cover a large area of ecoregions.

On the other hand, there are signals of a recolonisation regarding some riverine species which indicates improvements in the overall habitat quality and the ecological status. Awareness of the vulnerability and sensitivity of the large river ecosystem has risen and various restoration plans are put in praxis along the Danube. Linear systems like rivers are depending on processes within the entire catchment, and local efforts – despite their undoubted merits – can only marginally soften large-scale impairments. International cooperation is therefore required to monitor and improve the ecological status of the Danube and to conserve its fauna.

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Phytobenthos of the River Danube

Jarmila Makovinska and Dasa Hlubikova

Abstract Benthic algal flora of the River Danube is presented with implications for ecological status assessment. Structure of algal biofilms, species diversity, algal abundance, and biomass are described and discussed based on most recent algal investigations supplemented by methodological insight to community structure evaluation. Comparisons of literature data are provided. Seasonal and longitudinal changes of benthic algal assemblages are evaluated in terms of species abundance and biomass as well as community structure. In contrast to previous studies of Danubian periphyton that detected prevailing diatom abundance in the biofilms, recent research has found that cyanobacteria and green algae dominated almost along the whole Danube stretch. Ecological status of the entire Danube stretch is evaluated by means of the diatom-based "Indice de Polluosensibilité Specifique" (IPS), which showed distinct differences between the upper and middle section of the River Danube indicating longitudinal increase of general degradation of aquatic environment and increasing nutrient concentrations. The overall indication of ecological status varied between good and moderate.

Keywords Benthic diatoms, Danube, Large rivers, Nutrients, Phytobenthos

Contents

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Abbreviations

ICPDR	The International Commission for the Protection of the Danube River
IPS	"Indice de Polluosensibilité Specifique" (The polluo-sensitivity index)
JDS	Joint Danube Survey
SI	Saprobic index
WFD	The water framework directive

1 Introduction

Benthic algae (periphyton or phytobenthos) are the most successful primary producers in aquatic habitats. They are widely considered to be the main source of energy for higher trophic levels in many, if not most, unshaded temperate region streams (e.g., [1–3]). In addition to primary production, they are important chemical modulators transforming inorganic chemicals into organic forms [2, 3], participate at purification processes [4], function as stabilizers of substrata, and serve as an important habitat for many other organisms. All these features make them an essential component of aquatic ecosystems.

1.1 Phytobenthos in Aquatic Environment

Because benthic algal assemblages are attached to substrate, their characteristics are affected by physical, chemical, and biological disturbances that occur in the reach within a specific time period and thus reflect long-term conditions of aquatic environment. Development of the algal biofilm in rivers is governed by a complex array of factors and interactions. According to Biggs et al. [5], the benthic algal community structure is basically driven by sources availability (light and nutrients) and disturbances (mainly hydrological stress). At finer scale, a range of processes is operating to generate the diversity of algal biofilms and detailed knowledge of these factors has led to a development of numbers of methods using algal communities for bioindication. Compared to other groups of bioindicators such as macrophytes or benthic invertebrates, benthic algae cover nearly any type of substrate in the river bed and thus can be found in every type of water body. Contrary to secondary producers, they react directly to nutrient concentrations, and this makes them particularly interesting for use as indicator of changes related to eutrophication.

In large rivers, the leading role in primary production is governed by phytoplankton [6]. The specific conditions in such river types favor phytoplankton development, and the algal biofilms are often restricted to the littoral zone because of limited light availability and high turbidity of the flow. Therefore, studies on phytobenthos from large rivers naturally refer to the riverbank area, respectively, visible and suitable for collecting samples. Nevertheless, phytoplankton as bioindicator mirrors environmental conditions in flows in short terms, while attached benthic algae that are exposed to fluctuations of environmental factors and water chemistry within a period of time reflect a long-term status of aquatic health. In the Danube, where nutrients have been identified as an important anthropogenic pressure threatening the quality of the river water [7], benthic algae are an essential component of all bioassessment studies.

1.2 Phytobenthos in the River Bioassessment

Phytobenthos together with macrophytes are identified as the biological quality element under the European Water Framework Directive 2000/60/EC [8] and as such need to be monitored to identify anthropogenic influences on aquatic ecosystems. Especially in the rivers, phytobenthos are considered among most suitable indicators determining the impact of nutrient pollution. The methods for phytobenthos use in water quality monitoring and assessment have been evolving in two main streams using the whole phototrophic community on one hand and diatoms only on the other hand. The former holistic approach is adopted on routine basis in North America [9, 10] and New Zealand [11], while there are much fewer studies considering both diatoms and non-diatoms in Europe (e.g., [12–16]). However, considering all phototrophic organisms simultaneously can be problematic, because of the wide range of spatial scales and life histories encompassed within this term [17]. Also, the identification of non-diatoms is often impeded by complicated life cycles requiring in vitro cultivation and necessity of life material analysis, which requires greater effort for sampling and microscopic observations. Methods that use phytobenthos in bioindication have for reasons mentioned above tended to focus on diatoms, which often form a large part of the algal diversity in freshwaters [18] and often dominate in the periphyton. Due to short life cycles and fast proliferation, diatoms respond relatively rapidly to shifts in environmental conditions, but since they are attached to the substrate, they integrate impacts over certain period of time. Moreover, the presence of highly resistant frustule in diatoms is a significant advantage compared to other soft benthic algae, because the diatom sample can be fixed in high-resolution mountants on permanent slides allowing detailed examination without time limitations. Diatoms showed to be reliable indicators of trophy, organic pollution, acidification, salinity, or climate (summarized in, e.g., Stroemer and Smol [19]) and were proved to offer a similar insight into the pressures shaping the benthic flora, but in a more cost-effective manner than when the entire flora is examined [20]. For the reasons described above, the predominant approach adopted in mainland Europe was to consider diatoms as proxies for phytobenthos-based assessment of ecological status of rivers (see Kelly et al. [21] and Kelly [22]) and lakes [23–25]. A number of diatom indices were developed based on autecological requirements of diatoms [26–32] that were further successfully applied all around Europe [29, 33–43] and are routinely used in all European countries for standard monitoring of river ecological status (see Kelly et al. [21] for summary).

1.3 Historical Overview of Danube Phytobenthos Studies

The leading role of phytoplankton in large rivers is mirrored also by research interests and activities of algologists in Danubian algal flora. While the phytoplankton of the Danube has been surveyed regularly and extensively in Austrian, Slovak, and Hungarian parts (see, e.g., [44–68]), surveys of benthic algal flora were much less frequent. The intensity of research activities differed between countries, and it seems that the past century was more productive in terms of phytobenthic surveillance of the Danube compared to recent studies.

An exhaustive summary of the early studies of periphyton on the Danube from German, Austrian, Hungarian, and Slovak parts was compiled by Szemes [46, 69] and later by Kusel-Feltzman [70]. Despite the great changes and progress in algal taxonomy in the last decade, these data are a very valuable source of information for comparative purposes. The phytobenthic communities in the German stretches were studied by Backhaus [71–76]. More recent investigations were carried out as a part of a more complex survey of Ács et al. [77]. In the Austrian stretch, the first phytobenthic investigations are dated to 1914 and were carried by Handman in Linz region (compiled in Szemes [69]). Later Cholnoky [78] and Bursik [79] continued with algal investigations focusing on benthic diatoms. Weber [80] investigated the benthic algae at ten different sampling points, unfortunately a detailed species composition was provided only for one sampling station at Nussdorf (km 1,934.1). The Danube stretch in the region of Vienna was studied by Kann [81], but the presented taxalists did not specify the diatom composition. A detailed phytobenthic survey was finally carried out by Schagerl and Donabaum [82, 83] near Vienna who supplemented the standard microscopic analysis by evaluation of class-specific pigment markers and provide exhaustive taxalists of all periphytic algal groups [82, 83] comparing species composition on natural and artificial substrates. The benthic algal survey of the Slovak stretch was initiated by Juriš in 1969 [84, 85] who documented the periphytic algal composition on slides and later Ertl and Tomajka [86] studied primary production of algal biofilms. After these early investigations, all the further research activities focused on phytoplankton [53, 62, 63, 65, 66] until a regular diatom monitoring in surface waters had started in 2003 [43]. In the meantime the data on Danube benthic algal species could be only filtered out from taxalists obtained during planktonic surveys; nevertheless these are exhaustively documented by Hindák and Hindáková [66]. In the frame of the Slovak national monitoring of surface waters, a regular surveillance of benthic diatom assemblages was launched at three sites of the Slovak stretch in 2003, and the outcoming diatom taxalists from the period of 2003 to 2009 were presented in Hlúbiková et al. [87].

Periphytic algae in the Hungarian stretch of the Danube were contrary to other parts intensively studied since the nineteenth century [88], and the results of the early works were summarized by Tamás [89, 90] presenting all algal groups and Szemes [91] focusing on diatoms. Further research of periphyton was relaunched by the research team of Eva Ács who investigated taxonomical composition of benthic algal communities either in the branch system [92–96] or in the main stretch [97–100] together with methodological insights studying the colonization processes and differences between benthic algal communities from different natural and artificial substrates [101–104]. All these works utilized microscopy-based techniques and morphological criteria for taxa identification. Except for these classical determination approaches, also the potential of molecular methods for water quality monitoring purposes using phytobenthos was tested on benthic algae from Danube at Göd (km 1,669); molecular fingerprinting was applied to explore the diatom assemblages [105] and the whole microeukaryote community [106] comparing the results with microscopic observations.

The lower reach of the Danube was intensively studied in terms of algal composition of benthic communities in the Bulgarian section in the 80th by Stoyneva [107, 108] and Draganov and Stoyneva [109, 110], recently summarized by Stoyneva [61], and the Romanian stretch was mainly investigated in the Danube Delta [111–116]. More recently, benthic algal communities were surveyed and used for ecological status assessment of the River Danube in the Ukrainian section by Oksiyuk et al. [117].

All the research activities mentioned above explored or summarized mainly local algal flora by national research teams focusing on relatively short sections or several sampling points determined by state borders. Naturally, the scientific background, research methods (both of sampling and analysis), taxonomic depth, and way of results evaluation differed greatly among the published results also depending on the purpose of the different studies. There was, however, a lack of a global survey mapping the benthic algal communities along a longer reach applying harmonized approach to sampling and analysis with consistent taxonomic demands.

All these gaps were supposed to be filled within the Joint Danube Survey (JDS) research expedition coordinated by the International Commission for the Protection of the Danube River (ICPDR). Among others, the particular objective of the survey was to collect data readily comparable from the entire Danube from the spring down to Danube delta. In particular, taxonomic composition and abundance of benthic algae were surveyed every 6 years (2001, 2007, 2013) along the entire river from the spring down to the Danube delta [118, 119]. These results provide the most comprehensive overview of the Danubian benthic algal flora [120, 121] of both diatoms and non-diatoms together with outcomes for ecological status assessment based on diatom indices. The need of a broader survey alongside the Danube was later recognized also by Ács et al. [77], who investigated epilithic algal communities of the Danube from Ingolstadt (Germany) to Göd (Hungary) and from Bratislava to Mohács (Hungary) together with the main Danube tributaries. They analyzed and quantified both diatoms and non-diatoms and tested performance of several diatom indices in order to evaluate the water quality in addition to the taxonomic composition. Yet, the lower reach crossing Croatia, Bulgaria, Romania, and Ukraine was not involved. With regard to the investigations referred, the JDS remains the most complex and harmonized survey of biological, chemical, and hydromorphological elements carried along the whole Danube profile. The results presented are therefore mainly based on the outcomes of the JDS.

2 Material and Methods

The data on benthic algae discussed and presented below originated mainly from Joint Danube Surveys, which have been held in the years 2001 (JDS1) and 2007 (JDS2) [118, 119].

Sampling of phytobenthos for JDS1 and JDS2 has been performed by combining the methods for benthic diatoms and non-diatoms and cyanobacteria. In the frame of JDS1, all samples have been preserved and sent to the laboratory for identification and abundance estimation. During the JDS2, the sampling and analysis followed instructions of the European standards CEN 13946 [122] and CSN EN 15708 [123]. Additionally, fluorescence measurements for phytobenthos biomass determination were performed using Benthofluor® fluorometer (see below for details).

For sampling, a river segment with a suitable substrate was selected at each sampling site. Epilithon was sampled wherever possible by scrubbing a surface of at least at least five boulders or more pebbles at all sampling sites. Where hard substrata were absent, epiphyton was sampled. Samples were always taken from the euphotic zone, usually up to 1 m depth. After measurements of chlorophyll-*a*, an area of minimum 10 cm^2 was brushed thoroughly from each stone. The sample was transferred from tray to sample container and labeled. All field information needed have been recorded to the standardized field protocol. Samples used for benthic diatoms analyses were preserved in formaldehyde, and samples of non-diatom algae and cyanobacteria were analyzed in vivo directly after the sampling. Native samples were stored in the refrigerator before the analysis. If the macroscopic algae (e.g., *Cladophora*, *Hydrodictyon*) were present, separate sample container was used for easier determination.

The microscopic analysis has been performed using light microscopy at 400× to 1,000× magnification. All important determination characteristics of the species were recorded using image analysis. The determination was done as detailed as possible using actual determination keys for individual algal groups. Estimation of the quantity of the individual species in the scale 1–9 was used.

Based on the species diversity and estimation of quantity, the saprobic index was calculated during JDS1. In the frame of JDS2 based on sampling information together with microscopic analysis the estimation of the ratio of cyanobacteria, green algae, diatoms and other algal groups was performed. The preparation and quantification of samples of benthic diatoms followed the instructions of the European standard [124]. Diatoms were cleaned using 40% hydrogen peroxide and permanent slides were mounted using Naphrax. On average, 400 valves were counted on each slide in random transects with light microscope with DIC (differential interference contrast) at 1,000× magnification. Based on diatom inventories, 17 diatom indices were calculated using Omnidia 4.2 [125].

Measurement of phytobenthos biomass has been performed using the Benthofluor® fluorometer (bbe Moldaenke, Kiel, Germany) according to Aberle et al. [126]. On each of five or more stones, five subareas were measured, each measurement was done 3–4 times to obtain sufficient database of chlorophyll-*a* concentrations for statistical analysis. Three main algal groups were distinguished: diatoms, green algae, and cyanobacteria. For each of these groups and for total benthic algal biomass, the chlorophyll-*a* level was determined in μg/cm^2.

3 Results and Discussion

3.1 Algal Biofilm Structure and Biomass

The periphytic communities of the River Danube have usually been reported to be dominated by diatoms (Bacillariophyceae [77, 81–83, 91, 95, 100–102, 104, 105, 127]). Diatoms prevailed on both natural and artificial substrates [82, 83, 101] and were mostly represented by pennates (Penales). On the contrary, results of the JDS2 showed much lower abundance of diatoms compared to other algal groups within the collected samples (Fig. 1). According to these results, most of the sites contained prevailing numbers of cyanobacteria (Cyanophyta) and/or green algae (Chlorococcales), while diatoms reached an average relative abundance of only

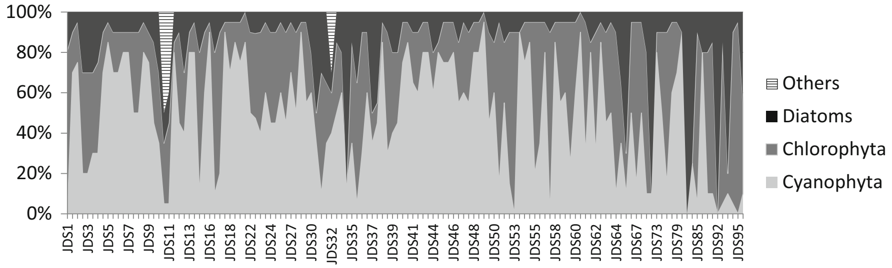

Fig. 1 Proportion of three different algal classes in the algal biofilms along the entire Danube stretch during the JDS2 (in 2007), from 2,600 riv. km down to the mouth based on algal relative abundances

16% within the whole dataset (Figs. 1 and 2). Cyanobacteria predominated at 65% of all sites (with an average relative abundance over 51%) and green algae at 28%, respectively. Diatoms were found predominant only at 9 out of 135 sites. Among them, the diatom-dominated assemblages occurred mostly in the lower reaches of the Danube (e.g., Upstream Timok 649 riv. km, Upstream Iskar 640 riv. km, Downstream Ruse/Giurgiu 488 riv. km, Upstream Arges 434, Reni 130 riv. km, Vilkova 18 riv. km) and at three sites of the middle Danube. Average relative abundance of cyanobacteria and green algae reached 49 and 34%, respectively, within the whole dataset.

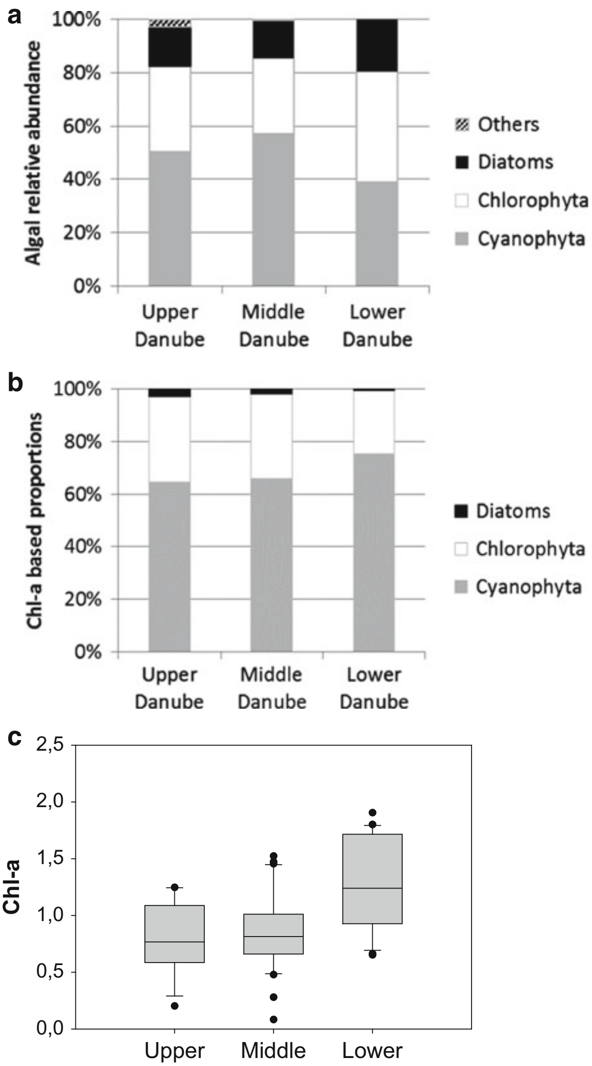

Fig. 2 Distribution of algal classes in the three different Danubian parts during the JDS2 (in 2007), upper Danube (2,600–1,852 riv. km), middle Danube (1,806–1,159 riv.km), lower Danube (1,151–0 riv.km): (**a**) based on their relative abundance (microscopic evaluation) and (**b**) chlorophyll-*a* measurements (fluorometric method) and (**c**) comparison of the total chlorophyll-*a* concentration in the respective parts

There was however considerable difference in algal proportions comparing the results of microscopic estimation of relative abundance and chlorophyll-*a* measurements (Fig. 2a, b). Microscopic evaluations have clearly led to higher diatom quantities, while according to chlorophyll-*a* measurements, diatoms reached considerably lower numbers and cyanobacteria higher. The overall match of the two evaluation methods reached 30%, while 70% of measurements were significantly different (Blond-Altman test reference). The bias was most evident in the lower Danube (Downstream Sava, 1,159 riv. km), where the average abundance of cyanobacteria was below 40% and the chlorophyll-*a* measurement indicated nearly 70% of proportion of cyanobacteria in the samples. The lower reach of the River Danube (Downstream Sava 1,159 riv. km) is typical by steep banks, where sampling is often impeded by the lack of hard substrata. Fine sediments usually cover the substrate's surface and might mask minute algae, which could be misestimated contrary to the sensitive fluorometric method. Nevertheless, both methods confirmed dominant position of cyanobacteria in the biofilm, which is in contrast with data previously published (e.g., [77, 91, 95, 100–102, 104, 105]). These studies basically quantified algae using the microscopic Utermöhl's technique [128] and mostly referred to artificial substrata (glass slides or tiles). The Utermöhl's technique is a standardized EU method for phytoplankton quantification, and we don't want to disparage its reliability in phytobenthos quantification. However, it must be noted that the nature and structure of benthic samples is different containing large and numerous filaments, and also a precise quantitative sampling is nearly impossible for benthic algae. Nevertheless, in the results interpretation, one must keep in mind that such microscopic quantification does not represent the real biomass of the algal groups, but only the numbers of individuals. Compared to biomass measurements (e.g., chlorophyll-*a*), these results might underestimate large or filamentous taxa, which also in JDS2 might have relatively underestimate cyanobacteria and overestimate diatoms during microscopic observations. Besides the possible bias related to the counting technique alone (see [129]), most of the studies mentioned above used artificial substrata and investigated different methodological aspects of phytobenthic surveys in the Danube, while the JDS2 dealt with communities on natural substrata. Nevertheless, the cyanobacteria prevailed over other algal groups according to both microscopic observations and biomass measurements in the JDS2.

The use of artificial substrates was often applied in the Danube to study different aspects of the colonization process and also due to the lack of adequate hard natural substrates that are sometimes not accessible in targeted areas. Studies focusing on colonization processes in the Danube refer that diatoms unlike other algal groups can quickly proliferate and colonize artificial substrates more rapidly and significantly dominate the communities on non-natural substrates [82, 83]. According to Schagerl and Donabaum [82, 83] (km 1,943.2–1,938.9), diatoms dominated on both artificial and natural substrates, but the natural substrates contained higher proportion of blue-greens. This was explained by short exposure time of artificial substrates and the dominance of diatoms in the phytoplankton that quickly inoculate the biofilm. On the other hand, the periphytic communities on artificial substrates in the Danube were proved to change significantly during the colonization process and showed temporal, seasonal, and spatial variations [101], although diatoms always dominated the community. The seasonal differences in algal communities were manifested by higher abundance of cyanophytes and chlorophytes and increased biomass and species diversity during the summer period [82, 83, 101], and also the periphyton abundance was reported to form more rapidly during summer colonization. The evident changes during summer periods were related to higher temperatures and low water levels. The discharge and current velocity, in particular, were proved to have the most immediate influence on the colonization process and algal biofilm structure in the Danube at Göd (1,699 riv.km.) [95, 103]. These factors form algal community by influencing the immigration rates, cause algae detachment (e.g., [130, 131]), and accelerate nutrient transportation within the biofilm [132]. In particular, low water levels and reduced discharge in the Danube were reported to increase algal biomass and abundance [95, 103].

In the light of the listed facts, the summer period and natural substrata are favorable for development of non-diatoms in the Danube, which eventually led to their dominance in the biofilm at most of the studied sites during the JDS2. Similarly to these results, Ács et al. [77] detected a dominant occurrence of a green algae *Protoderma viride* Kützing (Chlorococcales) in August 2001 at sites between Deggendorf (2,304 riv.km) and Göd (1,699 riv.km) reaching more than 70% of relative abundance. This phenomenon was explained by the ongoing flood events that reduced the algal biomass, while a firmly attached fast proliferating species such as *P. viride* throve.

With regard to the total biomass of the algal biofilm detected during the JDS2, values of chlorophyll-*a* concentration varied between 0.08 μg/cm^2 (Sio, 1,497 riv. km) and 1.90 μg/cm^2 (Irongate reservoir, 954 riv.km). The highest concentrations were in general detected in the lower Danube (Fig. 2c). The JDS2 identified significantly lower chlorophyll-*a* quantities than Schagerl and Donabaum [82, 83], whose average highest summer measurements on natural substrata approximated 40 μg/cm^2 and the minimum reached 4 μg/cm^2. As the algal biomass is greatly influenced by the shear stress, such significant difference might appear as a consequence of higher Danube discharges that occurred before the JDS2. The discharges at Regensburg during the 2 weeks prior to the survey almost reached a 1-year flood event [133]. The shear stressed involved might reduce algal biofilms in the upper Danube and cause lower biomass rates than detected by Schagerl and Donabaum [82, 83].

3.2 Species Diversity

While the quantity of *non-diatoms* was considerably higher at most of the sites investigated within both JDS expeditions, the species diversity showed to be significantly higher for diatoms. There were 341 algal species identified in the Danube during the first Danube expedition in 2001 [120], among them 264 diatom taxa. A total of 438 algal species were identified during the JDS2, among them 47 non-diatoms and 391 diatoms. These results are in general in large agreement with all studies dealing with benthic algae in the Danube so far (see references in the introduction). The most frequent groups among non-diatoms found during the JDS2 were Cyanobacteria (Cyanophyta), green algae (Chlorophyta), and red algae (Rhodophyta). Cyanobacteria were mainly represented by filamentous species *Heteroleibleinia fontana* (Hansgirg) Anagnostidis et Komarek, *H. kützingii* (Schmidle) Compère, *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; most common genera detected were *Chroococcus* sp., *Chamaesiphon* sp., and *Gloeocapsa* sp. Planktonic species as, e.g., *Pseudanabaena catenata* Lauterborn were also present. Species diversity of green algae was lower at individual sampling stations, in general, but they were more abundant in the shallow poles of the river (e.g., *Cladophora glomerata* (L.) Kützing, *Hydrodictyon reticulatum* (L.) Lagerheim, *Spirogyra* sp., *Stigeoclonium tenue* (Aghard) Kützing). *Cladophora glomerata* was often observed to accompany water macrophytes. Contrary to Ács et al. [77], the reported dominant green alga *Protoderma viride* was detected. However, *Protoderma* species were shown to highly resemble *Stigeoclonium tenue* (Aghard) Kützing in cultures [134], which was found rather frequently during the JDS2.

Among red algae, *Hildenbrandia rivularis* (Liebmann) Aghard was found upstream dam Abwinden-Asten (2,120 riv.km) and later upstream dam Greifenstein (1,950 riv.km) together with *Bangia atropurpurea* (Roth) Aghard similarly to the results of JDS1.

Concerning the *diatom species* diversity, numerous diatom taxalists were published in the literature with vast species numbers but usually lacking any abundance data. The species diversity is therefore difficult to compare as the diatom assemblages usually contain significant proportion of rare species with only few predominant taxa. Makovinská et al. [121] refer to significantly high similarity of samples at sites comprised in the JDS2. However, benthic diatom assemblages from the upper Danube and the beginning of the middle Danube (Upstream Iller, Germany 2,600 riv. km – Bratislava, Slovakia 1,869 riv. km) were distinctly separated from diatom downstream Bratislava 1,869 riv. km. In general, the species composition at sampling sites was changing gradually, depending on confluence of tributaries (apart of others abiotic descriptors). Within the total of 391 diatom species detected during the JDS2, 75 taxa were found with frequency higher than 20%, and only 13 diatom taxa showed frequency of more than 50%. With regard to the relative abundance, only 21 taxa reached average relative abundance higher than 1% (Table 1) indicating homogenous species composition with low variability among the dataset. Among them, *Navicula recens* (Lange-Bertalot) Lange-Bertalot and *Navicula tripunctata* (O.F. Müller) Bory were the most abundant and most frequent and occurred at 83 and 74% of sites, respectively (Table 1). Generally, species from the genera *Amphora*, *Cocconeis*, *Eolimna*, *Gyrosigma*, *Luticola*,

Table 1 List of most abundant and frequent diatom species observed during the JDS2 based on results of Makovinská et al. [121]

The most frequent and abundant taxa (>50% sites)	Average relative abundance (%)	Frequency (%)
Navicula recens (Lange-Bertalot) Lange-Bertalot	22.90	83.73
Navicula tripunctata (O.F. Müller) Bory	7.53	74.10
Cocconeis placentula Ehrenberg var. *lineata* (Ehrenberg) Van Heurck	4.34	68.07
Cyclotella meneghiniana Kützing	4.28	42.17
Amphora pediculus (Kützing) Grunow	3.55	68.07
Nitzschia inconspicua Grunow	3.53	38.55
Navicula viridula (Kützing) Ehr. var. *rostellata* (Kützing) Cleve	3.41	66.27
Navicula cryptotenella Lange-Bertalot	2.96	71.08
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	2.81	62.65
Amphora copulata (Kützing) Schoeman and Archibald	1.95	61.45
Navicula capitatoradiata Germain	1.93	57.23
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann	1.79	24.70
Diatoma vulgaris Bory	1.63	36.75
Navicula erifuga Lange-Bertalot	1.61	53.01
Eolimna minima (Grunow) Lange-Bertalot	1.35	43.98
Navicula antonii Lange-Bertalot	1.28	51.20
Nitzschia palea (Kützing) W. Smith	1.15	42.17
Reimeria uniseriata Sala, Guerrero and Ferrario	1.14	39.76
Cocconeis pediculus Ehrenberg	1.08	37.35
Nitzschia amphibia Grunow f. amphibia	1.02	34.34
Gyrosigma nodiferum (Grunow) Reimer	1.02	40.96
Navicula germainii Wallace	0.99	50.00
Ulnaria ulna (Nitzsch.) Compère	0.59	54.82
Taxa abundant only at one or few sites (max 10 samples, max relative abundance >10%)	**Max relative abundance (%)**	**Frequency (%)/ number of samples**
Navicula kotschyi Grunow	43.22	0.038/6
Fragilaria capucina Desmazières sensu lato	27.51	0.035/6
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	15.12	0.044/7
Bacillaria paxillifera (O.F. Müller) Hendey var. *paxillifer*	13.14	0.056/9
Meridion circulare (Greville) C.A. Agardh var. *circulare*	10.53	0.025/4
Navicula schroeteri Meister var. *schroeteri*	10.03	0.019/3

Navicula, *Nitzschia*, *Rhoicosphenia*, and *Reimeria* were among the most abundant and common at the sites studied. Regarding the frequency of the taxa, 200 diatom taxa appeared at more than 1 sampling location, 75 taxa were found with frequency higher than 20%, and only 13 diatom taxa showed frequency of more than 50%. There were several taxa with unknown species identity, so far identified to the genera level that reached the relative abundance higher than 5%. With regard to autecological preferences of the most frequent and dominant species, the sites were mostly dominated by eutrophic to hypertrophic species, e.g., *Amphora pediculus* (Kützing) Grunow, *Navicula tripunctata* (O.F. Müller) Bory, *Navicula viridula* (Kützing) Ehrenberg var. *rostellata* (Kützing) Cleve, *Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann [135], *Navicula recens* (Lange-Bertalot), *Navicula erifuga* Lange-Bertalot, *Nitzschia inconspicua* Grunow, *Nitzschia clausii* Hantzsch, and *Nitzschia palea* (Kützing) W. Smith referring to beta-mesosaprobic to polysaprobic conditions. Most of the taxa were alcaliphilous.

Compilation of literature data showed that the final taxalists greatly depend on the successional stage of algal biofilms. The composition of diatom assemblages in the Danube showed diverse successional models with significant shifts of species depending on the length of colonization and disturbance. Patterns of the periphyton diversity in the Danube have shown certain periodical features in the formation of algal coating [102] caused by assemblages collapse. Sudden decrease in algal density appears regularly due to large flood waves causing deterioration in living conditions (shear stress, lower transparency, mechanical abrasion) leading to recolonization of substrates and thus diverse species composition and abundance. As, for example, benthic algal composition at Göd (1,669 riv.km), especially in terms of diatoms, has been intensively studied since 1984 [95] and exhaustively documented (see [95, 101–104]). Significant differences were manifested for both the relative abundance and species composition depending on the phase of colonization and season. Different diatom strategies were manifested by Ács and Kiss [101] during the years 1985 and 1986, who found *Gomphonema olivaceum* (Hornemann) Bréb., *G. angustatum* (Kütz.) Rabenh., and *Achnanthidium minutissimum* (Kütz.) Czarnecki as pioneer species dominating at the beginning of the colonization, while *A. minutissimum* prevailed during the summer period and all disappeared during further biofilm development. On the other hand, *Reimeria sinuata* (Gregory) Kociolek and Stoermer, *Cocconeis placentula* Ehrenb., and *Amphora pediculus* (Kütz.) Grunow remained dominant in the biofilms. On the contrary, studies performed in 1984 [95, 102] and in 1992 [103] reported *Gomphonema angustatum* and *G. olivaceum* as intermediate or late colonists, and *Achnanthidium minutissimum* did not appear among the dominant taxa at all in 1984. Later Ács et al. [77] and Ács et al. [127] found again that *A. minutissimum* was the most abundant diatom species in August 2001 among the entire stretch studied from Germany to Hungary (1,887–2,622 riv.km). During the JDS2, *A. minutissimum* appeared only in four samples (out of 166) with relative abundance above 1% and in contrast to the previous study of Ács et al. [77] was among the rare species.

Except for pennate diatoms, several workers have detected a relatively high proportion of centric diatoms (Centrales) in Danubian biofilms [77, 104, 105], which typically dominate in the Danube phytoplankton [136]. In general, the periphyton in large rivers appears to be an important refuge for planktonic species, and many can survive and even proliferate in the periphyton and inoculate the plankton [77]. However, their abundance largely depends on the discharge regime (see [77]). Ács et al. [77] reported several abundant centric species in the river stretch from 2,622 to 1,887 riv. km such as *Cyclotella cyclopuncta* Håk et Carter, *C. comta* Kütz., *Stephanodiscus invisitatus* Hohn et Hellerman, *S. neoastrea* Håk et Hickel, *Thalassiosira guilardii* Hasle, *T. pseudonana* Hasle et Heimdal, and *T. weissflogii* (Grunow) G.A. Fryxell. Compared to the results of JDS2, centric diatoms were rare and reached a relative abundance of more than 5% only in 17 out of 166 samples. Among the species detected, *Cyclotella meneghiniana* Kütz. was the most frequent and abundant taxa, other centrics were rare with low abundances. Interestingly, two sites at Velika Morava (1,103–1,097 riv. km) contained nearly a monoculture of *C. meneghiniana* that reached respectively 92 and 85% of relative abundance in samples.

In summary, the comparisons indicated that in addition to essential environmental parameters that determine the composition of algal biofilms, the species composition during different research studies greatly depends on the stability of aquatic conditions and the successional stage of the biofilm.

3.3 Diatom-Based Assessment of Ecological Status

The first JDS held in 2001 used the algal taxalists for saprobic evaluation of the water quality using the saprobic index (SI) of Zelinka and Marvan [137]. The values of the saprobic index ranged in the Danube from 1.77 to 2.11. This phytobenthic results would characterize a beta-mesosaprobic conditions for all JDS samplings sites. The Danube Delta (Reni Chilia arm, Vilkova Chilia arm) had the highest saprobic values within the Danube stations. There was only very slight increase of the SI values in the Danube section of river km 1,800–1,100 and downstream of river km 641. However in the longitudinal profile of the Danube, the differences within the saprobic indices were low, thus generally not significant, indicating that the saprobic index for the phytobenthos community is evidently less sensitive and responsive compared to the saprobic evaluation based on macrozoobenthos. Results of the JDS2 were applied to calculate different diatom indices using Omnidia 4.2 [125, 138], which were further tested for correlations with nutrients and conductivity to choose the best performing index. Among all, the polluo-sensitivity index ("Indice de Polluosensibilité Specifique" – IPS) of Coste (in CEMAGREF [27]) showed to perform the best in terms of reflecting the "general degradation and pollution." Moreover, it is widely and successfully being applied in European waters [22, 28, 33, 34, 38, 39, 43, 77, 139, 140] and was successfully used in the common intercalibration exercise of ecological status assessment of European rivers [21]. The IPS index was developed by Coste (in CEMAGREF [27]) and it is based on the weighted average equation of Zelinka and Marvan [137]. In general, the index was established to reflect a general pollution gradient extending from unpolluted to heavily polluted rivers of different types in France and was based on a large diatom database of French water quality monitoring. The IPS itself was adapted and adopted by the Agence de l'Eau Artois-Picardie in northern France as part of their routine environmental assessments [140]. The great advantage and popularity of the index lies in the great number of taxa involved in the calculation and the regular updates of the database on the level of diatom taxa and related ecological values. This makes the IPS the most up-to-date diatom index available. All these arguments led to the selection of the IPS for preliminary status indication of the Danube based on the results of JDS2 as shown at Figs. 3 and 4. Values of IPS

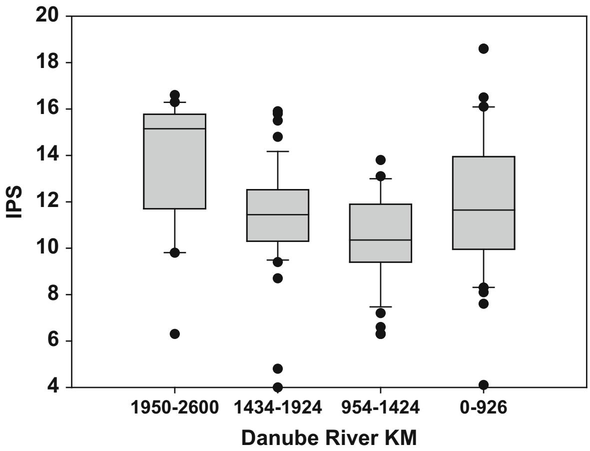

Fig. 3 Comparison of IPS values in the longitudinal profile of the Danube River during the JDS2 in 2007. Different *box plots* refer to different river sections identified by the respective river kilometers. *Box 1*: Upstream Iller – Upstream dam Greifenstein ($N = 20$). *Box 2*: Klosterneuburg – Batina ($N = 48$). *Box 3*: Upstream Drava – Starapalanka-Ram ($N = 52$). *Box 4*: Banatska Palanka/ Bazias – Sulina – Sulina arm ($N = 40$)

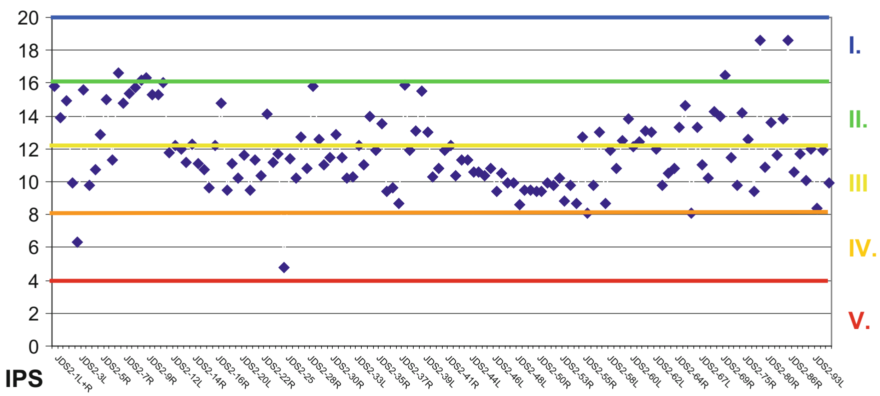

Fig. 4 Evaluation based on IPS index values. Results from the JDS2 in 2007

index seem to decrease downstream indicating the longitudinal increase of pollution and ranged between 6.3 and 18.6. The lowest IPS value (6.3) was calculated for the Danube at Geisling power plant (2,354 riv.km) mainly due to a dominance of *Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann that reached 52 and 91% of the relative abundance in the samples from the right and left side of the river bank, respectively. Comparisons of the IPS values in different parts of the longitudinal profile showed that there are four groups of sites distinctly separated depending on the level of pollution (Fig. 3), showing best quality at sites from Upstream Iller (Germany, 2,600 riv. km) to Greifenstein (Austria, 1,950 riv.km) (Group 1), showing change of water quality in the manner of higher level of pollution from Klosterneuburg (Austria, 1,942 riv.km) to Batina (Croatia, 1,424 riv.km) (Group 2), showing worst level of pollution at sites from Upstream Drava (Croatia, 1,384 riv.km) to Starapalanka – Ram (Serbia, 1,077 riv.km) (Group 3), and showing large variability of index values at sites downstream Banatska Palanka/Bazias (1,071 riv.km) probably due to multiple factors that besides pollution form the structure of benthic diatom communities and thus significantly increase the uncertainty of diatom-based assessment (Group 4). Basically, most of the upper Danube sites fall into good status, and starting with the Hungarian stretch, the status becomes moderate (middle and lower section) (Fig. 4), which is in agreement with similar evaluation of Ács et al. [77]. However, as Ács et al. [127] showed, also the diatom-based assessment might be subjected to significant seasonal changes. In their study the IPS values varied between 8 and 16 in the Danube at Göd (1,669 riv. km) from May to December 2003 reaching the lowest value in August 2003. These results indicate that a preciseness of a diatom-based assessment in such river type might be very sensitive to seasonal changes. Assessment tools combining diatoms with other algal groups might probably help to buffer against the distinct seasonal variability observed in diatom assemblages. Therefore despite the advantages of such purely diatom-based assessment, it is obvious that diatoms alone do not ensure a comprehensive indication of the whole range of processes that govern the status and diversity of all members of the periphytic community (see Yallop and Kelly [17]). Yet results of Kelly [20] indicated that diatoms should be adequate in situations where nutrients and organic pollution are the most important stressors, which is the case of the Danube River.

4 Conclusions

Large rivers are unique systems, heavily influenced by pressures and specific hydraulic regimes involving great sampling challenges and methodological limitations in studying of aquatic communities. Surveys of benthic algae are particularly influenced by the methodological obstacles as the sampling is restricted to the riverbanks with favorable light conditions. Despite of these limitations, phytobenthos shows to be an abundant and divers element of aquatic ecosystems of large rivers. Moreover, the applicability of phytobenthos, mostly represented by diatoms, in assessment of ecological status proves that it is also an important indicator of environmental conditions and degradation. The successful application of diatoms in assessment of the Danube confirms that they can significantly contribute to overall assessment surveys, especially regarding the WFD. However, the often exclusive use of diatoms reduce the "biological answer" of the whole algal biofilm to expression of a scale of chemical gradients (mostly nutrients) indicated by diatom species. Algal biofilms in large rivers are however influenced by a large scale of interacting factors, other than chemical, that play an important role in shaping the community structure and also relate to the level of degradation of aquatic environment. Only an evaluation of the whole scale of environmental factors present can provide the WFD required assessment of "ecosystem functioning." Further research should be therefore devoted to development of tools evaluating not only the values of indices developed on the base of the relationships between algae and water chemistry but also involving the relationships with other biological communities and hydrological aspects. There is also a high need of effective methods comprising non-diatoms in the final assessment. Nevertheless, the purely diatom-based assessment has shown to be sufficiently reliable and precise so far, but in situations where diatoms do not dominate in the biofilm, other algae could greatly contribute to the final assessment.

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Macrophytes in the Danube River

Georg A. Janauer, Brigitte Schmidt, and Udo Schmidt-Mumm

Abstract Recording and assessment of aquatic macrophytes was a request for the Joint Danube Survey 2 (JDS2). New insight regarding occurrence, abundance and specific distribution of macrophytes was based on methodological adaptations better adjusted to the size of this large European river and permitted more appropriate statistical interpretation. Regarding the ecological status of sampling stretches, an intentional, preliminary way of interpretation is provided, respecting trendsetting new international literature. Due to longer river stretches recorded, a higher number of species was detected in JDS2. Each of the ten official river sections showed an individual character of the macrophyte vegetation. Results of JDS2 macrophyte survey are put in relation with international literature and side effects are discussed, which are of relevance when assessing macrophytes in large rivers for purposes of science, European Water Framework Directive or regarding conservation issues.

Keywords Danube River, Ecological status, European Water Framework Directive, Large rivers, Macrophytes

Contents

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1 Introduction

A somewhat casual but rather accurate explanation of the term ‘aquatic macrophytes’ was given by Westlake [1], who described them as the aquatic plants that can often be determined to species level with the unaided eye. Most scientists rank the following plant groups as macrophytes: macro algae, aquatic bryophytes and, among the vascular plants, water ferns and angiosperms. The European Water Framework Directive (WFD) introduced ‘macrophytes’ as one of the four biological quality elements to be applied in assessing the ecological status of surface waters. Therefore, macrophytes were part of the analytical programme for examining the ecological quality of the Danube River under the lead of ICPDR during the JDS2 survey, and Birk et al. [2] provided substantial reason for following this requirement. Based on experience gained during the first Joint Danube Survey in 2001, ICPDR adapted resources, sampling sites, methodology and organisation for the second macrophyte survey (JDS2, August 12–September 28, 2007), which provided deeper insight on the aquatic macrophyte vegetation of the Danube River. JDS2 defined the methodological adaptation of macrophyte survey for large rivers like the Danube. Regarding reference, conditions where near-natural examples are absent were first discussed during the preparation of JDS2 (Birk and Janauer, personal communication, 2008, Senec meeting) and – based on extensive statistical work – were recently published by Birk et al. [2].

Aquatic macrophytes are not only biological quality elements for assessing the ecological quality of water bodies, they also play an important role in the aquatic ecosystem: macrophytes add to total biodiversity as such, gain importance within the scope of the EU Habitats Directive, add spatial structure to the water body and provide niches and habitats for countless other aquatic organisms [3, 4]. Water chemistry is influenced especially with respect to the oxygen regime during photosynthetic periods and by the uptake of plant nutrients, keeping proliferous algae at bay.

The abiotic conditions of a large river like the main channel of the Danube restrict macrophyte growth to areas of decreased flow velocities and to water depth usually less than 1.5–2 m. But in side channels and floodplain water systems, macrophytes can become the dominant plant group [5].

This contribution puts the most important results of the macrophyte survey of Joint Danube Survey 2 in perspective and highlights associated relevant findings from the Danube catchment.

2 Methods

2.1 Macrophyte Survey

The aquatic macrophyte vegetation was assessed in the Danube main channel and in some mouth sections of important tributaries at all sampling sites of JDS2. Helophytes (reed and some bank species) were considered of importance when growing on the midwater line directly at the banks. Individual survey units (SU) were of 1 km length, and 3 river kilometres (rkm) were recorded on each side of the main river channel by boat, resulting in a total recorded length of 6 rkm at each sampling site. Abundance assessment followed the European Standard EN14184, recording all macrophyte species present in each survey unit and their abundance [6]. In the Danube countries, this approach is the most widely used for national macrophyte assessment in the context of the WFD. It features five estimator levels which are phrased – by literal translation of the German original [7] – 'very rare, not more than five individuals' (1), 'rare' (2), 'frequent' (3), 'abundant' (4) and 'very abundant or mass development' (5).

The estimator scale is of exponential character, which was posted first by Melzer et al. [8] and was numerically proved for running waters by Janauer and Heindl [9]. It integrates the vertical development of the plant stands, which is determined by environmental characteristics. Usually, several survey units are combined in a contiguous group to provide a more representative data set of species occurrence and abundance. This is in accordance with the related European Standard EN 14184 mentioned above, where 'stretches of defined river lengths' and 'adapted to the scale and purpose of the study' are recommended. Field workers with even little experience are able to assess the plant abundance estimates correctly and reproducibly after a very short learning period [6, 7], and this method was validated in 2008 with a group of 35 employees of Apele Romane (Romanian National Water Agency) during a quality assurance test (Janauer, personal communication).

The relative abundance of individual species relates to the total abundance of all species recorded in a river reach and is weighted by the length of the individual survey units (1 km in JDS2). Regarding this metric, see Pall and Janauer [10].

2.2 Multivariate Data Analysis

Multi Response Permutation Procedure (MRPP) was used to test the null hypothesis of no significant differences in the floristic and quantitative composition of survey units among the ten section types of JDS2. MRPP is the non-parametric analogue of discriminant function analysis but without many of the associated assumptions. Bray-Curtis distance measures and a natural weighting (n/sum (n)) was used in the MRPP [11].

MRPP provides the test statistic, a measure of 'effect size' (A-values) and a p-value. Differences among section types were described by indicator species analysis (ISA, [12]). A Monte Carlo simulation test with 1,000 randomised runs, assigning survey units randomly to types, was used to determine the significance ($P \leq 0.05$) of the indicator values [11]. MRPP and ISA were conducted with PC-ORD version 5.1 [13].

2.3 Assessment of the Ecological Status

The procedure for providing a provisional ecological status assessment complied with the Austrian Directive for Running Waters – Macrophytes (ADR-M 2007 [14]). The following calculation method was used (Table 1):

Table 1 Calculation for assessing ecological status 'macrophytes'

Species	Abu	Class				# Classes
		1	2	3	4	
Species 1	PM_1	PM_1				1
Species 2	PM_2	$PM_2 / 3^2$	$PM_2 / 3^2$	$PM_2 / 3^2$		3
Species 3	PM_3	PM_3				1
Species 4	PM_4	$PM_4 / 2^2$	$PM_4 / 2^2$			2
Species 5	PM_5	PM_5 x 0	PM_5 x 0	PM_5 x 0	PM_5 x 0	4
...						
Sum PM*G		Sum_1	Sum_2	Sum_3	Sum_4	Cross sum A
Sum PM*G*CL		Sum_1*1	Sum_2*2	Sum_3*3	Sum_4*4	Cross sum B
Index Value						Cross sum A/ Cross sum B
Ecological Status Class						Index rounded to integer

Abu: abundance; # Classes: number of marks in different classes. PM1: abundance estimate of species 1 (Abundance estimates according to Kohler et al. [7]). Each PM is divided by G, the square of the number of classes in which a species occurs. This puts more weight on species with a narrow ecological amplitude. Species occurring in all classes are excluded as 'ubiquistic' species, which are supposed to have no specific indicative value. Therefore, their PM is multiplied by zero. Then the sum is calculated for each class, and the values are summed to produce cross sum A. In the next step, the PMxG sum is multiplied by the class identification, which then produces cross sum B. Cross sum A over cross sum B produces the final index value, which is rounded to integer.

Reference conditions were adapted to the conditions of the Danube River in the different section type reaches. For the Danube River, neither historical quantitative data nor modelling approaches are available to produce a priori macrophyte reference conditions. Therefore, with respect to differentiating 'good' from 'moderate' status in analogy to Birk et al. [2], various sources had to be used, including historical maps of the river course, results from side channels, saprobity maps of the Danube of various dates, JDS1 and JDS2 data on chemical components and macrophyte data from the whole-river macrophyte survey of the MIDCC project [15] to create reference conditions by expert judgement, including information on the ecological characterisation of macrophyte species.

3 Results

3.1 General Characteristics of the Danube Macrophyte Survey

During the JDS2 macrophyte survey, 96 sites were sampled, 3 rkm on each side (Table 2). The accumulated length was 556.5 km (c. 21% of the navigable part of the Danube River) and covered aquatic macrophytes as well as bank-side 'helophytes' (e.g. common reed). Results show that this spatial expansion was a minimum requirement for collecting sufficient data for a survey of rivers the size of the Danube and large tributaries.

Sixty-nine aquatic species, three macro algae and 60 helophyte species were detected in 485 survey units (87% of all sampled rkm). When compared with JDS1, the number of aquatic species increased by 57%. This is due to – at least in part – the extension of sampled river length. Among the species found, some are rarely recorded on the main channels of large rivers, e.g. *Wolffia arrhiza* (L.) Horkel ex Wimm., *Lemna turionifera* Landolt, *Riccia fluitans* L. emend Lorb., *Azolla filiculoides* Lam., *Utricularia vulgaris* L., *Trapa natans* L. and *Stratiotes aloides* L.

Aside from river regulation and bank protection installations, a series of power stations in Germany and Austria, the Gabcikovo hydroelectric plant in Slovakia and the two impoundments of the Iron Gates affect the habitats of macrophytes, deviating conditions of flow velocity, sediment type, water temperature and turbidity.

The greater number of aquatic species recorded in JDS2 (Table 3) may be caused in part by different bryophyte species agglomerated within individual patches and cushions, as well as on the strategy of surveying both sides of the river for 3 km each, regarding the non-bryophyte species, or on natural long-term variation.

Table 2 Representative information of the JDS2 macrophyte survey

Survey	Geomorphologic sections	Sampling sites	rkm per site	Accumulated length (rkm)	Aquatic species
JDS1	9	98	2	313	44
JDS2	10	96	6	556	69

Table 3 Comparison of macrophyte species richness: JDS1 and JDS2 result

Genus name	Species name	Author	present at JDS1	present at JDS2
Alisma	*gramineum*	LEJEUNE	x	
Alisma	*lanceolatum*	With.		x
Alisma	*plantago-aquatica*	L.		x
Amblystegium	*varium*	(Hedw.) Lindb.	x	
Azolla	*filiculoides*	Lam.		x
Brachythecium	*rivulare*	Schimp.	x	
Bryum	*capillare*	Hedw.		x
Bryum	*klinggraeffii*	Schimp.		x
Bryum	*pallescens*	Schleich. ex Schwägr.	x	
Butomus	*umbellatus*	L.		x
Callitriche	*brutia*	Petagna		x
Callitriche	*sp.*	-		x
Ceratophyllum	*demersum*	L.	x	x
Cinclidotus	*fontinaloides*	(Hedw.) P. Beauv.		x
Cinclidotus	*riparius*	(Host ex Brid.) Arnott	x	x
Cratoneuron	*filicinum*	(Hedw.) Spruce	x	x
Didymodon	*tophaceus*	(Brid.) Lisa	x	
Drepanocladus	*aduncus*	(Hedw.) Warnst.		x
Drepanocladus	*fluitans*	(Hedw.) Warnst.		x
Eichhornia	*crassipes*	(Mart.) Solms		x
Elodea	*canadensis*	Michx.	x	x
Elodea	*nuttallii*	(Planch.) H.St.John	x	x
Enteromorpha	*intestinalis*	(L.) Link		x
Eurhynchium	*crassinervium*	(Taylor) Schimp.	x	
Fissidens	*rufulus*	B.S.G.	x	x
Fontinalis	*antipyretica*	Hedw.	x	x
Funaria	*hygrometrica*	Hedw.		x
Homalothecium	*nitens*	(Hedw.) Robins		x
Hydrocharis	*morsus-ranae*	L.	x	x
Hydrodictyon	*reticulatum*	(L.)		x
Hygroamblystegium	*fluviatile*	(Hedw.) Loeske		x

(continued)

Table 3 (continued)

Hygroamblystegium	*tenax*	(Hedw.) Jenn.
Hygrohypnum	*eugyrium*	(Schimp.) Broth.
Hygrohypnum	*luridum*	(Hedw.) Jenn.
Lemna	*gibba*	L.
Lemna	*minor*	L.
Lemna	*trisulca*	L.
Lemna	*turionifera*	Landolt
Leptodictyum	*riparium*	(Hedw.) Warnst.
Leskea	*polycarpa*	Hedw.
Limosella	*aquatica*	L.
Myriophyllum	*spicatum*	L.
Myriophyllum	*verticillatum*	L.
Najas	*marina*	L.
Najas	*minor*	All.
Nitellopsis	*obtusa*	(Desv. in Loisel.) J.Groves
Nuphar	*lutea*	(L.) Sibth. & Sm.
Nymphea	*alba*	L.
Polygonum	*amphibium*	L.
Potamogeton	*acutifolius*	Link
Potamogeton	*alpinus*	BALB.
Potamogeton	*crispus*	L.
Potamogeton	*friesii*	Rupr.
Potamogeton	*gramineus*	L.
Potamogeton	*lucens*	L.
Potamogeton	*natans*	L.
Potamogeton	*nodosus*	Poir.
Potamogeton	*pectinatus*	L.
Potamogeton	*perfoliatus*	L.
Potamogeton	*praelongus*	WULF.
Potamogeton	*pusillus*	L.
Potamogeton	*trichoides*	Cham. & Schltdl.

(continued)

Table 3 (continued)

Potamogeton	*x zizii*	Koch ex Roth
Ranunculus	*fluitans*	Lam.
Rhynchostegium	*confertum*	(Dicks.) B.S.G.
Rhynchostegium	*riparioides*	(Hedw.) Cardot
Riccia	*fluitans*	L. emend Lorb.
Sagittaria	*sagittifolia*	L.
Salvinia	*natans*	(L.) All.
Schistidium	*apocarpum*	(Hedw.) B.S.G.em. Poelt
Schistidium	*rivulare*	(Brid.) Podp.
Sparganium	*emersum*	Rehmann
Sparganium	*erectum*	L.
Spirodela	*polyrhiza*	(L.) Schleid.
Stratiotes	*aloides*	L.
Trapa	*natans*	L.
Utricularia	*vulgaris*	L.
Vallisneria	*spiralis*	L.
Veronica	*beccabunga*	L.
Wolffia	*arrhiza*	(L.) Horkel ex Wimm.
Zannichellia	*palustris*	L.

3.2 Species Richness and Floristic Composition

Species richness and relative abundance (RPM) of dominant species recorded during JDS2 in each river section are presented in Fig. 1.

The most conspicuous distribution of species groups along the Danube relates to bryophytes and rheophile *Ranunculus* species, which are important elements of the aquatic vegetation only in the Upper Danube, and the progress of *Ceratophyllum* and *Myriophyllum* species in the lower reach of the river. Especially in the middle reach, *Potamogeton* sp. and the duckweeds *Lemna* and *Spirodela* were recorded in higher abundance.

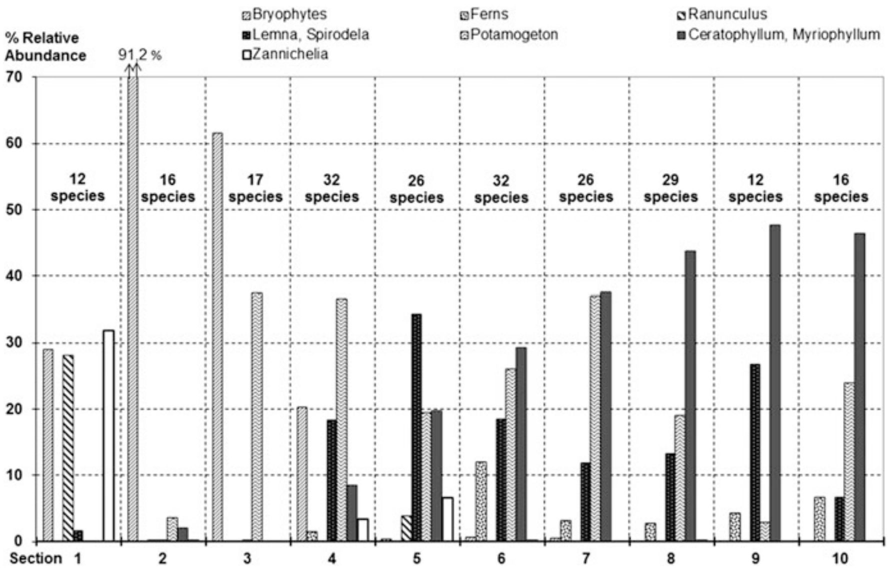

Fig. 1 Species richness and relative abundance of dominant species determined for each Danube River section (according to JDS2 classification). Columns from *left* to *right*: bryophytes, ferns, *Ranunculus* sp., *Lemna* spp. and *Spirodela* sp., *Potamogeton* sp., *Ceratophyllum* and *Myriophyllum* spp., *Zannichellia* sp.

3.3 Statistical Comparison of JDS2 River Sections

MRPP and ISA were used to describe significant differences of the macrophyte species groups characteristic for the river sections of the Danube. Section limits were determined using macro-invertebrate and geomorphology features of the river course (see [16]).

MRPP results show that the different sections of the Danube River are habitats of significantly different macrophyte species compositions, underlining the ecological richness of this second largest river of Europe but also raising attention regarding the definition of reference conditions for ecological status assessment (Table 4).

Regarding ISA, the results revealed some special features. Except for Section 4, all other sections had at least one specific indicator species (Table 5) though Sections 9 and 10 – see below – have to be regarded as special, too. Section 4 is located between Greifenstein (AT) and the mouth of the Mosoni Duna (HU): the ISA seems to indicate that this reach is a kind of an ‘ecotone’, a boundary reach, between two possibly different sections. A stepwise analysis of the macrophyte species revealed that the point of separation of these new sections could be close to the inflow of the Morava River near Bratislava. In Sections 9 and 10, helophyte species were the indicator species. While reeds like *Typha* sp. and *Phragmites* sp. are regarded as natural near to the Delta, *Xanthium strumarium* L. indicates a disturbed riparian zone. In all other sections, indicator species were among the aquatic macrophytes.

Table 4 Multi-response permutation procedure (MRPP) for Danube River sections (Sec.)

SEC.	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**	-

Bold print indicates statistical significance, which was the case for each of the compared pairs of river stretches.

Table 5 Indicator species per river section

Section 1 *Ranunculus fluitans* Lam. *Zannichellia palustris* L.	Section 6 *Spirodela polyrhiza* (L.) Schleid. *Salvinia natans* (L.) All.
Section 2 *Cinclidotus riparius* (Host ex Brid.) Arnott *Phalaris arundinacea* L.	Section 7 *Potamogeton perfoliatus* L. *Potamogeton nodosus* Poir. *Ceratophyllum demersum* L.
Section 3 *Fontinalis antipyretica* Hedw. *Lycopus europaeus* L.	Section 8 *Potamogeton pusillus* L.
Section 4 –	Section 9 *Xanthium strumarium* L.
Section 5 *Lemna gibba* L.	Section 10 *Phragmites australis* (Cav.) Trin. ex Steud *Typha latifolia* L.

4 Discussion

4.1 Species Distribution and Richness

Along the more than 2,600 km of river course covered by JDS2 (c. 91% of the total length of the Danube, from mouth to source), ten official river sections revealed significantly diverse macrophyte species assemblages.

An important feature of the aquatic vegetation is the concentration of bryophytes in the upper river reach, where hard substrates are lining the banks of the German and Austrian Danube (Sections 1 to 4, Fig. 1). Regulated river reaches and hydroelectric power plant impoundments, both mainly lined by rip-rap, provided extremely favourable conditions for bryophyte colonisation. Gravel and sand deposits within groyne fields are only sparsely colonised or avoided by higher aquatic plant species. In the middle and lower reaches of the Danube, essentially no bryophytes were detected on the sediments of the main river channel.

Zannichellia palustris was found in the first five sections but was dominant only in the first. This species is often classified as indicating eutrophic conditions, but in contrast to this opinion, it is widespread in mesotrophic rivers, growing in closest distance to species like *Ranunculus trichophyllus* or *Groenlandia densa*, even intermingled with these species in the same plant stand [17, 18].

Regarding species richness, Fig. 1 indicates river Sections 4 and 6 as showing the most abundant composition of macrophytes. Section 4 was located between Krems (AT) and Gönyü (HU). It included the most eastern part of the alpine reach of the Danube, covering also three hydropower installations, as well as the macrophyte-rich Čunovo reservoir (SK). Based on macro-invertebrate data, these two very different water bodies were merged for JDS2 purposes [19]. But Birk et al. [2] clearly divided JDS-Section 4, merging its upper part with the traditional upper reach and its lower part with the Middle Danube reach, as already discussed in the final JDS2 report [20].

A prime hydromorphological feature of the Upper Danube is the great number of hydropower installations. The Bavarian impoundments were more species rich (average species number, 1.70 per km; mean length, 8.22 km; adapted from Pall and Janauer [10]) than the much longer reservoirs in Austria (average species number per km, 0.33; mean length, 27.61 km; adapted from Janauer and Jolankai [4]). This may be related to the greater hydrological monotony of the longer reservoirs. The situation in the Gabcikovo impoundment (Middle Danube reach, SK) is quite different due to the wide, slow flowing and silted Čunovo part (maximum width: 3.04 km), providing favourable conditions for macrophyte development. The middle reach ends at the Iron Gate I reservoir, which is the longest in the Danube River (145 km). Its hydrophyte species number per km (Romanian riverside) was only 0.17 (Sarbu, survey 2000–2003, in [21]).

Groundwater upwelling possibly causing fast water flow in the outlet of the Rackeve-Soroksar side channel probably supported the occurrence of *Ranunculus fluitans* in Section 5.

Stronger aquatic plant development was also found in the reach between Novi Sad and Belgrade, where the head section of the Iron Gate reservoir is located: two aquatic ferns, *Salvinia natans* and *Azolla filiculoides* were detected in noticeable abundance. In this reach, the large tributaries Drava, Tisza and Sava merge with the Danube; their extensive floodplain waters could serve as the source of these free-floating ferns.

Section 7, the Iron Gate, holds a special position between the middle and lower Danube. In the three narrow gorge stretches, rock-lined banks exist, whereas in the wider parts of the Iron Gate reservoirs, calm waters and finer substrates prevail. Due to these heterogeneous conditions, natural moss stands were found on the rock face in the gorges and a moderate diversity of other macrophytes occurred in the wider valley parts.

Lemnaceae (*L. minor*, *L. gibba*, *Spirodela polyrhiza*) were found in the whole Danube in 2007. Surprisingly, high abundances of these free-floating species were recorded in survey units with flow faster than would be expected (e.g. Section 5), while their occurrence was sparse in rather slow flowing water (e.g. upper Iron Gate reservoir, Danube Delta).

Enhanced macrophyte growth possibly triggered by nutrient enrichment was recorded several kilometres down-river of the mouth sections of the rivers Timok and Olt and down-river of the cities Ruse (BG), Oltenita (RO) and Tutrakan (BG), resulting in higher species numbers and abundance (all in Section 8).

Pondweeds like *Potamogeton crispus*, *P. friesii*, *P. gramineus*, *P. lucens*, *P. natans*, *P. nodosus*, *P. pectinatus*, *P. perfoliatus*, *P. pusillus* and *P. trichoides* were rather evenly distributed across the middle and lower reaches (Sections 3 to 10). This is mainly due to the wide ecological amplitude of these species. Particularly *P. pectinatus* (synonym: *Stuckenia pectinata* (L.) Börner) is tolerant to a wide range of habitat properties, e.g. nutrient load, flow velocity or shading by 'aufwuchs'.

Ceratophyllum demersum and *Myriophyllum spicatum* occurred almost everywhere in the Danube. They influence the dominance relationship of aquatic plant species, but they have characteristically different values in occurrence in individual parts of the river. Such species even develop into indicator species when such species assemblages are analysed with statistical methods and therefore have their – variable – imprint on the whole macrophyte community. As, e.g. *C. demersum* increases in importance when proceeding from the upper reach to the lower reach of the Danube, the elimination of such 'ubiquistic' elements from ecological status assessment procedures seems like a fallacy, especially with respect to producing statistically reliable results. Neglecting such species leads, of course, to significantly higher separation of species groups, when species with a wider ecological amplitude are deleted. But statistical relevance is then much reduced. Between Chiciu (RO)/Silistra (BG) and Reni, *Ceratophyllum demersum* dominated the aquatic vegetation to a great extent, but the species number decreased to 12 (Section 9).

In the Danube Delta, only the Vilkova-Chilia arm was rich in aquatic plants, especially along some of the small settlements situated on its banks, but the rare species *Stratiotes aloides* was also detected there (Section 10).

The statistically significant differentiation of macrophyte assemblages in the river sections of the Danube was also reported for different large water bodies of the lower Danube reach in Romania [22]. Two successive reaches of the main river channel, two large side channels in parallel location and three Delta channels display a highly significant set of different macrophyte compositions (Table 6).

The water bodies of the Danube River corridor between rkm 375 near Calarasi and the mouth of the three Delta channels are clearly individualised by their indicating macrophyte species, despite their close connectivity.

Results like that of the JDS2 river sections and that of the Romanian water bodies in the Danube River corridor (Table 6) show the need to survey river reaches in enough detail to enable distinguishing between seemingly similar and potentially different macrophyte assemblages, which would otherwise not be detected.

Table 6 Individual character of macrophyte assemblages in running water bodies of the Danube River corridor [22]

	Cal	Bra	Bor	Mac	Chi	Sul	Sf.G
Species richness (S)	65	60	45	73	39	50	57
Total number of indicator species (IS)[a]	1	4	5	17	14	12	3
IS: hydrophytes and amphiphytes only	0	0	0	4	8	6	2
Top IS	None	None	None	Pot luc	Pot pec	Sal nat	Tra nat
Top non-IS species	Myr spi	Pol amp	Azo fil	Oen aqu	Pot cri	Hyd mor	Myr ver

River reach codes: *Cal* Danube main channel between Calarasi and Giurgeni, *Bra* Danube main channel between Giurgeni and Braila, *Bor* Borcea side channel (parallel to Cal), *Mac* Macin side channel (parallel to Bra), *Chi* Chilia Delta arm, *Sul* Sulina Delta channel, *Sf.G* Sfântu Gheorghe Delta arm. Species codes: Pot luc *Potamogeton lucens* L., Pot pec *Potamogeton pectinatus* L., Sal nat *Salvinia natans* (L.) All, Tra nat *Trapa natans* L., Myr spi *Myriophyllum spicatum* L., Pol amp *Polygonum amphibium* L., Azo fil *Azolla filiculoides* Lam., Oen aqu *Oenanthe aquatica* (L.) Poir., Pot cri *Potamogeton crispus* L., Hyd mor *Hydrocharis morsus-ranae* L., Myr ver *Myriophyllum verticillatum* L.

[a]According to indicator species analysis (ISA): Dufrêne and Legendre [12]. Data basis: Sarbu et al. [22]

JDS2 Section 6, equally species rich as Section 4, was located in the HR/RS and RS/RO river reach, from the Hungarian border to the head water of the Iron Gate I reservoir. Influence from the large and floodplain-rich tributaries Tisza and Sava as well as the reduction in water flow velocity [23, 24] in this middle reach of the Danube may have caused the rich macrophyte development.

Despite the notable increase in especially *Ceratophyllum demersum* and *Myriophyllum spicatum*, overall species richness decreased in the lower Romanian Danube and towards the Danube Delta channels (Fig. 1), when considering typical hydrophytes. The widening of the river channel and the increase in bare sandy sediment in the shallow river littoral where macrophytes would sustain may influence this negative development.

4.2 Ecological Status Assessment and Determinant Side Effects to be Considered

The assessment of the ecological status of river reaches lies within the competence of each European Union Member State. Therefore only an ‘intentional ecological status’ was worked out for the JDS2 report. The following conditions were respected when applying the metrics described in the methods section of this contribution: (a) practically no fully near-natural conditions can be found along the whole Danube River corridor; (b) when considering ecological quality for ‘good status', and its differentiation from 'moderate status', we followed a 'short-cut way' quite similar to the approach of Birk et al. [2]; (c) bryophytes occurring on rock-face-dominated banks and in constrained reaches indicated close-to-natural conditions; bryophyte occurrence on rip-rap or other hard anthropogenic surfaces was considered moderate ecological conditions, at best; (d) vascular aquatic species occurrence in regulated river reaches was weighted against undisturbed historical flow and morphology conditions; and (e) in the lower river reaches, the influence of the large catchment with regard to natural nutrient enhancement was considered close-to-natural conditions, but noticeable pollution influence was considered moderate status or worse. In addition, e.g. historical river maps or differential saprobic data were also integrated. Following this procedure, a considerable number of JDS2 sites were considered as 'good ecological conditions', but many, especially those in hydropower impoundments, were classified 'moderate' [16].

However, with that experience in mind, it became explicitly clear that a singular assessment of ecological status can be determined by several pressures not necessarily related to negative human influence.

Natural seasonal and interannual variation of aquatic macrophyte composition is common in running water systems. Such temporal changes have been studied in the German and Austrian catchment of the Danube. Short-term fluctuation of species composition but also the recovering process after reduction or increase of water pollution is reflected by the macrophyte population.

Table 7 shows that the sites of the two JDS in the main river channel, in the upper impoundment of the flood relief channel in Vienna and in the flood exposed oxbow system of Rosskopf, are characterised by a rather similar ratio of constant and variable hydrophyte species, respectively. The high ratio of variable species in the lower impoundment of the New Danube channel may be due to the intensive use as recreational area for water sports and leisure activities, which influence the near-bank areas throughout the summer season. The other extreme of c. 2/3 of constant species was reported for the groundwater-dominated Slovak river, which guarantees extremely constant flow and temperature conditions. Similar effects were

Table 7 Ratio of constant and variable hydrophyte species in different water bodies of the Danube catchment

Water body	Tno[a]	% constant	% variable
JDS1 (2001)–JDS2 (2007)	48	47.9	52.1
New Danube UI (1995–2007)	17	41.2	58.8
New Danube LI (1988–2007)	22	27.3	72.7
Rosskopf (1987/1993/1994/2009)	31	41.9	58.1
Klatovske rameno (1996/2005)	27	70.4	29.6

JDS1–JDS2, main Danube River channel; New Danube UI, upper impoundment of the flood protection side channel located in Vienna; New Danube LI, lower impoundment (Wychera, personal communication); Rosskopf, oxbow series in the active Danube floodplain of the Eastern Austrian Danube reach (Jäger, courtesy); Klatovske rameno, groundwater-fed stream located in the alluvial cone of the Zytný ostrov (SK) [25]

[a]Tno: total number of hydrophytes

recorded in student field courses on the Fischa River at Siegersdorf (Austria), where 60% of the species occurred annually in a 30-year period (Janauer, personal communication).

Other examples are recorded from the Bavarian (Germany) Danube catchment. An extensive time-series study was carried out at Moosach (Germany), a tributary to the Isar River of c. 31 km length, fed by groundwater and rich in carbonate and in macrophytes. Schweinitz et al. [26] aggregated the results of eight macrophyte surveys between 1970 and 2010. Aside from recording river reaches with or without changes in species composition, e.g. an increase in the eutrophic species group, evidence was provided of how to conserve ecologically sensitive, rather pristine macrophyte assemblages in parts of the water body system.

A similar study was conducted at Friedberger Au (Germany), which is 33 km long and also rich in carbonate and macrophytes. It merges with the Danube near Marxheim. Veit et al. [27] reported 53% constant and 47% variable hydrophyte species based on survey campaigns between 1972 and 1996. Seibold's recent results (1972–2012) are in press [28].

Results of long-term investigations should be considered when assessing the ecological status of the biological quality element 'macrophytes', as natural, non-anthropogenic interannual variation between constant and 'fluctuating' macrophyte species can affect the critical determination of 'good' or 'moderate' ecological status.

Many parts of the present Danube River and its floodplain corridor are no longer in near-natural condition due to human activities. Impounded stretches and reaches with embankments and other 'hard' types of regulation may fall under the category of 'heavily modified' river parts. Mitigation measures to reach 'good ecological potential' are then requested by the WFD. In many cases, fish passes are built to reconstruct longitudinal connectivity at least to some extent. But usually, no measures are considered practical for enhancing the potential of the reservoir part of hydropower plants (HPPs). In the reservoir of Freudenau HPP (Vienna, AT), considerable effort was put into ecological improvement of the reservoir stretch by constructing 'compensation structures' along its left bank. Different man-made side-channel environments improved habitat conditions for natural colonisation by aquatic plant species, predominantly submersed macrophytes. This increase in structural diversification of the narrow but up to several-kilometres-long side channels triggered the accumulation of many fish species covering rheophilic to eurytopic to stagnophilic species [29], which resulted in a considerable increase in ecological quality of the impoundment.

5 Conclusions

The JDS2 survey of the second largest river in Europe was of great importance for assessing the most determinant abiotic and biotic parameters along the navigable reach a second time. Regarding macrophytes, the survey method could be adapted to the exceptional spatial dimension of the Danube River. The quality of our results is mirrored by extensive statistical analyses carried out by scientists working on intercalibration exercises. Added value is provided in using the macrophyte information when assessing the ecological status of Danube reaches in the future and in case of meeting ecological potential requirements in some river parts. As a side effect, information for conservational management was also provided.

In JDS2, macrophytes were detected in almost 90% of the survey units, but abundance was usually low, as expected for a large river. Assessing species occurrence and abundance over the full length of each survey unit provided information on the total basic population and not only on test squares possibly biased by subjective selection. The statistically individual character of the macrophyte composition in each river section was clearly shown. The linkage of species or species groups to different river reaches, e.g. bryophytes to the rip-rap-protected banks of the upper reach, and less flow-sensitive vascular species to the middle and lower reach are substantial to provide a background for correctly estimating the boundary between good and moderate ecological status of sampling sites.

Finally, deeper insight is requested regarding the natural temporal variation of macrophyte composition in running waters: particularly interannual species variation may erroneously create negative influence on the results of ecological status assessment according to the WFD, as well as on the appointment of conservation status in, e.g. Natura 2000, protected floodplain areas.

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Current Status of Fish Communities in the Danube

Vladimír Kováč

Abstract The Danube is a river with the highest fish species richness (102 species ever reported) in Europe. Nevertheless, it is also a river that faces various human pressures with serious negative impacts on its ecosystems, including fish communities. In this chapter, data from both the Joint Danube Survey 2 (2007) and the Gabčíkovo Hydroelectric Scheme Monitoring (1991–2011) are reanalysed briefly (data from JDS3 - 2013 are not included). A total of 69 species of fishes were recorded within the recent surveys of the Danube, a number that still suggest a high diversity of the Danubian fish community. However, as many as 12 of these species were not native in the Danube, at least not in its whole course, and a total of 18 non-native species have been ever recorded in the Danube. Concerning native species, cyprinids, especially bleak, highly predominated along the whole course of the Danube, though invasive species, such as gobies in the Upper and Middle Danube and gibel in the Lower Danube, were found to be extremely abundant. Biological invasions not only indicate deterioration of environments but also may result in an overall decline in biodiversity. Therefore, a predictive risk assessments and management strategies for introductions and invasions of non-native fishes should be developed for the Danube and applied subsequently at an international level. Human impacts on fish communities of the Danube are also briefly illustrated, with the Gabčíkovo Hydroelectric Scheme used as an example.

Keywords Diversity, Fishes, Gabčíkovo monitoring, Human impacts, Invasive gobies, Joint Danube Survey 2

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Contents

1 Introduction

With as many as 102 species of fishes recorded, the Danube is a river with the highest species richness in Europe. The first comprehensive review of the Danubian ichthyofauna was provided by Balon [1, 2] who also defined the Danube as the major migration route for a diverse Central Asian and Ponto–Caspian fauna [3]. Thanks to a high habitat diversity and dense ecotonal structure, the Danube provides diverse combinations of environmental conditions suitable for a great variety of different fish species [4, 5].

Nevertheless, the Danube is also a river with great international importance as a route for transport of goods across Europe, a vital resource for water supply, a strong source of hydro-energy, as well as a base for agriculture, industry, recreation, tourism and both recreational and commercial fisheries. Therefore, there have been various environmental pressures resulting from diverse human activities that have had serious negative impacts on the Danubian ecosystems, including its fish communities. That is why it is important to pay a constant attention to what is going on in the Danubian ecosystems, as well as what are the trends in the dynamics of fish communities. The ecological status and problems of the Danube and its fish fauna were recently reviewed by Schiemer et al. [5]. In the meantime, the Joint Danube Survey 2 (JDS2), which took place from 13 August to 28 September 2007, brought the most detailed and most comprehensive data on fish communities ever collected from the Danube [6, 7] (data from JDS3 2013 were not available when writing this chapter). Furthermore, since 1990, a continuous monitoring of fish fauna has been carried out in order to evaluate the impacts of the Gabčíkovo Hydroelectric Scheme (GHS) on fish communities in the Čunovo–Sap section (Middle Danube), including sidearms.

In this chapter, data from both JDS2 and GHS monitoring are reanalysed briefly, in order to provide the most recent update of the status of fish communities in the Danube. Of course, the Danube is a really large river, and thus the methodological constraints in the sampling protocols of both these sources of data [6, 8] do not allow to make scientifically undisputable conclusions. Nevertheless, a collection of samples taken within a short period of time from 45 sampling sites all along the Danube, combined with a collection of samples taken over a 20-year-long period but from sampling sites situated at one stretch of the Danube, provides a unique chance to get at least an overall picture on what is the current status of fish communities in the Danube.

2 Fish Community of the Upper Danube (JDS 2)

The upper section of the Danube runs from the Black Forest (Germany) to the Devín Gate (Slovakia, river km 1880), where the River Morava enters the Danube [5]. During the Joint Danube Survey sampling that took place in 2007, a total of 45 species of fishes were found in the Upper Danube [6]. Among these, 39 species were native, and 6 species were allochthonous, with 4 species considered invasive (Table 1).

Two species were found to be eudominant (relative density >10%), with an extremely high predominance of bleak (*Alburnus alburnus*) that covered more than 60% of all fish individuals collected in the upper section of the Danube. Bleak was followed by round goby (*Neogobius melanostomus*), a species that has recently invaded not only the whole Danube but also the River Rhine, as well as several other river systems in Europe (e.g. Copp et al. [9]). The following 9 species formed a slightly more than one fifth of the Upper Danube fish community, and the remaining 34 species were represented by less than 1% of relative density (Table 1).

Concerning native species, cyprinids, especially bleak, followed by nase (*Chondrostoma nasus*), roach (*Rutilus rutilus*), chub (*Leuciscus cephalus*), ide (*Leuciscus leuciscus*), common bream (*Abramis brama*) and barbel (*Barbus barbus*), highly predominated. Two further species – perch (*Perca fluviatilis*) and eel (*Anguilla anguilla*) – also exceeded 1% of relative density. Two non-native invasive gobies (round and bighead) formed a relevant part (13.1%) of the Upper Danube fish community, whereas the relative density of the other four non-native species attained only 1.5% (Table 1).

Most of the species recorded in the upper section of the Danube demonstrated high affinity to current velocity – 31 species were rheophilous. Nevertheless, these rheophils did not cover more than 27.53% of all individuals, because of bleak, which is eurytopic, and together with other ten eurytopic species formed as much as 72.4% of all fish specimens collected in the upper section of the Danube. Only three species, which represented together just 0.1% of the Upper Danube fish community, were limnophilous (Table 1).

Table 1 Species of fishes collected in the Upper Danube during JDS2 ([7], data reanalysed)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Alburnus alburnus	Nat	EU	A.1.4	60.60
Neogobius melanostomus	Inv	RB	B.1.3	10.76
Chondrostoma nasus	Nat	RA	A.1.3	3.50
Rutilus rutilus	Nat	EU	A.1.4	3.09
Leuciscus cephalus	Nat	EU	A.1.3	2.67
Leuciscus idus	Nat	RB	A.1.4	2.49
Neogobius kessleri	Inv	RB	B.1.3	2.31
Perca fluviatilis	Nat	EU	A.1.4	2.30
Abramis brama	Nat	RB	A.1.4	1.84
Anguilla anguilla	Nat	EU	N/A	1.67
Barbus barbus	Nat	RA	A.1.3	1.50
Leuciscus leuciscus	Nat	RA	A.1.4	0.90
Aspius aspius	Nat	RB	A.1.3	0.79
Carassius gibelio	Inv	EU	A.1.5	0.79
Gasterosteus aculeatus	Non	EU	B.2.4	0.69
Lota lota	Nat	RB	A.1.2	0.64
Alburnoides bipunctatus	Nat	RA	A.1.3	0.61
Gymnocephalus cernuus	Nat	RB	A.1.4	0.44
Esox lucius	Nat	EU	A.1.5	0.36
Sander lucioperca	Nat	RB	B.2.5	0.36
Vimba vimba	Nat	RB	A.1.3	0.32
Blicca bjoerkna	Nat	RB	A.1.5	0.20
Silurus glanis	Nat	EU	B.1.4	0.16
Gymnocephalus schraetser	Nat	RA	A.1.4	0.11
Abramis sapa	Nat	RA	A.1.3	0.10
Proterorhinus marmoratus	Nat	EU	B.2.7	0.10
Zingel zingel	Nat	RB	A.2.3	0.09
Rutilus pigus	Nat	RA	A.1.5	0.08
Scardinius erythrophthalmus	Nat	LI	A.1.5	0.07
Cyprinus carpio	Nat	EU	A.1.5	0.06
Salmo trutta m. fario	Nat	RA	A.2.3	0.05
Barbatula barbatula	Nat	RA	A.1.6	0.03
Cottus gobio	Nat	RA	B.2.7	0.03
Hucho hucho	Nat	RA	B.2.3	0.03
Lepomis gibbosus	Inv	LI	B.2.2	0.03
Zingel streber	Nat	RA	A.2.3	0.03
Gobio albipinnatus	Nat	RA	A.1.6	0.02
Gymnocephalus baloni	Nat	RA	A.1.4	0.02
Rhodeus amarus	Nat	EU	A.2.5	0.02
Sander volgensis	Nat	RB	B.2.5	0.02
Thymallus thymallus	Nat	RA	B.2.3	0.02
Oncorhynchus mykiss	Non	RA	A.2.3	0.01

(continued)

Table 1 (continued)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Phoxinus phoxinus	Nat	RA	A.1.3	0.01
Tinca tinca	Nat	LI	A.1.5	0.01
Gobio gobio	Nat	RA	A.1.6	0.01

Nat native species, *Non* non-native species, *Inv* invasive species, *EU* eurytopic species (i.e. without specialised affinity to current velocity), *RA* rheophils A (i.e. species that live in lotic habitats throughout their life circle), *RB* rheophils B (i.e. species that prefer lotic habitats but make seasonal habitat shifts between the river and backwaters), *LI* limnophils (i.e. species that prefer stagnant water). Reproductive guilds [10]: *A* nonguarders, *A.1* open substrate spawners, *A.1.1* pelagophils, *A.1.2* lithopelagophils, *A.1.3* lithophils, *A.1.4* phytolitophils, *A.1.5* phytophils, *A.1.6* psammophils, *A.2* brood hiders, *A.2.2* phytolitophils, *A.2.3* lithophils, *A.2.5* ostracophils, *B* guarders, *B.1* substrate choosers, *B.1.3* lithophils, *B.1.4* k phytophils, *B.2* nest spawners, *B.2.2* polyphils, *B.2.3* lithophils, *B.2.4* ariadnophils, *B.2.5* phytophils, *B.2.7* speleophils, *C* bearers, *C.1.5* pouch bearers. Relative density is expressed in percent of individuals of a species from the total number of individuals in the community

Concerning the affinity to spawning substrate, phyto-lithophilous fishes represented by nine species were the most abundant in the Upper Danube (71.8%), though lithophilous species prevailed in number (16 species covering 22.8% of relative density), followed by phytophils that were represented by ten species but covered only 2.1% of the Upper Danube fish community. The remaining ten species (3.2% of relative density) demonstrated affinity to various other substrata; three of them were psammophilous (Table 1).

3 Fish Community of the Middle Danube (JDS 2)

The middle section of the Danube starts just below the Devín Gate, where it still has a character of a submontane river, and ends at the Iron Gate reservoir (river km 1075; [5]). In 2007, a total of 51 species of fishes were recorded in this section of the Danube [6], though only 40 species belonged to native fauna, whereas 11 species were non-native, with 9 species considered invasive (Table 2).

Two species were found to be eudominant, again with an extremely high predominance of bleak that covered more than 44% of all fish individuals collected in the middle section of the Danube, followed by the Ponto–Caspian invader, round goby. The subsequent ten species formed approximately one third of the Middle Danube fish community, and as many as 37 species were represented by less than 1% of relative density (Table 2).

Concerning native species, cyprinids, such as bleak, followed by roach, asp (*Aspius aspius*), dace (*Leuciscus idus*), silver bream (*Blicca bjoerkna*) and common bream highly predominated, accompanied with burbot (*Lota lota*) and perch in the group of species exceeding 1% of relative density. However, almost one quarter of the Middle Danube fish community was found to be formed by non-native species,

Table 2 Species of fishes collected in the Middle Danube during JDS2 ([7], data reanalysed)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Alburnus alburnus	Nat	EU	A.1.4	44.13
Neogobius melanostomus	Inv	RB	B.1.3	10.90
Rutilus rutilus	Nat	RA	A.1.5	7.93
Neogobius kessleri	Inv	RB	B.1.3	5.43
Aspius aspius	Nat	RB	A.1.3	4.45
Carassius gibelio	Inv	EU	A.1.5	3.86
Lota lota	Nat	RB	A.1.2	3.17
Leuciscus idus	Nat	RB	A.1.4	2.39
Blicca bjoerkna	Nat	RB	A.1.5	2.20
Neogobius fluviatilis	Inv	RB	B.1.3	1.68
Perca fluviatilis	Nat	EU	A.1.4	1.58
Abramis brama	Nat	RB	A.1.4	1.19
Gobio albipinnatus	Nat	RA	A.1.6	1.00
Esox lucius	Nat	EU	A.1.5	1.00
Lepomis gibbosus	Inv	LI	B.2.2	0.92
Chondrostoma nasus	Nat	RA	A.1.3	0.77
Gymnocephalus schraetser	Nat	RA	A.1.4	0.75
Sander lucioperca	Nat	RB	B.2.5	0.75
Neogobius gymnotrachelus	Inv	RB	B.1.3	0.72
Barbus barbus	Nat	RA	A.1.3.	0.69
Rhodeus amarus	Nat	EU	A.2.5	0.67
Gymnocephalus baloni	Nat	RA	A.1.4	0.42
Proterorhinus marmoratus	Nat	EU	B.2.7	0.40
Ameiurus melas	Inv	LI	B.2.3	0.39
Eudontomyzon mariae	Nat	RA	A.2.3	0.39
Leuciscus cephalus	Nat	EU	A.1.3	0.30
Gymnocephalus cernuus	Nat	RB	A.1.4	0.27
Cyprinus carpio	Nat	EU	A.1.5	0.26
Scardinius erythrophthalmus	Nat	LI	A.1.5	0.26
Abramis sapa	Nat	RA	A.1.3	0.25
Vimba vimba	Nat	RB	A.1.3	0.21
Rutilus pigus	Nat	EU	A.1.4	0.11
Silurus glanis	Nat	EU	B.1.4	0.09
Sander volgensis	Nat	RB	B.2.5	0.08
Pseudorasbora parva	Inv	EU	A.2.2	0.07
Zingel zingel	Nat	RA	A.2.3	0.07
Pelecus cultratus	Nat	EU	A.1.1	0.05
Alburnoides bipunctatus	Nat	RA	A.1.3	0.04
Abramis ballerus	Nat	RB	A.1.4	0.03
Tinca tinca	Nat	LI	A.1.5	0.03
Anguilla anguilla	Nat	EU	N/A	0.02
Gobio gobio	Nat	RA	A.1.6	0.02

(continued)

Table 2 (continued)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Cobitis elongatoides	Nat	RB	A.1.5	0.02
Misgurnus fossilis	Nat	LI	A.1.5	0.02
Leuciscus leuciscus	Nat	RA	A.1.4	0.01
Sabanejewia sp.	Nat	RA	A.2.3	0.01
Acipenser ruthenus	Nat	RA	A.1.2	0.01
Ameiurus nebulosus	Non	LI	B.2.7	0.01
Carassius carassius	Nat	LI	A.1.5	0.01
Hypophthalmichthys molitrix	Non	LI	A.1.1	0.01
Perccottus glenii	Inv	LI	B.2.5	0.01

Nat native species, *Non* non-native species, *Inv* invasive species, *EU* eurytopic species (i.e. without specialised affinity to current velocity), *RA* rheophils A (i.e. species that live in lotic habitats throughout their life circle), *RB* rheophils B (i.e. species that prefer lotic habitats but make seasonal habitat shifts between the river and backwaters), *LI* limnophils (i.e. species that prefer stagnant water). Reproductive guilds [10]: *A* nonguarders, *A.1* open substrate spawners, *A.1.1* pelagophils, *A.1.2* lithopelagophils, *A.1.3* lithophils, *A.1.4* phytolitophils, *A.1.5* phytophils, *A.1.6* psammophils, *A.2* brood hiders, *A.2.2* phytolitophils, *A.2.3* lithophils, *A.2.5* ostracophils, *B* guarders, *B.1* substrate choosers, *B.1.3* lithophils, *B.1.4* k phytophils, *B.2* nest spawners, *B.2.2* polyphils, *B.2.3* lithophils, *B.2.4* ariadnophils, *B.2.5* phytophils, *B.2.7* speleophils, *C* bearers, *C.1.5* pouch bearers. Relative density is expressed in percent of individuals of a species from the total number of individuals in the community

and almost every fourth specimen collected was invasive (Table 2). Among these, Ponto–Caspian gobies, especially round goby, bighead goby (*Neogobius kessleri*) and monkey goby (*Neogobius fluviatilis*), formed a major part of the invaders, providing together 18% of the total fish community.

The submontane character of the middle section of the Danube was also reflected in the species composition, concerning their affinity to current velocity. A majority of the 51 species (30 species represented by 38.1% of all individuals) were rheophilous, followed by 12 eurytopic species (60.3% of all individuals) and 9 limnophilous species (only 1.6% of all individuals). Unfortunately, approximately a half of the rheophils was covered by invasive gobies.

Thanks to the predominance of bleak, phyto-lithophilous species were the most abundant in the Middle Danube (58.8%, 11 species), though lithophils were represented by the highest number of species (15 species, 26.3%), followed by phytophils (14 species, 8.7%). Other reproductive guilds were represented by 11 species contributing by 6.3% of relative density from the total fish community (Table 2).

4 Fish Community of the Lower Danube (JDS 2)

The lower section of the Danube starts below the Iron Gate reservoir and continues up to the delta, where the Danube enters the Black Sea [5]. A total of 46 species of fishes were found in the Lower Danube in 2007 [6]. In contrast to the previous two sections of the Danube, the species composition in this fish community contained a highest proportion of native species (41), and only five species were non-native, with four species being invasive (Table 3).

Two species were found to be eudominant, with the same leader as in the upper and middle sections (bleak) that covered more than 40% of all fish individuals, though the population of the second most abundant species (gibel; *Carassius gibelio*) was also very dense (24.8%). Fifteen other species with more than 1% of relative density contributed to the Lower Danube fish community with 29% of all individuals, and the remaining 29 species were represented by less than 1% of relative density (Table 3).

Similar to the previous two sections, cyprinids, and especially bleak, again, highly prevailed among the native species. Silver bream (*Blicca bjoerkna*), roach, white-eye bream (*Abramis sapa*), bitterling (*Rhodeus amarus*), common bream, white-finned gudgeon (*Romanogobio vladykovi*), asp and ide also exceeded 1% of the Lower Danube fish community. Further five species – sterlet (*Acipenser ruthenus*), pikeperch (*Sander lucioperca*), round goby, perch and monkey goby (*Neogobius fluviatilis*) – also contributed to the Lower Danube fish community with more than 1% of all individuals. Three non-native invasive species (gibel, pumpkinseed and topmouth gudgeon) formed a considerable part (27.4%) of the Lower Danube fish community, whereas the relative density of the other two non-native species was rather negligible (only 0.1%; Table 3).

Even in the lower section of the Danube, most species (24) demonstrated high affinity to current velocity, though the cumulative relative density of the rheophils covered only 23.2% of the fish community. On the other hand, eurytopic fishes, represented by 15 species, prevailed, since almost three quarters of all fish specimens collected in the lower section of the Danube were indifferent to current velocity. Finally, seven species, that represented 2.5% of the Lower Danube fish community, were limnophilous (Table 3).

Approximately a half all of the fishes (49%) collected in the Lower Danube (represented by ten species) were phyto-lithophilous. Concerning species composition, lithophils prevailed with 13 species that covered 9.1% of relative density, followed by phytophils that were represented by 12 species and, thanks to the invasive gibel, covered about one third (33.6%) of the Lower Danube fish community. Other reproductive guilds were represented by 11 species contributing by 8.4% of relative density from the total fish community (Table 3).

Table 3 Species of fishes collected in the Lower Danube during JDS2 ([7], data reanalysed)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Alburnus alburnus	Nat	EU	A.1.4	40.03
Carassius gibelio	Inv	EU	A.1.5	24.80
Blicca bjoerkna	Nat	RB	A.1.5	4.94
Rutilus rutilus	Nat	EU	A.1.4	2.87
Abramis sapa	Nat	RA	A.1.3	2.39
Rhodeus amarus	Nat	EU	A.2.5	2.31
Acipenser ruthenus	Nat	RA	A.1.2	2.11
Abramis brama	Nat	RB	A.1.4	1.93
Sander lucioperca	Nat	RB	B.2.5	1.80
Gobio albipinnatus	Nat	RA	A.1.6	1.64
Neogobius melanostomus	Nat	RB	B.1.3	1.56
Lepomis gibbosus	Inv	LI	B.2.2	1.49
Aspius aspius	Nat	RB	A.1.3	1.35
Perca fluviatilis	Nat	EU	A.1.4	1.35
Neogobius fluviatilis	Nat	RB	B.1.3	1.15
Pseudorasbora parva	Inv	EU	A.2.2	1.11
Leuciscus idus	Nat	RB	A.1.4	1.00
Neogobius kessleri	Nat	RB	B.1.3	0.69
Gymnocephalus schraetser	Nat	RA	A.1.4	0.67
Scardinius erythrophthalmus	Nat	LI	A.1.5	0.65
Leuciscus cephalus	Nat	EU	A.1.3	0.58
Cyprinus carpio	Nat	EU	A.1.5	0.57
Neogobius gymnotrachelus	Nat	RB	B.1.3	0.49
Neogobius eurycephalus	Nat	RB	N/A	0.41
Esox lucius	Nat	EU	A.1.5	0.36
Chondrostoma nasus	Nat	RA	A.1.3	0.33
Barbus barbus	Nat	RA	A.1.3.	0.31
Pelecus cultratus	Nat	EU	A.1.1	0.16
Vimba vimba	Nat	RB	A.1.3	0.15
Perccottus glenii	Inv	LI	B.2.5	0.14
Silurus glanis	Nat	EU	B.1.4	0.14
Proterorhinus marmoratus	Nat	EU	B.2.7	0.09
Syngnathus abaster	Nat	LI	C.1.5	0.09
Carassius carassius	Nat	LI	A.1.5	0.09
Cobitis elongatoides	Nat	RB	A.1.5	0.06
Benthophiloides brauneri	Nat	EU	B.2.3	0.04
Benthophilus nudus	Nat	EU	B.1.3	0.03
Gymnocephalus cernuus	Nat	RB	A.1.4	0.03
Tinca tinca	Nat	LI	A.1.5	0.02
Acipenser stellatus	Nat	A	A.1.2	0.01
Mugil cephalus	Nat	EU	A.1.6	0.01
Zingel zingel	Nat	RA	A.2.3	0.01

(continued)

Table 3 (continued)

Species	Origin	Habitat preference	Reproductive guild	Relative density
Abramis ballerus	Nat	RB	A.1.4	0.01
Ameiurus nebulosus	Non	LI	B.2.7	0.01
Gymnocephalus baloni	Nat	RA	A.1.4	0.01
Sander volgensis	Nat	RB	B.2.5	0.01

Nat native species, *Non* non-native species, *Inv* invasive species, *EU* eurytopic species (i.e. without specialised affinity to current velocity), *RA* rheophils A (i.e. species that live in lotic habitats throughout their life circle), *RB* rheophils B (i.e. species that prefer lotic habitats but make seasonal habitat shifts between the river and backwaters), *LI* limnophils (i.e. species that prefer stagnant water). Reproductive guilds [10]: *A* nonguarders, *A.1* open substrate spawners, *A.1.1* pelagophils, *A.1.2* lithopelagophils, *A.1.3* lithophils, *A.1.4* phytolitophils, *A.1.5* phytophils, *A.1.6* psammophils, *A.2* brood hiders, *A.2.2* phytolitophils, *A.2.3* lithophils, *A.2.5* ostracophils, *B* guarders, *B.1* substrate choosers, *B.1.3* lithophils, *B.1.4* k phytophils, *B.2* nest spawners, *B.2.2* polyphils, *B.2.3* lithophils, *B.2.4* ariadnophils, *B.2.5* phytophils, *B.2.7* speleophils, *C* bearers, *C.1.5* pouch bearers. Relative density is expressed in percent of individuals of a species from the total number of individuals in the community

5 Twenty Years of Monitoring the Čunovo–Sap Section (Middle Danube)

Since 1990, a continuous monitoring of fish fauna has been carried out in order to evaluate the impacts of the Gabčíkovo Hydroelectric Scheme (GHS) on fish communities in the Čunovo–Sap section (river km 1851–1815, including sidearms). Electroshocking with a handheld anode, both wading and from a boat, has been used to collect the samples three times per year, usually in April–May, July–August and September–October [8].

In total, 41 species of fishes were recorded in this stretch of the Danube (Table 4) during the period 1991–2011. Two eudominant species (roach and bleak) were the most abundant, followed by pumpkinseed, tubenose goby, perch and gibel. Nevertheless, the fish community has been undergoing changes over the two decades after the GHS was put into operation. To evaluate these changes, the Fish Index of Slovakia (FIS) developed in terms of Water Framework Directive has been used. FIS is a multimetric index that calculates the deviation of observed values from the expected values. For each stream type, a hypothetical reference fish community has been defined based on a thorough analysis of historical data. Such a reference community provides the expected values for each metric of FIS. Most of these metrics are based on the classification of fishes into ecological guilds [11]. Trends and changes in the Middle Danube fish community can be best illustrated by the following seven metrics expressed in relative abundance (deviation of observed from expected values): phytophilous species, lithophilous species, benthic species, rheophilous species, potamodromous species, piscivorous species and invasive species.

Table 4 Species of fishes collected over 20 years of monitoring the Čunovo - Sap section (Middle Danube; data by J. Černý [8])

Species	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Total
Rutilus rutilus	14.35	34.08	34.34	1.61	20.35	18.71	14.55	15.44	16.91	12.13	9.20	15.98
Alburnus alburnus	0.00	10.33	0.64	15.40	24.19	11.66	20.96	6.84	8.05	7.46	4.36	13.53
Lepomis gibbosus	0.11	3.78	2.48	13.61	11.80	9.61	2.52	4.30	2.50	3.41	28.07	9.69
Proterorhinus marmoratus	2.16	2.99	3.51	0.72	2.36	12.68	14.89	26.08	28.53	36.03	19.35	9.61
Carassius auratus	19.42	7.43	12.27	3.94	3.54	8.26	6.19	8.86	9.93	10.24	13.24	8.05
Perca fluviatilis	7.80	8.99	27.53	20.05	16.81	13.39	7.33	9.11	11.90	6.06	4.92	7.82
Rhodeus sericeus	0.00	3.05	0.25	0.90	4.42	4.23	5.38	1.77	1.16	7.60	4.60	4.62
Neogobius melanostomus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.87
Leuciscus idus	1.17	0.81	0.71	28.11	0.88	1.54	8.02	5.82	5.28	3.34	2.06	3.32
Esox lucius	1.11	5.55	1.08	3.76	6.78	7.56	7.10	5.82	2.86	3.55	3.17	3.30
Ameiurus melas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.08
Scardinius erythrophthalmus	0.00	2.82	0.28	0.72	0.00	1.41	2.29	0.25	0.27	3.07	0.24	2.29
Leuciscus cephalus	5.02	2.83	0.14	6.44	3.54	2.43	1.83	1.77	2.77	1.11	1.11	2.16
Cottus gobio	23.00	11.15	6.96	0.00	0.00	0.83	0.57	2.03	0.54	0.00	0.71	2.08
Blicca bjoerkna	7.08	0.63	4.54	2.86	0.88	3.01	3.89	4.05	4.20	3.69	1.43	1.88
Neogobius kessleri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.54	1.76
Chondrostoma nasus	0.80	0.14	0.00	1.18	0.29	0.19	0.57	0.51	2.24	0.00	0.08	1.52
Aspius aspius	0.08	0.04	0.43	0.72	0.29	0.77	0.92	0.76	0.00	0.00	0.32	1.30
Lota lota	1.17	0.57	2.54	0.00	0.00	0.38	0.34	0.76	0.45	0.56	2.70	1.27
Barbus barbus	6.24	0.79	0.21	0.00	0.88	0.77	1.37	0.51	1.43	0.00	0.08	0.57

(continued)

Table 4 (continued)

Species	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Total
Gymnocephalus baloni	5.11	1.00	1.21	0.00	0.29	0.13	0.11	0.51	0.00	0.00	0.08	0.42
Gasterosteus aculeatus	0.00	0.00	0.00	0.00	0.29	0.58	0.34	0.76	0.81	0.35	0.56	0.29
Cyprinus carpio	2.11	0.00	0.00	0.00	0.29	0.32	0.00	0.76	0.09	0.28	0.00	0.22
Leuciscus leuciscus	0.00	0.00	0.00	0.00	1.18	0.13	0.11	0.25	0.00	0.00	0.95	0.21
Silurus glanis	0.33	0.00	0.57	0.00	0.00	0.51	0.23	0.51	0.00	0.00	0.24	0.20
Gymnocephalus cernuus	0.08	0.25	0.00	0.00	0.59	0.70	0.34	1.27	0.00	0.00	0.00	0.17
Abramis brama	0.22	0.81	0.11	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00	0.15
Vimba vimba	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
Misgurnus fossilis	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.11	0.00	0.10
Gobio albipinnatus	0.19	1.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
Gobio kessleri	1.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Tinca tinca	0.00	0.00	0.00	0.00	0.29	0.19	0.11	0.25	0.00	0.00	0.00	0.06
Zingel streber	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Zingel zingel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Stizostedion lucioperca	0.97	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
Abramis ballerus	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Cobitis taenia	0.00	0.14	0.11	0.00	0.00	0.00	0.00	0.25	0.09	0.00	0.00	0.03
Barbatula barbatula	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Carassius carassius	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stizostedion volgense	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neogobius gymnotrachelus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Species	2002	2003	E2004	2004	2005	2006	2007	2008	2009	2010	2011	Total
Rutilus rutilus	18.09	14.34	3.39	1.76	13.59	15.49	25.62	20.54	13.71	20.58	12.83	15.98
Alburnus alburnus	12.77	17.46	1.69	18.64	32.07	15.40	24.95	18.68	18.97	13.89	13.20	13.53
Lepomis gibbosus	9.19	14.77	0.00	0.59	16.04	20.38	9.88	17.60	16.34	13.27	12.91	9.69
Proterorhinus marmoratus	17.66	13.64	8.90	2.85	8.07	3.94	1.15	0.72	1.23	1.12	2.80	9.61
Carassius auratus	2.48	5.99	41.95	1.85	2.66	4.23	1.62	2.78	1.86	4.65	3.69	8.05
Perca fluviatilis	4.59	6.69	3.81	9.24	2.76	3.00	1.86	0.87	1.54	2.67	1.18	7.82
Rhodeus sericeus	12.77	8.51	5.08	18.89	0.51	1.31	0.33	2.01	7.81	5.27	5.75	4.62
Neogobius melanostomus	0.00	0.00	4.66	6.21	5.41	5.07	8.97	14.72	10.35	14.26	15.56	3.87
Leuciscus idus	2.19	2.26	2.12	0.00	0.61	0.38	1.34	0.67	0.82	2.60	2.43	3.32
Esox lucius	2.99	3.21	3.39	2.69	2.76	3.00	1.48	1.18	1.18	1.55	0.81	3.30
Ameiurus melas	0.00	0.00	0.00	0.00	4.09	17.00	9.69	7.21	14.16	3.41	12.32	3.08
Scardinius erythrophthalmus	3.65	4.60	1.27	0.34	2.96	4.60	1.38	3.91	7.22	3.60	5.53	2.29
Leuciscus cephalus	2.55	0.61	8.05	1.26	0.31	0.09	0.76	0.98	0.00	3.22	0.66	2.16
Cottus gobio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08
Blicca bjoerkna	1.60	1.13	0.00	0.00	0.00	0.00	0.62	0.82	0.00	0.87	0.00	1.88
Neogobius kessleri	6.42	4.00	5.93	1.68	4.70	2.72	2.15	2.06	0.95	2.98	2.51	1.76
Chondrostoma nasus	0.00	1.04	0.42	24.94	0.00	0.00	0.00	0.62	0.00	0.25	0.07	1.52
Aspius aspius	0.14	0.00	0.85	1.60	3.06	1.78	6.20	1.90	1.68	3.53	3.47	1.30
Lota lota	1.09	0.09	1.27	7.47	0.00	0.47	1.00	1.03	1.32	1.36	3.32	1.27
Barbus barbus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.57
Gymnocephalus baloni	0.00	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42

(continued)

Table 4 (continued)

Species	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	Total
Gasterosteus aculeatus	0.22	0.43	2.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29
Cyprinus carpio	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.31	0.15	0.22
Leuciscus leuciscus	0.07	1.13	0.85	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.21
Silurus glanis	0.14	0.00	0.00	0.00	0.31	0.38	0.24	0.62	0.14	0.12	0.15	0.20
Gymnocephalus cernuus	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17
Abramis brama	0.14	0.00	0.00	0.00	0.10	0.00	0.29	0.21	0.32	0.12	0.29	0.15
Vimba vimba	1.09	0.00	0.00	0.00	0.00	0.00	0.38	0.87	0.00	0.19	0.00	0.12
Misgurnus fossilis	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.06	0.15	0.10
Gobio albipinnatus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
Gobio kessleri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Tinca tinca	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Zingel streber	0.00	0.00	1.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Zingel zingel	0.00	0.00	1.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Stizostedion lucioperca	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.05
Abramis ballerus	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.03
Cobitis taenia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Barbatula barbatula	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Carassius carassius	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stizostedion volgense	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neogobius gymnotrachelus	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00

E2004 special expedition [9]

During 1991–2011, the most important changes in the fish community of the Čunovo–Sap section were observed in relative abundance of benthic, rheophilous, lithophilous and invasive species (Figs. 1, 2, 3 and 4). In contrast to benthic, rheophilous and lithophilous species, in which the trend was mainly decreasing

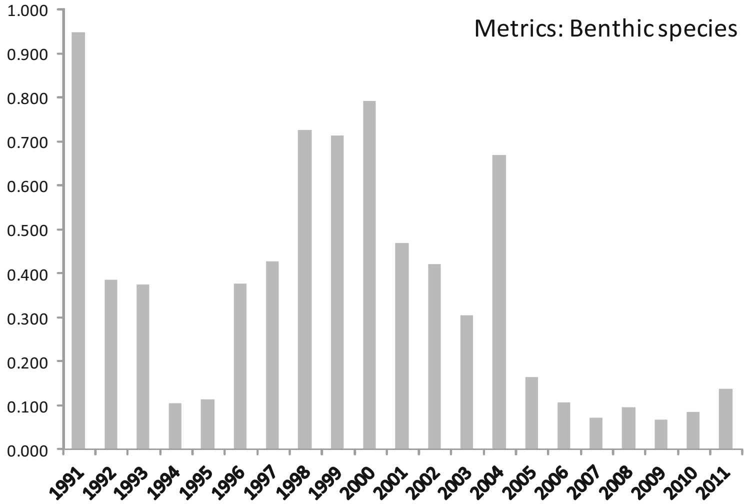

Fig. 1 Variation of benthic species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated from relative density and express the deviation from the expected value, i.e. 1.000. Native species are considered only

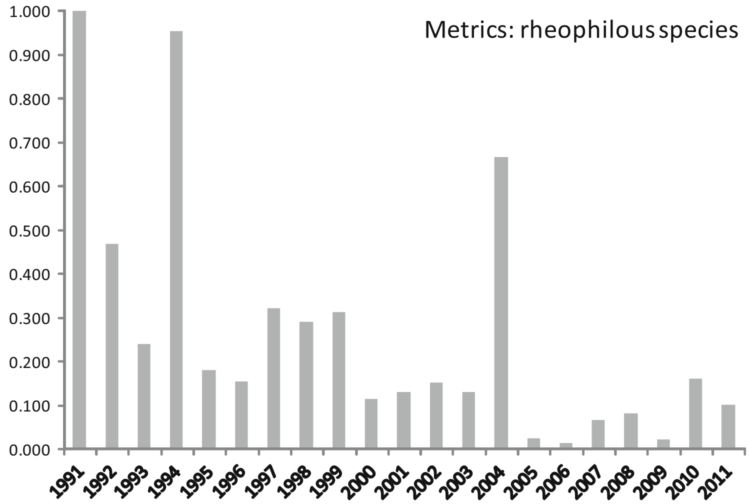

Fig. 2 Variation of rheophilous species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated as described in Fig. 1

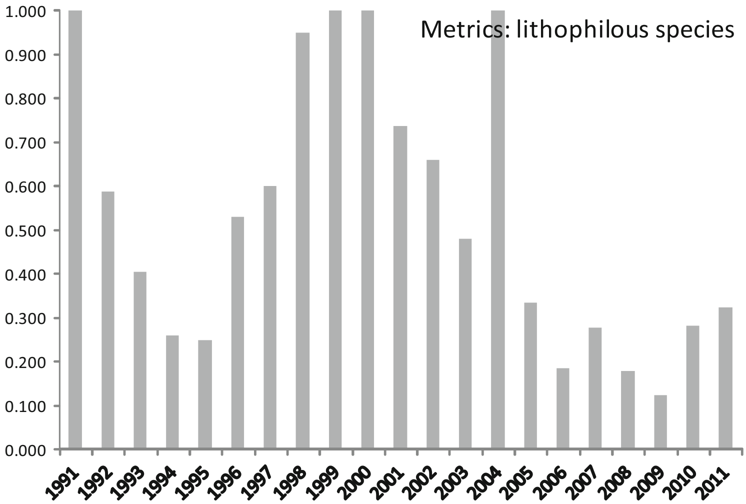

Fig. 3 Variation of lithophilous species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated as described in Fig. 1

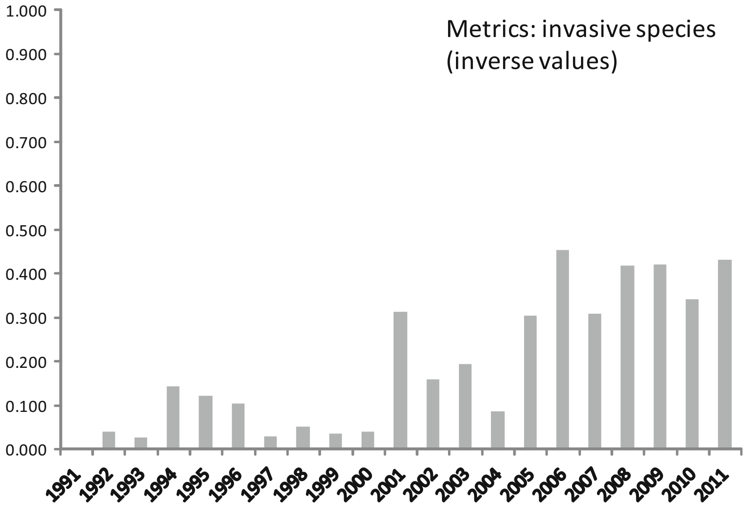

Fig. 4 Variation of invasive species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated from relative density and express the deviation from the expected value, i.e. 0.000

during the second half of the monitoring period, the relative abundance of invasive species was increasing. Since 2005, i.e. soon after the appearance of round goby in this section of the Danube, the inverse value of this metric oscillated around 0.4 (Fig. 4), which indicates a very serious contamination of native fish community with invasive species.

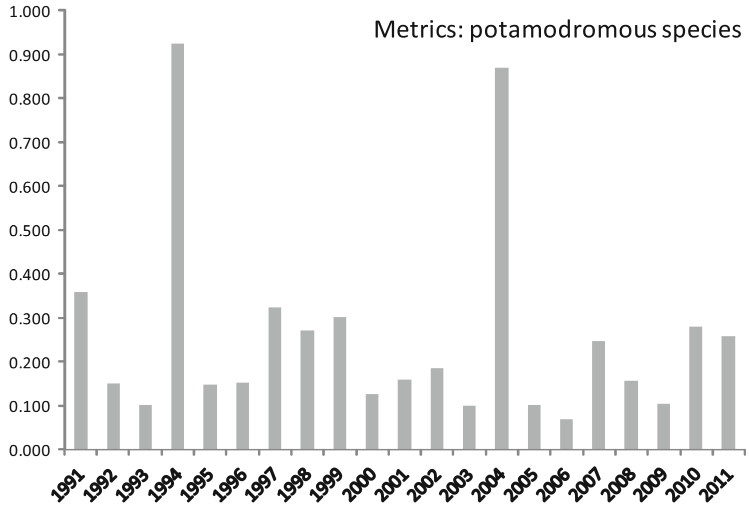

Fig. 5 Variation of potamodromous species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated as described in Fig. 1

Concerning potamodromous species, no apparent changes and/or trends were observed between 1991 and 2011, because even before GHS was put into operation, the relative abundance of this ecological group was rather low, ranging from 15 to 35% of the expected values. Nevertheless, some temporary fluctuations were also observed, especially in 1994 and 2004, when their relative abundance jumped to 92.5% and 87%, respectively (Fig. 5). On the other hand, phytophilous species appear to have been present in the fish community in expected relative abundances (metrics = 1.000) throughout the whole period, except 1993, when the metric of phytophilous species decreased to 0.59, temporarily.

Piscivorous species passed through apparent fluctuations, peaking in a periodicity of 10 years, approximately (Fig. 6). In 1990s (1994–1998), the peak resulted from increasing abundance of pike (*Esox lucius*), whereas asp (*Aspius aspius*) became the most abundant piscivorous species in 2007 (Table 4).

At the species level, the changes in the Čunovo–Sap fish community resulted mainly from the fact that such rheophilous species as bullhead (*Cottus gobio*), wild carp (*Cyprinus carpio*), white-finned gudgeon (*Gobio albipinnatus*) and/or Kessler's gudgeon (*Gobio kessleri*) disappeared from the eupotamal habitats monitored, and the abundance of Balon's ruffe (*Gymnocephalus baloni*) and breams (*Abramis brama*, *A. sapa*, *A. ballerus* and even *B. bjoerkna*) also reduced considerably. All these species appear to have been replaced by more plastic, especially invasive species, such as pumpkinseed (*Lepomis gibbosus*) and round goby (Table 4).

Nonetheless, these trends can be related exclusively to the littoral habitats of the main channel and sidearms of the Danube that have been monitored, since the limitations of the sampling protocol applied in the monitoring of fish community must be considered. Most of the species present in eupotamal of the Danube before

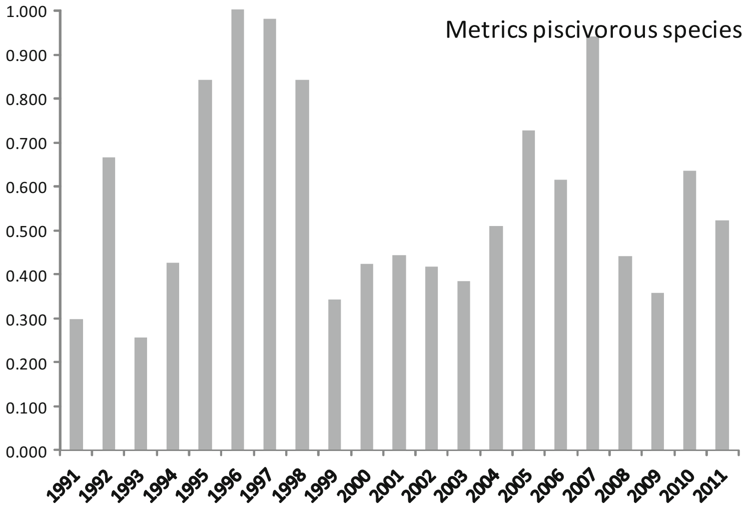

Fig. 6 Variation of piscivorous species in the Čunovo–Sap fish community in the 1991–2011 period. Values of the metrics are calculated as described in Fig. 1

GHS operation have not disappeared completely, though their abundance has reduced. This is obvious from the JDS 2 results (see Table 2). Reophilous species still find their habitats in the middle of the main channel of the Danube, as well as in deeper parts of its littoral zone (personal observation, August 2011, electroshocking bottom trawling performed by *B. Csány* and his team). The only exception seems to be the bullhead that attained as high relative abundance as 23% in 1991, but its population declined afterwards to disappear completely from this stretch of the Danube in 2002 (Table 4; see also Table 2).

The overall abundance of the Čunovo–Sap fish community appears to have stabilised, but the Catch per unit effort (CPUE) values remain very low. Another problem is the absence of individuals from older age classes, as well as the reduction of economically important species, such as perch, pike, pikeperch and wels, especially larger individuals [12].

All the negative trends in the Čunovo–Sap fish community are clearly reflected in the FIS values over the period 1991–2011. Since 1991, the FIS values dropped down from 0.731 (indicating class 1 of ecological status) up to values oscillating around 0.200 after 2005 (indicating class 5 of ecological status Fig. 7). Interestingly, FIS demonstrated an increasing tendency soon after the GHS began working, and this tendency persisted for a period of 4 years (1995–1998). However, since 1998, FIS started declining to reach class 5 in 2005. The only exception occurred in 2004, when FIS jumped up to 0.574 (class 2). This is very likely to be associated with the extremely high discharge of the Danube in August 2002, which overflooded the whole sidearm system and increased thus the spawning and nursery grounds for most of the fish species. On the other hand, low FIS values corresponding to bad ecological status of this stretch of the Danube coincide with

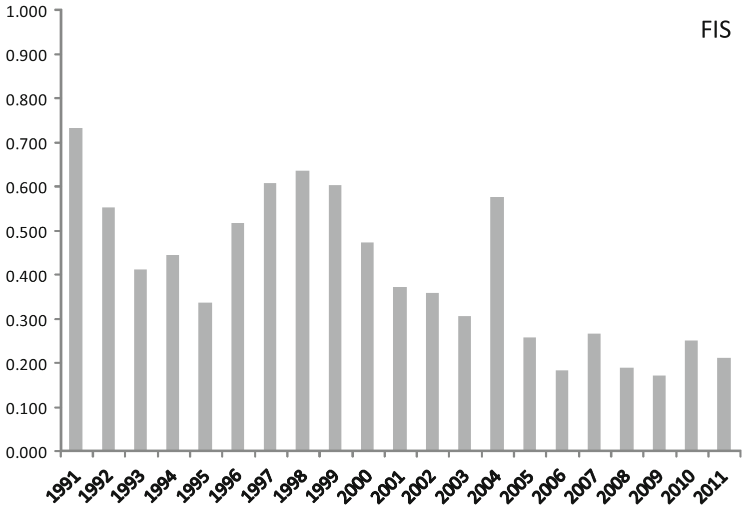

Fig. 7 Variation of the Fish Index of Slovakia in the Čunovo–Sap fish community in the 1991–2011 period. Values of FIS express the deviation from the expected value, i.e. 1.000. Please note that FIS values as well as values of all metrics are biased by the constraints of the sampling methods used during the GHS monitoring that do not meet the WFD requirements, because the Danube falls into the category of large rivers. Therefore, the ecological status, in terms of WFD, cannot be derived from the presented FIS values, though they illustrate the trends and changes in the Čunovo–Sap fish community over the last two decades

the increase of invasive species that form a separate metric of FIS and as such have the power to push FIS values to lower levels.

6 Human Impacts

Major impacts of human activities on the ecosystems of the Danube comprise pollution, i.e. deterioration of water quality, regulations, construction of dams and reservoirs and navigation. As reviewed recently by Schiemer et al. [5], fish communities can also be heavily affected by inappropriate management of fisheries and illegal fishing (see also Černý [12]). Water quality of the Danube is a subject of other chapters; therefore, it is not discussed here.

6.1 River Regulations

Hydromorphological alterations, including regulations of rivers, have been identified as one of the four basin-wide significant water management issues that result in substantial environmental impacts. Hydromorphological alterations result, for example, in the decline of both species diversity and species abundance, in alterations in the structure of populations and/or in limited migrations, which make reproduction of some species impossible [13].

Regulations of the River Danube have not been an issue of recent times, exclusively. For example, already in the thirteenth century, local dams in the form of circular forts were built in separate villages at the middle section of the Danube. In 1424, the King Žigmund (Sigismund of Luxemburg) ordered to interconnect these dams. In the seventeenth century, organised construction of dams along the inundation zone of the Danube began, followed by intensive damming in the nineteenth century, arranged by water unions in the cities of Šamorín and Komárno. As a result, the original course of the Danube, with a dense network of anastomosing sidearms and waste inundation area, was channelised into an area that was 1–3 km wide, and most of the discharge was concentrated in one main stream.

In the Upper Danube, the process of intensive engineering also began in the nineteenth century, with the aim to create a single, straightened channel, stabilised by riverside embankments and rip-raps [5]. Similar to the Middle Danube, the sidearms of the original braided system were cut off. To retain the water level in wetlands, weirs were built in the sidearms. These regulations changed the hydromorphology of the Danube considerably, especially its slopes and transport of bed sediments, as well as runoff characteristics [5].

One of the main reasons for these regulations, both in the Upper and Middle Danube, was to improve navigation. As the upper and lower ends of the sidearms were closed, a new main channel of the river with a more straight stream formed, which was further supported by construction of weirs to direct the stream into a channel suitable for navigation. This has had detrimental impacts on the original habitats – the inshore habitats reduced considerably, large floodplain areas disappeared and the connectivity between the river and floodplains became limited [5, 12]. The geomorphological processes also altered as the erosive forces were suddenly concentrated in the main channel that resulted in deepening of the riverbed. In the 1980s and early 1990s, massive excavation of gravel at Bratislava just speeded up these processes, and as a result not only the bottom of the main channel sank down but the communication between the sidearm system of the inundation area and the main channel got very limited. The last natural flood in the inundation area below Bratislava, so important for reproduction of fishes, occurred in 1992 [12].

Thus, the river regulations initiated trends that still continue: lowering of the water table and deepening of the riverbed, combined with sedimentation processes in sidearms leading to permanent changes and a loss of aquatic habitats [5].

6.2 Dams and Reservoirs

Another ecological concern is associated with the construction of hydropower dams. The Danube has a high hydroelectric potential that has been largely exploited by 52 power dams in the Upper Danube and three major barrages in the Middle Danube – Gabčíkovo, Iron Gates 1 and 2 [5].

The Gabčíkovo Hydroelectric Scheme (GHS) has affected the fish communities in the Slovak stretch (Middle Danube), considerably. However, GHS was not the first construction that had such a detrimental impact on the Danubian fish communities. Iron Gate (Serbia/Romania), as well as numerous dams in Germany and Austria, had been built up earlier. Nevertheless, diverting the former Danube main stream into the new artificial canal in 1992 interrupted and/or damaged natural processes in the inland delta of the Danube. Soon after the GHS started to work, the communication between the sidearm system and the former main stream was blocked and the hydrological regime changed, dramatically. The network of sidearms was divided into isolated sections and their character changed from lotic to lenitic. A number of smaller oxbows also became isolated. At present, some of the main branches are permanently fed by water from the new channel and thus have again a predominantly lotic character. However, a uniform littoral zone has emerged in the original riverbed of the Danube, with strongly reduced water levels. The littoral zone has shifted towards the middle of the riverbed, and therefore, many natural shelters as well as spawning grounds disappeared. As a result, both abundance and species diversity have decreased in the littoral zone of the former main stream [12]. The relative abundance of eurytopic, mostly phytophilous species with a wide ecological tolerance, increased. In contrast, rheophilous species, predominant in the sidearms in the past, have become subdominant or recedent. Abundance of almost all species of fishes, especially predators, has decreased considerably.

However, this is not only a consequence of the environmental changes associated with GHS but also a consequence of high pressure from anglers and poachers violating the fishery legislation in 1990s. The age structure of many populations has also changed – specimens of higher age classes are now very rare and the rate of reproduction rather low. The relative abundance declined mainly in such species as pike, pikeperch and wels. In contrast, species as burbot (*Lota lota*) and/or zingel (*Zingel zingel*) benefit from the presence of invasive gobies that have become their dominant prey. Recently, there have been several attempts to revitalise the former sidearm system of the inland delta, and there is a hope that many of the former habitats will be restored. Nonetheless, it will take decades to re-establish or at least to approach the original diversity in local fish communities.

In Lower Danube, construction of Iron Gate 1 (river km 942.5) in 1970 and Iron Gate 2 (river km 863) in 1984 interrupted longitudinal connectivity of the river and resulted in a physical separation from the Middle Danube [5]. Subsequently, side levees separated the main channel of the river from its floodplain, which lead to a serious impact on the overall environmental situation and fisheries. The area of the former floodplain saturated by natural floods was reduced to 15% of its original size (approximately 5000 km^2). The discharge regime, the transport of suspended sediments and bed load as well as the daily water level fluctuation in the Bulgarian and Romanian stretches of the Danube also changed, considerably. The negative impacts of these changes as well as impacts of other dams in the Danube on fish communities and fisheries have been discussed by Schiemer et al. [5].

6.3 Navigation

Several recent studies from all the three sections of the Danube have addressed the essential role of the littoral zone, including the shoreline, for long-term survival of native fish communities and for keeping their natural composition, diversity and structure [14–16].

The results from the JDS2 suggest that navigation has a negative impact on fish community, and this is probably most intensive in the Upper Danube [7]. Moving vessels generate large waves that can drift larvae or beach out juveniles and affect thus the reproductive success of some species, such as barbel and nase. Heavy navigation can therefore result in changes in population structure of species that depend on the littoral zone. For example, Wiesner et al. [7] have reported clear differences between the population structure of barbel and nase at Kelheim (a site without navigation) and Jochenstein (a site with a narrow channel and navigation). It also appears, however, that further downstream, the negative impact of navigation decreases, probably due to the increasing width of the Danube [7].

7 Non-native and Invasive Species

A total of 69 species of fishes were recorded within the recent surveys of the Danube [6, 12], a number that suggests a high diversity of the Danubian fish community. However, as many as 12 of these species are not native in the Danube, at least not in its whole course, and a total of 18 non-native species have been ever recorded in the Danube (Table 5).

All of these species have been introduced to the Danube by humans, some of them intentionally, but most of them unintentionally. When assessing ecological status of a river, it is important to distinguish between non-native species and those that have become invasive. There have been numerous debates on what it means to be invasive, and various definitions have been proposed (e.g. Copp et al. [9]). Very often, invasive organisms are considered “native or alien species that spread, with or without the aid of humans, in natural or seminatural habitats, producing a significant change in composition, structure, or ecosystem processes, or cause severe economic losses to human activities” (see Paunović et al.). However, to assess whether a change in composition, structure or ecosystem processes is significant or not depends on how we define what is “significant” and what is not, which is often a subjective judgement rather than a scientific analysis. Similarly, what is the boundary between severe and less than severe economic losses to human activities may also vary from opinion to opinion. Finally, assessments of the impacts of non-native species to ecosystems and/or economies, supported by scientific data, are not always available. Therefore, in this chapter, non-native species are considered those that had not occurred in the Danube prior to introduction by humans, whereas invasive species are considered only those from them that

Table 5 A list of species of fishes (+) ever reported from the Danube (data from Schiemer et al. [5]), found recently in the Austrian, Hungarian and Romanian parts of the Danube [5], found during JDS2 in the Upper, Middle and Lower Danube [7], found during the monitoring of the Čunovo–Sap section in 1991–2011 (data from J. Černý [8]) and confirmed recently, i.e. collected recently, in any section of the Danube (results from JDS2 and monitoring together)

Species	reported ever	Sch. et al. (2004)	JDS2 Upper	JDS2 Middle	JDS2 Lower	1999-2011	confirmed recently
Abramis ballerus (Linnaeus, 1758)	+	+	-	+	+	+	+
Abramis brama (Linnaeus, 1758)	+	+	+	+	+	+	+
Abramis sapa (Pallas, 1814)	+	+	+	+	+	-	+
Acipenser gueldenstaedtii Brandt et Ratzeburg, 1833	+	+	-	-	-	-	-
***Acipenser nudiventris* Lovetzky, 1828**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Acipenser ruthenus Linnaeus, 1758	+	+	-	+	+	-	+
Acipenser stellatus Pallas, 1771	+	+	-	-	+	-	+
***Acipenser sturio* Linnaeus, 1758**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Alburnoides bipunctatus (Bloch, 1782)	+	+	+	+	-	-	+
Alburnus alburnus (Linnaeus, 1758)	+	+	+	+	+	+	+
Alosa immaculata Bennett, 1835	+	+	-	-	-	-	-
Alosa maeotica (Grimm, 1901)	+	+	-	-	-	-	-
Alosa tanaica (Grimm, 1901)	+	+	-	-	-	-	-
Ameirurus melas (Rafinesque, 1820)	+	+	-	+	-	+	+
Ameiurus nebulosus (Lesueur, 1819)	+	+	-	+	+	-	+
Anguilla anguilla (Linnaeus, 1758)	+	+	+	+	-	-	+
Aspius aspius (Linnaeus, 1758)	+	+	+	+	+	+	+
Atherina boyeri Risso, 1810	+	+	-	-	-	-	-
Barbatula barbatula (Linnaeus, 1758)	+	+	+	-	-	+	+
Barbus barbus (Linnaeus, 1758)	+	+	+	+	+	+	+
Barbus peloponnesius Valenciennes, 1842	+	+	-	-	-	-	-
Benthophiloides brauneri Belling et Iljin, 1927	+	+	-	-	+	-	+
Benthophilus stellatus (Sauvage, 1874)	+	+	-	-	+	-	+
Blicca bjoerkna (Linnaeus, 1758)	+	+	+	+	+	+	+
Carassius carassius (Linnaeus, 1758)	+	+	-	+	+	+	+
Carassius gibelio (Bloch, 1782)	+	+	+	+	+	+	+
Clupeonella cultriventris (Nordmann, 1840)	+	+	-	-	-	-	-
Cobitis elongatoides Bacescu et Mayer, 1969	+	+	-	+	+	+	+
Coregonus albula (Linnaeus, 1758)	+	+	-	-	-	-	-
Coregonus peled (Gmelin, 1788)	+	+	-	-	-	-	-
Coregonus renke (Schrank, 1783)	+	+	-	-	-	-	-
Cottus gobio Linnaeus, 1758	+	+	+	-	-	+	+
***Cottus poecilopus* Heckel, 1836**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Ctenopharyngodon idella (Valenciennes, 1844)	+	+	-	-	-	-	-
Cyprinus carpio Linnaeus, 1758 (wild form)	+	+	+	+	+	+	+
Esox lucius Linnaeus, 1758	+	+	+	+	+	+	+
Eudontomyzon mariae (Berg, 1931)	+	+	-	+	-	-	+
Gasterosteus aculeatus (Linnaeus, 1758)	+	+	+	-	-	+	+
Gobio albipinnatus Lukasch, 1933	+	+	+	+	+	+	+
Gobio gobio (Linnaeus, 1758)	+	+	+	+	-	-	+
Gobio kesslerii Dybowski, 1862	+	+	-	-	-	+	+
Gobio uranoscopus (Agassiz, 1828)	+	+	-	-	-	-	-
***Gobius ophiocephalus* (Pallas, 1814)**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Gymnocephalus baloni Holčík et Hensel, 1974	+	+	+	+	+	+	+
Gymnocephalus cernuus (Linnaeus, 1758)	+	+	+	+	+	+	+
Gymnocephalus schraetser (Linnaeus, 1758)	+	+	+	+	+	-	+
Hucho hucho (Linnaeus, 1758)	+	+	+	-	-	-	+
Huso huso Linnaeus, 1758	+	+	-	-	-	-	-
Hypophthalmichthys molitrix (Valenciennes, 1844)	+	+	-	+	-	-	+
Hypophthalmichthys nobilis (Richardson, 1845)	+	+	-	-	-	-	-
***Chalcalburnus chalcoides* (Gueldenstaedt, 1772)**	**+**	**-**	**-**	**-**	**-**	**-**	**-**
Chondrostoma nasus (Linnaeus, 1758)	+	+	+	+	+	+	+
Ictalurus punctatus (Rafinesque, 1818)	+	+	-	-	-	-	-
Knipowitschia cameliae Nalbant et Otel, 1995	+	+	-	-	-	-	-
Knipowitschia caucasica (Berg, 1916)	+	+	-	-	-	-	-
Lepomis gibbosus (Linnaeus, 1758)	+	+	+	+	+	+	+
Leucaspius delineatus (Heckel, 1843)	+	+	-	-	-	-	-
Leuciscus borysthenicus (Kessler, 1859)	+	+	-	-	-	-	-
Leuciscus cephalus (Linnaeus, 1758)	+	+	+	+	+	+	+
Leuciscus idus (Linnaeus, 1758)	+	+	+	+	+	+	+

(continued)

Table 5 (continued)

Leuciscus leuciscus (Linnaeus, 1758)	+	+	+	+	-	+	+
Liza aurata (Risso, 1810)	+	+	-	-	-	-	-
Liza saliens (Risso, 1810)	+	+	-	-	-	-	-
Lota lota (Linnaeus, 1758)	+	+	+	+	-	+	+
Micropterus salmoides (La Cepéde, 1802)	+	+	-	-	-	-	-
Misgurnus fossilis (Linnaeus, 1758)	+	+	-	+	-	+	+
Mugil cephalus Linnaeus, 1758	+	+	-	-	+	-	+
Neogobius eurycephalus (Kessler, 1874)	+	+	-	-	+	-	+
Neogobius fluviatilis (Pallas, 1814)	+	+	-	+	+	+	+
Neogobius gymnotrachelus (Kessler, 1857)	+	+	-	+	+	+	+
Neogobius kessleri (Gunther, 1861)	+	+	+	+	+	+	+
Neogobius melanostomus (Pallas, 1814)	+	+	+	+	+	+	+
Neogobius syrman (Nordmann,1840)	+	+	-	-	-	-	-
Oncorhynchus mykiss (Walbaum, 1792)	+	+	+	-	-	-	+
Pelecus cultratus (Linnaeus, 1758)	+	+	-	+	+	-	+
Perca fluviatilis Linnaeus, 1758	+	+	+	+	+	+	+
Percarina demidoffi (Nordmann, 1840)	+	+	-	-	-	-	+
Perccottus glenii Dybowski, 1877	+	-	-	+	+	-	+
Phoxinus phoxinus (Linnaeus, 1758)	+	+	+	-	-	-	+
Platichthys flesus (Linnaeus, 1758)	+	+	-	-	-	-	+
Proterorhinus Marmoratus (Pallas, 1814)	+	+	+	+	+	+	+
Pseudorasbora parva (Temminck et Schlegel, 1842)	+	+	-	+	+	-	+
Pungitius platygaster (Kessler, 1859)	+	+	-	-	-	-	-
Rhodeus sericeus (Pallas, 1776)	+	+	+	+	+	+	+
Rutilus meidingeri (Heckel, 1851)	+	+	-	-	-	-	-
Rutilus pigus (La Cepéde, 1803)	+	+	+	+	-	-	+
Rutilus rutilus (Linnaeus, 1758)	+	+	+	+	+	+	+
Sabanejewia sp.	+	+	-	+	-	-	+
Sabanejewia bulgarica Drensky, 1928)	+	+	-	-	-	-	-
Salmo trutta m. fario (Linnaeus, 1758)	+	+	+	-	-	-	+
Salvelinus fontinalis (Mitchill, 1814)	+	+	-	-	-	-	-
Sander lucioperca (Linnaeus, 1758)	+	+	+	+	+	+	+
Sander volgensis (Gmelin, 1788)	+	+	+	+	+	+	+
Scardinius erythrophthalmus (Linnaeus, 1758)	+	+	+	+	+	+	+
Silurus glanis Linnaeus, 1758	+	+	+	+	+	+	+
Syngnathus abaster Risso, 1859	+	+	-	-	+	-	+
Thymallus thymallus (Linnaeus, 1758)	+	+	+	-	-	-	+
Tinca tinca (Linnaeus, 1758)	+	+	+	+	+	+	+
Umbra krameri Walbaum, 1792	+	+	-	-	-	-	-
Vimba vimba (Linnaeus, 1758)	+	+	+	+	+	+	+
Zingel streber (Siebold, 1863)	+	+	+	-	-	+	+
Zingel zingel (Linnaeus, 1766)	+	+	+	+	+	+	+
Total number	102	96	45	51	46	42	69

Species in bold are considered extinct or not present in the Danube any longer, light grey indicates non-native species and their presence and dark grey indicates invasive species and their presence have established viable populations, achieved high abundance and tend to spread actively into new areas of distribution. Thus, the current list of invasive species of fishes in the Danube contains nine species (black bullhead, gibel, pumpkinseed, monkey goby, racer goby, bighead goby, round goby, Amur sleeper and topmouth gudgeon), though not all of them demonstrate the above invasive attributes in their Danubian habitats, and not all of them are invasive throughout the whole course of the river.

The black bullhead (*Ameiurus melas*) was introduced to Europe in the late nineteenth and early twentieth centuries and is now established in many European countries [9, 17]. With its tendency to overpopulate and spread rapidly, black bullhead is considered to be a species with a notable invasive potential [18]. Nevertheless, a recent study suggests that, in the Danubian area, black bullhead has been spreading, thanks to increased propagule pressure (i.e. continuing introductions by humans) rather than on its own [19]. This is also supported by the fact that during JDS2 black bullhead was recorded in the Middle Danube but not in the Upper or Lower Danube (Tables 1, 2 and 3). On the other hand, once a population has been established locally, its abundance has the potential to grow very fast (see Table 4, 2005–2011).

Gibel (*Carassius gibelio*) is reported to have appeared in the Lower Danube in the first quarter of the last century and to have invaded the Middle and Upper Danube, afterwards [20]. Its invasion was facilitated by its gynogenetic reproduction – the entire population contained females, exclusively. Gibel females used males of other cyprinid species for reproduction (e.g. Balon [21] and Holčík [22]). In the Slovak part of the Danube, the first males were observed in 1992, and since then the population of gibel, being well established, consists of both sexes. It appears that in the Middle Danube the abundance of gibel has stabilised at much lower levels compared to Lower Danube, where the species still keeps extremely high relative abundance (Tables 2 and 3).

One of the most successful of the non-native fish species in Europe is pumpkinseed *Lepomis gibbosus* [23, 24], which was first introduced around 1880 [25] and during the nineteenth and twentieth centuries became established in many European countries [26]. Pumpkinseed has been reported to be very common in the Slovak stretch of the Danube, especially the floodplain areas and the lower parts of the Danube's tributaries [27, 28]. Indeed, pumpkinseed appears to be the third most abundant species of fish in the Gabčikovo–Sap section (Table 4), and it has been recorded in all three sections of the Danube during JDS2 (Tables 1, 2 and 3).

Over the last two decades, four species of gobies, native to Lower Danube, have invaded the Middle and Upper Danube: bighead goby, racer goby, monkey goby and round goby. The expansion of these species was facilitated by human activities (e.g. Wiesner [29]), and all these species spread rapidly [9].

Bighead goby originally inhabited the brackish zone in northern and western shores of the Black Sea and lower parts of rivers entering the sea between the rivers Danube and Dnepr [30]. The species appeared in the Middle Danube in the early 1990s, first found in Hungary [31] and then (in 1994) in eastern Austria [32, 33].

The first records of bighead goby in the Slovak part of the Danube were in June 1996 [34, 35].

However, bighead goby, the first Ponto–Caspian gobiid invader of the Middle and Upper Danube and previously the most abundant and widely distributed of the invading gobiids, has been recently outnumbered in both abundance and distribution dynamics by a subsequent invader, the round goby [36]. Indeed, round goby has recently greatly extended its native range from the Black Sea, Caspian Sea and surrounding waters and invaded not only the Middle and Upper Danube but also the River Moscow and ultimately the Baltic Sea [9] as far as the German coast [37]. Round goby has invaded not only across Europe but also Great Lakes in North America. In the Danube, Bănărescu [20] reported the upriver expansion of round goby since the 1960s, but it had been known earlier as far upstream as Vidin [38, 39]. In 1997, the species was found for the first time in the Serbian part of the Danube [40]. By 2000, it was present in the main Danube near Vienna, Austria. Since then, it has been observed in modest abundance, mainly in industrial harbours and to a lesser extent along the banks of the main channel [29]. In 2003, round goby was detected as the fourth new gobiid species in the Slovak catchment of the Danube [9, 41].

Monkey goby established populations in Hungary already in the 1980s (Lake Balaton and River Tisza; [42]). In Slovakia, monkey goby was first observed in 2001 in the Danube and its tributaries, including the River Hron [43]. However, the current distribution and habitat preferences of monkey goby in the Middle and Upper Danube differ from the other two invasive goby species [44]. Also, monkey goby has not achieved the same high densities as the bighead and round gobies [45], and because of high habitat specialisation, it appears that monkey goby will not be so widespread and abundant as round and/or bighead goby.

Racer goby has also invaded both the Middle and Upper Danube and, similar to round goby, has reached the River Rhine, where it was first recorded in 2010 [46]. In the Danube, both distribution and abundance of racer goby have been rather limited, especially compared to round and bighead gobies (Tables 1, 2, 3 and 4).

Amur sleeper (*Perccottus glenii*), highly invasive elsewhere (e.g. Grabowska et al. [47]), has been recorded only sporadically in the middle section of the Danube (Table 2). Nevertheless, its extremely high invasive potential makes Amur sleeper a hot candidate to become a highly invasive species in the inundation area across all sections of the Danube. Therefore, this species deserves special attention, with the emphasis to risk assessment and prevention.

One of the most successful invasive species in Europe in recent times has been the topmouth gudgeon *Pseudorasbora parva*, a cyprinid native to East Asia that has achieved an almost pan-Eurasian distribution within less than 40 years [48, 49]. The species was accidentally introduced as a contaminant of imported fish consignments, such as grass carp, which arrived in Romania in 1961 and 1962 [50]. Topmouth gudgeon subsequently dispersed through most of Europe, again as a contaminant of fish consignments and by natural dispersal via watercourses [9, 51, 52]. A detailed recent review has even assigned topmouth gudgeon to be the most compelling fish invasion in the world [53]. Therefore, even if this species was recorded only in the Lower Danube during JDS2, it is also present in the Upper and Middle Danube and certainly represents a great risk for the native fish communities, especially because topmouth gudgeon is a host of two highly pathogenic parasites – *Anguillicola crassus* and the rosette agent *Sphaerothecum destruens* [54, 55].

Biological invasions may lead to the extirpation of native species, resulting in an overall decline in biodiversity [56]. Indeed, for example, in the Slovak part of the Middle Danube, invasive species of fishes, especially two species of gobies (round and bighead), topmouth gudgeon and black bullhead, have become a major problem for native fish communities. Small benthic native species, e.g. bullhead, white-finned gudgeon and stone loach, virtually disappeared from the local fish communities [12]. However, a wide-scale analysis of the impact of invasive species on Danubian fish communities is still lacking. Accidental introductions, which should be regarded as biological pollution [57], can often lead to irreversible ecological impacts on native ecosystems [53]. Therefore, a predictive risk assessments and management strategies of introductions and invasions of non-native fishes should be developed for the Danube and applied subsequently at an international level.

8 Conclusions

A total of 69 species of fishes that were recorded within the recent ichthyological surveys of the Danube may seem to demonstrate a high diversity of the current Danubian fish community. However, the structure of this community, especially species composition (high predominance of bleak and high relative densities of invasive species), does not provide an ideal picture at all. Indeed, environmental pressures resulting from human activities have serious negative impacts on the Danubian ecosystems. River regulations, constructions of dams and reservoirs, deterioration of water quality, navigation, etc., these all have reduced most of the native populations of fishes, and several species have been even extinct. To prevent further deterioration of the Danubian fish community, the human activities with potential negative impacts should be reconsidered, and programmes of restorations should be developed. Occasional high water levels, such as that in August 2002, clearly demonstrate that the sidearm systems have vital importance for fish in the Danube, as they serve as spawning and nursery grounds for most species. Therefore, special attention should be paid to restoration of both longitudinal and transversal connectivities between the sidearms and the main channel of the river, especially in the Upper and Middle Danube. Finally, because of serious ecological as well as economic and social threats posed by biological invasions, a predictive risk assessments and management strategies for introductions and invasions of non-native fishes should be developed for the Danube and applied subsequently at an international level.

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Invasive Alien Species in the Danube

Momir Paunović, Béla Csányi, Predrag Simonović, and Katarina Zorić

Abstract Invasive alien species (IAS) have been recognized as one of the major threats to native biodiversity in the Danube Basin. The aim of this paper is to present the state of the art regarding IAS along the Danube River and its main tributaries. The work is mainly based on the results of the Danube research expeditions, Joint Danube Survey 2 (2007), Joint Danube Survey 1 (2001) and AquaTerra Danube Survey (2004), but other recent data on IAS were taken into consideration, as well. The complexity of the problem with IAS could be illustrated by the fact that six species of neophytes, 19 alien macroinvertebrates and 15 - non-native fish species were recorded during JDS2. The total number of alien species recorded, as well as their frequency and abundance along the Danube, indicates high level of biological contamination. Despite the fact that IAS have been in the focus of the research in the Danube Basin for the last 15 years, we still do not have enough data on their exact distribution and migration. A lot of additional work concerning detection, monitoring, assessment of their impacts and management is necessary in order to deal with the IAS problem properly in river basin management planning.

Keywords Aquatic invasive species, Biological contamination, Biological invasions, Danube River, Neophyte, Neozoa

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Contents

1 Introduction

Historical change in the environment, especially the fluctuations in climate, led to a change in the distribution of organisms. Those processes have been accelerated by human influence. Pollution, hydromorphological degradation and the invasive alien species (IAS) strongly influence the aquatic ecosystems.

Humans have served as both accidental and deliberate dispersal agents of organisms for over 500 years. During the last century, there is an increasing cognizance in relation to the mainly human-aided dispersal of species beyond their natural range of distribution. The consequences of biotic invasions are diverse and complex, since invaders can alter fundamental ecological properties such as dominant species in a community, productivity and nutrient cycling and thus can alter the structure and function of the ecosystem. Anthropogenic distribution of plants and animals is considered within the major threats to the biodiversity [1–3]. Aquatic ecosystems are not an exception concerning this aspect of disturbance. Ballast water of ships, fish stocking and aquaculture were pointed as prospective agents of dispersal of nonindigenous species.

Human-mediated introductions of invasive alien species in European inland and coastal waters are considered as a serious environmental issue which requires development of relevant management approaches [4–6]. In the context of the EU Water Framework Directive [7], IAS represent a significant biological pressure.

Parts of European inland waterways that are highly biologically contaminated are probably irreversibly changed with respect to fauna composition. Some communities are now dominated by alien species. In some waterbodies alien-dominated communities have shown very stable composition of dominant species for over a decade. According to Arbačiauskas et al. [8] such newly established communities may be defined as xenocommunities, in analogy to xenodiversity (sensu [9]).

IAS have been recognized as one of the major threats to native biodiversity within the Danube Basin [10–14].

Canals can provide conduits for species to spread between previously separate biogeographic regions either by active movement and drift or as a result of ship transport [15, 16]. After the construction of the Rhine-Main-Danube channel, the Danube became an important invasion route. Several authors recorded the spread of nonindigenous species along the Danube (in both directions, upstream and downstream), as well as the expansion of neobiota from the Danube to its tributaries.

The Danube River is a part of the so-called Southern Invasive Corridor [15] and a part of the European Invasion Network [12, 17].

Categorization of species as indigenous (i.e. native) or nonindigenous (i.e. alien) is not a routine work. Owing to the huge and long-term historical global movements of the biota (human aided and natural) as well as due to the lack of relevant data that would either support or disprove classification of particular taxa as native or alien, it is clear that there are many species that cannot be reliably assigned to either category. Alien species are those that take up residence in a biogeographical area, such as a river catchment, where they were previously unknown [8].

Clout et al. [18] defined the invasive alien species as an alien that becomes established in natural or seminatural ecosystems or habitat that is an agent of change and threatens the native biological diversity. In their native habitat, where they have genetically and ecologically evolved, these organisms may not be a high-risk proposition. However, when aquatic and terrestrial species are transported to ecosystems outside their established range, problems can be caused for native organisms, disturbing the natural ecosystems by altering population, community and ecosystem structure and function.

The species of native or introduced character that cannot be defined is named as cryptogenic [19]. Richardson et al. [20] summarized:

- Introduction is the deliberate or unintentional (accidental) transfer and/or release, by direct or indirect human agency, of an organism(s) into the wild or into locations not completely isolated from the surrounding environment, by humans in geographical areas where the taxon (species, subspecies, race or variety) is not native.
- Invasion is a collection of events and processes related to appearance and impacts on communities and ecosystems of alien species.
- Translocation is the introduction of a species from one part of a political entity (country) in which it is native to another part of the same country in which it is not native.
- Native or indigenous refers to a taxon that occurs naturally in a geographical area, with dispersal occurring independent of human intervention, whether direct or indirect, intentional or unintentional.
- Non-native or nonindigenous refers to a taxon that does not occur naturally in a geographical area, i.e. it did not previously occur there or its dispersal into the area was mediated or facilitated directly or indirectly by humans, whether deliberately or unintentionally.
- Invasive organisms are native or alien species that spread, with or without the aid of humans, in natural or seminatural habitats, producing a significant change in composition, structure or ecosystem processes, or cause severe economic losses to human activities.
- Acclimatized species (or taxa) are those that are able to complete part or most of their life cycle in the wild in an alien environment or climate, but are unable to reproduce and sustain a population without the support of humans; naturalized refers to a non-native taxon that, following introduction, has established self-

sustaining populations in the wild and has been present of sufficient duration to have incorporated itself within the resident community of organisms, achieving or overcoming geographical, environmental and reproductive barriers.

- Vagrant refers to a taxon that, by natural means, moves from one geographical region to another outside its usual range or away from usual migratory routes and that does not establish a self-sustaining population in the visited region.
- Casual refers to introduced species that is unable to sustain without human aid despite its obvious ability to reproduce in the novel environment.

Principal pathways of aquatic IAS spread in Europe and qualitative descriptors of principal human activities involved in the spread of IAS have been identified recently (see in [17]).

Considering the current gap in addressing invasive alien species in European river basin management, our goal was also to contribute to the knowledge on the issue of biological invasions, to raise public awareness regarding the problem of biological invasions.

In relation to the growing concern about biological invasions in the Danube River and interconnected ecosystems [13, 21–23], the aim of this work is to provide information on the recent status of nonindigenous species that are observed in the Danube River and investigated tributaries, to give brief comments on their distribution, as well as to discuss agents of introduction and factors that influence their successful dispersal and naturalization.

The quantitative assessment of negative impacts of alien species is difficult and requires a comprehensive research and database efforts [24]. Quantitative estimates of "biological pollution" in aquatic ecosystems sensu Elliott [25] are lacking [26]. A more practical approach for assessing the impact of IAS on the aquatic communities, therefore, may be to assume that their effect is proportional to their occurrence and abundance within the invaded community. In such case, alien species would be considered as biological contaminants rather than biological pollutants, and "biological contamination" (i.e. biocontamination) means the presence of alien taxa regardless of their abilities to cause negative ecological and/or socio-economic impacts (see also [27]). Therefore, the procedure for evaluation of "biological contamination" proposed by Arbačiauskas et al. [8] was used in this chapter.

2 Methods

The considerations on IAS in this work are primarily based on the results of the Joint Danube Survey 2 (JDS2) from 2007 [28], but also taking into the account the results of other Danube Surveys – the Joint Danube Survey 1 [29], the International Tisza Survey [30] from 2001 and the AquaTerra Danube Survey [31]. In addition, recent data on IAS have been used [23, 32–35] in order to provide more complete

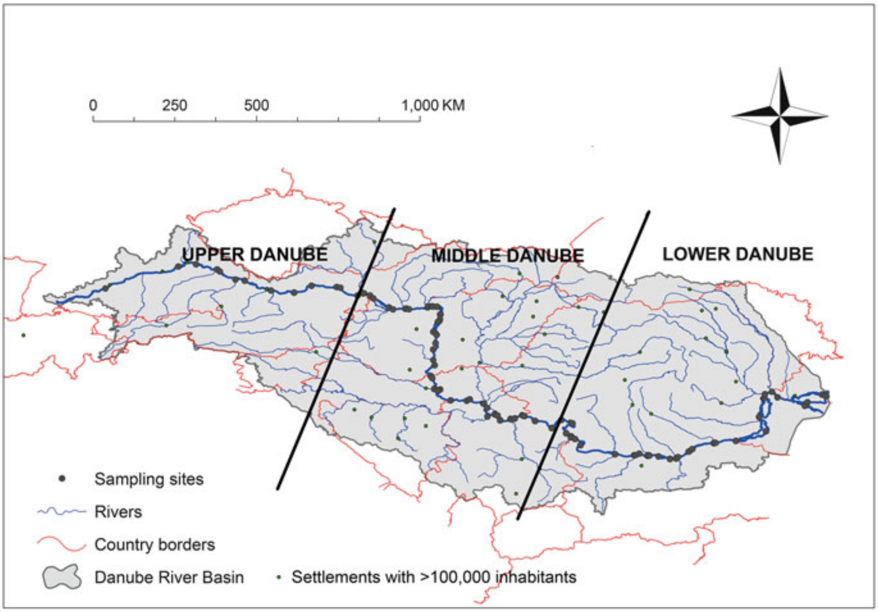

Fig. 1 Stretch of the Danube River covered by the work with the main Danube stretches and sampling sites indicated

information on IAS, especially in the largest tributaries that are also part of the Southern Invasive Corridor [17].

Altogether 2,580 km of the Danube River is covered by the data, including the mouths of 17 major tributaries. Sampling was performed at 96 sites (Fig. 1).

The list of IAS has been prepared based on several available datasets.

The evaluation of the relative abundance and pressure of nonindigenous species on the Danube Basin has been done based on the JDS2 results, in regard to sampling sites, as well as in respect to Danube sections, being considered as assessment units (sensu [17]).

In general, the term Assessment and Management Unit (AU) [25] could be applied to each unit of evaluation of biological contamination or pollution and could be used to describe each part of an aquatic habitat. The selection method for appropriate AUs depends mainly on two elements – the aim of the assessment and the type of waterbody [17]. With regard to assessment aims, three main levels can be identified: management, monitoring and research.

Having in mind the main Danube sections that are different in respect to overall natural character [29], three AUs have been selected – Upper, Middle and Lower Danube (Fig. 1). The selection has been based also on the fact that species that are native for the lower part of the Danube (Ponto-Caspian species) could be non-native or cryptogenic for the middle or upper stretch. The latter occurrence of some taxa in particular stretches could be also the consequence of recolonization phenomenon. Namely, for the majority of the Ponto-Caspian taxa, we do not have the data on its historical distribution along the Danube River. So, their absence in newer history could be the consequence of pollution and of a high level of hydromorphological pressures.

The evaluation based on larger areas has been selected in order to reduce the possible uncertainties about natural distribution of Ponto-Caspian taxa. In addition, larger assessment areas have been pointed as the main target of the management level of assessment of biological contamination and pollution. Large-scale assessment units are in the focus of conceptual model of risk assessment of invasive alien species introductions via European inland waterways developed by [17], and in the same time they are suggested to be the object of management in order to deal with IAS.

3 IAS: The Danube Situation

Within the ALARM project (European Commission Sixth Framework Programme Integrated Project ALARM, contract GOCE-CT-2003-506675), the database on Alien Invasive Species within Southern Invasive Corridor (AISSIC database) has been developed by the University of Belgrade, Institute for Biological Research, Belgrade.

At present, the AISSIC database contains 3,600 records, with total of 129 alien and cryptogenic taxa covered (24 aquatic macrophytes, 70 macroinvertebrates, 26 fish, 1 amphibian and 8 fish parasite species) within the Southern Invasive Corridor, which includes the main tributaries of the Danube, as well.

The situation with IAS along the Danube River could be assessed based on the results of three Danube expeditions. JDS1 [29] confirmed that the building of the Main-Danube Canal and its opening in 1992 removed a natural biogeographical barrier between the Rhine and the Danube. Since that time a mutual fauna transfer occurs. Among the aquatic macroinvertebrates collected during the JDS1, there are frequent and abundant neozoa species, which today already inhabit the Rhine river system. The JDS1 results especially emphasized the occurrence and migration of crustaceans. The taxon list contains several Amphipoda species that are native at the Ponto-Caspian area: *Dikerogammarus haemobaphes*, *D. villosus*, *Obesogammarus obesus*, *Echinogammarus trichiatus*, *E. ischnus*, *Chelicorophium curvispinum*, the Mysidacea *Limnomysis benedeni*, *Hemimysis anomala* and the Isopoda species *Jaera istri*. Further, the JDS1 data confirmed the spreading of the mussel species *Corbicula fluminea* along the Danube River. According to Literáthy et al. [29], *C. fluminea* migrated into the Danube River from the Rhine River. The species was recorded for the first time in the Danube River in 1998 in the Hungarian stretch [36] in the vicinity of the nuclear power plant of Paks (1,533 rkm). Several new sites of *C. fluminea* in the Danube River were revealed in 2000 during the Bioindicators Study [37] from the Lower Serbian Danube: Smederevo (rkm 1,115), Mala Vrbica (rkm 924) and Radujevac (rkm 849). This invasive species proved to be more widespread on the Lower Danube section including the Danube Delta in 2001 during the JDS1 survey [29].

The abundant presence of *Sinanodonta woodiana* (Bivalvia: Unionidae) was repeatedly reported by JDS1 [29] in the lower Hungarian stretch having a mass production at the cooling water outlet of the Paks Nuclear Power Plant. During JDS1, *Dreissena polymorpha* was also found in high relative abundance along the entire Danube stretch, having a peak in the lower section of Ráckevei-Soroksári Danube arm within the middle stretch. JDS1 dataset also confirmed the presence of Ponto-Caspian species *Dendrocoelum romanodanubiale* (Turbellaria) and *Hypania invalida* (Polychaeta), which have spread since 1993 in the opposite direction: from the Danube into the Main and the Rhine rivers via the Main-Danube Canal.

Further, the AquaTerra Danube Survey [21, 31] provided the data on high relative abundance of nonindigenous macroinvertebrates along the sector of the Danube between Klosterneuburg (Austria, 1,942 rkm) and Vidin-Calafat (Bulgaria-Romania, 795 rkm). Thus, *Dikerogammarus villosus*, *D. bispinosus*, *Chelicorophium curvispinum*, *Jaera istri and Limnomysis benedeni* were reported to be abundant along this considerable stretch of the Danube River.

The JDS2 provided more data on nonindigenous taxa compared to previous investigations. During the JDS2 [28] 6 alien macrophytes (Table 1), 20 alien and cryptogenic macroinvertebrates (Table 2) and 15 fish taxa were recorded (Table 3).

In regard to alien aquatic macrophytes, *Elodea nuttallii* had invaded the Danube River Corridor from the west to the east, replacing the earlier neophyte *Elodea canadensis*. According to JDS2 data [40] *Vallisneria spiralis* occurs in the stretch near Vienna only in floodplain, but in the Lower Danube, it was detected in the mainstream. *Lemna turionifera* was found in impounded reaches in Bulgaria. *Azolla* was native before the Ice Age and repopulated Europe from America. *Eichhornia crassipes* known as an overgrowing invader chocking everything underneath has to be classified as a “human impact”, but does not survive winter in the Danube Basin.

The helophyte *Chamaesyce glyptosperma* invaded the Danube Corridor up to Novi Sad, and *Xanthium strumarium* covers large areas along the banks in Romania and Bulgaria squeezing out most of the other helophytes. How climate change effects triggered, or enhanced such effects, is not completely clear at present, but other invaders are already present in Southern France and in Germany, with

Table 1 Neophytes recorded during JDS2

Genus name	Species name	Author	Origin
Azolla	*filiculoides*	Lam.	subtropic America
Eichhornia	*crassipes*	(Mart.) Solms	South America
Elodea	*canadensis*	Michx.	North America
Elodea	*nuttallii*	(Planch.) H. St. John	North America
Lemna	*turionifera*	Landolt	North America, East Asia
Vallisneria	*spiralis*	Linnaeus	Tropics, subtropics

Table 2 Alien macroinvertebrate species recorded during JDS2

Species	Status			Origin
	Upper Danube	Middle Danube	Lower Danube	
Dugesia tigrina	A	A	A	North America
Lithoglyphus naticoides[a]	C	C	N	Ponto-Caspian
Potamopyrgus antipodarum	NF	A	NF	Australia
Corbicula fluminalis	A	A	A	Asia
Corbicula fluminea	A	A	A	Asia
Sinanodonta woodiana	A	A	A	Asia
Dreissena cf. *bugensis*	NF	NF	A	Ponto-Caspian
Dreissena polymorpha	A	C	N	Ponto-Caspian
Hypania invalida	A	C	N	Ponto-Caspian
Branchiura sowerbyi	A	A	A	Asia
Hemimysis anomala	A	A	N	Ponto-Caspian
Limnomysis benedeni	A	A	N	Ponto-Caspian
Chelicorophium curvispinum	A	C	N	Ponto-Caspian
Dikerogammarus bispinosus	A	C	N	Ponto-Caspian
Dikerogammarus haemobaphes	A	C	N	Ponto-Caspian
Dikerogammarus villosus	A	C	N	Ponto-Caspian
Echinogammarus ischnus	A	C	N	Ponto-Caspian
Obesogammarus obesus	A	C	N	Ponto-Caspian
Jaera istri	A	C	N	Ponto-Caspian
Orconectes limosus	A	A	A	North America

A alien, *C* cryptogenic, *N* native, *NF* not found
[a]According to Adámek et al. [38], it is considered as nonindigenous for some tributaries of the Danube – the Morava and Dyje rivers

possible ways of propagation along the canal systems between western and eastern river basins.

It should be underlined that certain Ponto-Caspian species, e.g. gobies, pipefish, etc., could be native for the Lower Danube, but non-native for its upper part. In the

Table 3 Nonindigenous fish status in the Danube River drainage area (JDS2 dataset – [39] and unpublished data)

Species	Status			Origin
	Upper Danube	Middle Danube	Lower Danube	
Ameiurus melas	A	A	NF	North America
Ameiurus nebulosus	A	A	NF	North America
Ameiurus punctatus	NF	NF	A	North America
Anguilla anguilla	C	C	C	East Atlantic
Gasterosteus aculeatus	A	C	N	Ponto-Caspian
Hypophthalmichthys molitrix	A	A	A	Asia
Hypophthalmichthys nobilis	A	A	A	Asia
Lepomis gibbosus	A	A	A	North America
Neogobius fluviatilis	A	A	N	Ponto-Caspian
Neogobius gymnotrachelus	A	A	N	Ponto-Caspian
Neogobius kessleri	A	A	N	Ponto-Caspian
Neogobius melanostomus	A	A	N	Ponto-Caspian
Oncorhynchus mykiss	A	C	C	Aquaculture
Perccottus glenii	A	A	A	Asia
Polyodon spathula	NF	C	A	North America
Proterorhinus semilunaris	A	A	N	Ponto-Caspian
Pseudorasbora parva	A	A	A	Aquaculture
Syngnathus abaster	NF	A	N	Ponto-Caspian

A alien, *C* cryptogenic, *N* native, *NF* not found

case of the Middle Danube, due to lack of the data on exact distribution in the past, some species are considered as cryptogenic (indicated in Tables 2 and 3).

Based on the results of JDS2, nonindigenous taxa represent 4.53% of the total number of macroinvertebrate taxa. Alien taxa originating from Ponto-Caspian area, Asia, Australia and North America were found. According to JDS2 results, Ponto-Caspian species are the most prominent invaders (13 out of 20 taxa).

The ratio of alien macroinvertebrate taxa in regard to total number of macroinvertebrate taxa observed at each sampling site is presented at Fig. 2.

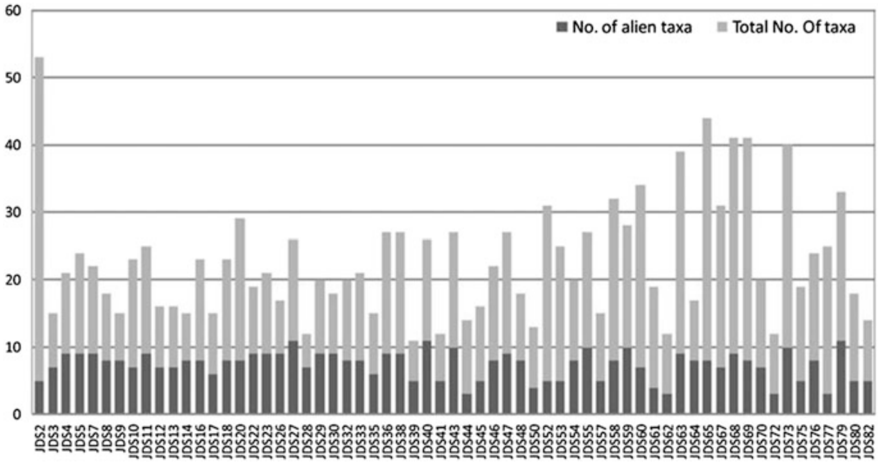

Fig. 2 The total number of macroinvertebrate taxa and number of alien macroinvertebrate taxa at sampling sites along the Danube based on JDS2 results

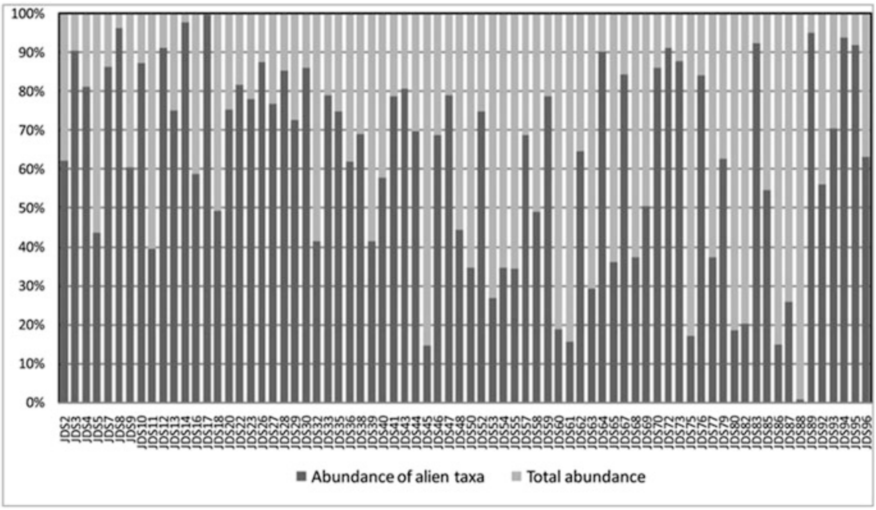

Fig. 3 The share of alien macroinvertebrate taxa in the total relative abundance of the benthic community at sampling sites along the Danube based on JDS2 results

At Fig. 3 the ratio of nonindigenous macroinvertebrates within the total macroinvertebrate community, based on JDS2 dataset, is presented.

According to Figs. 2 and 3, alien taxa are important components of the benthic community in regard to both the number of represented taxa and their relative abundance.

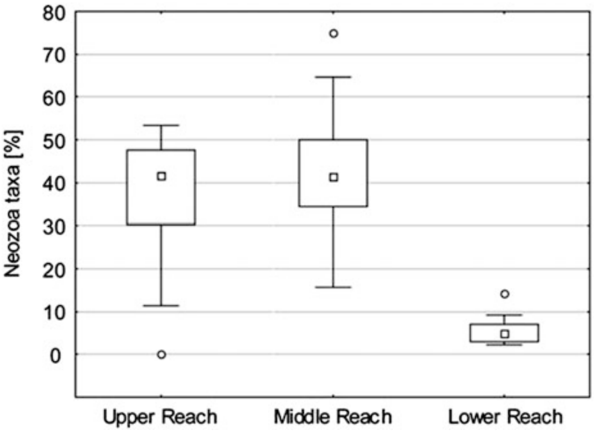

Fig. 4 Percentage participation of neozoa taxa in three main Danube sectors – the number of taxa

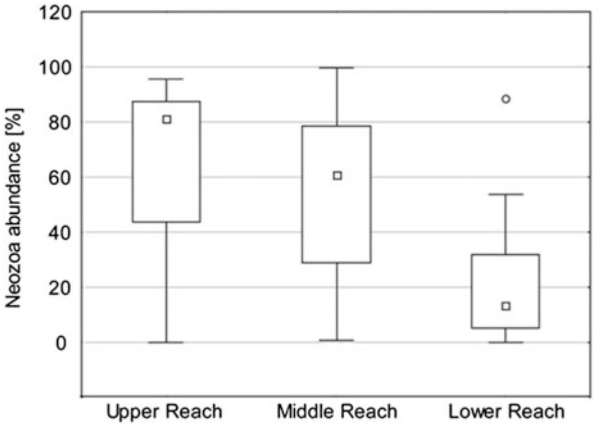

Fig. 5 Percentage participation of neozoa taxa in three main Danube sectors – relative abundance

If we consider the number of taxa and relative abundance, the taxa originating from the Ponto-Caspian region and Asia have the major influence to the Danube.

The distribution of neozoa taxa within the main Danube sectors is presented at Figs. 4 and 5.

The abundance and percentage of species of neozoa along the three reaches of the Danube indicate their essential importance for the ecosystem. Due to the abundance up to 90% within the Upper Reach or even 100% within the Middle Reach of the Danube samples, their impact on each applied assessment system becomes evident.

From what is presented in Figs. 2, 3, 4 and 5, it could be seen that neozoa dominate the Danube, not only locally, but they are distributed along the entire stretch.

Alien mollusc species *Corbicula fluminea*, *Anodonta woodiana*, *Dreissena polymorpha* and *D. bugensis* were found to be important components of the macroinvertebrate community along the Danube River (Figs. 6, 7 and 8) [22].

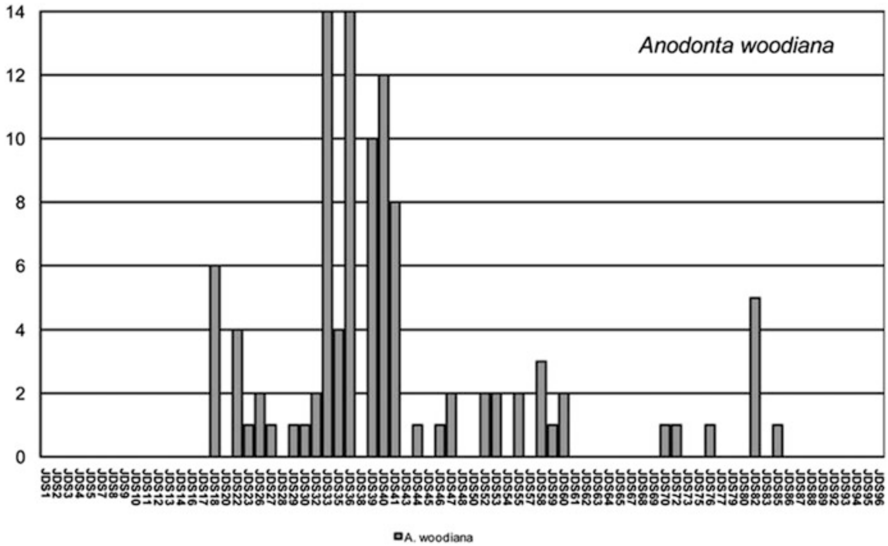

Fig. 6 The distribution of *S. woodiana* along the Danube River (JDS2 dataset)

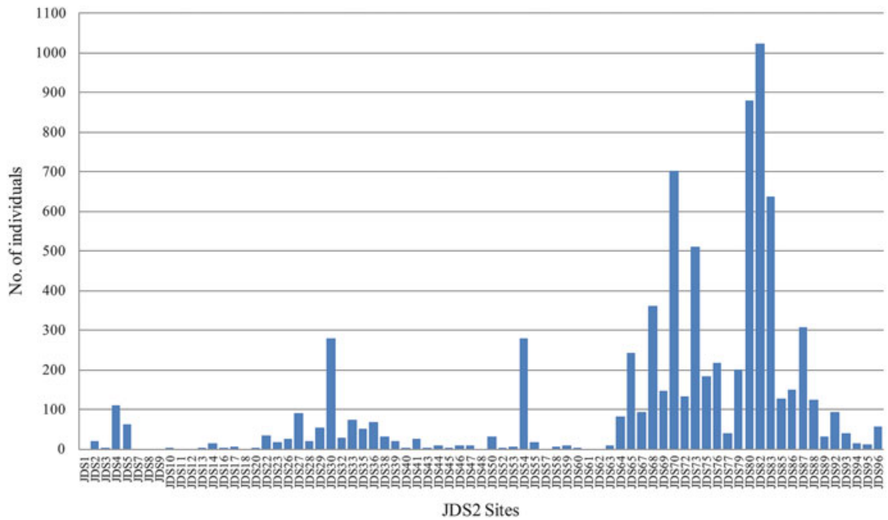

Fig. 7 The distribution of *C. fluminea* along the Danube River (JDS2 dataset)

In regard to previous Danube Surveys JDS1 [29] and ADS [21, 31], the fast dispersal of *D. bugensis* was recorded in 2007 [22]. *D. bugensis* was found in the Lower Danube, downstream the Iron Gate, while *D. polymorpha* was abundant in the upper stretch (Fig. 8). It should be emphasized that *D. bugensis* started to be dominant species in the Lake Balaton since 2009, as well.

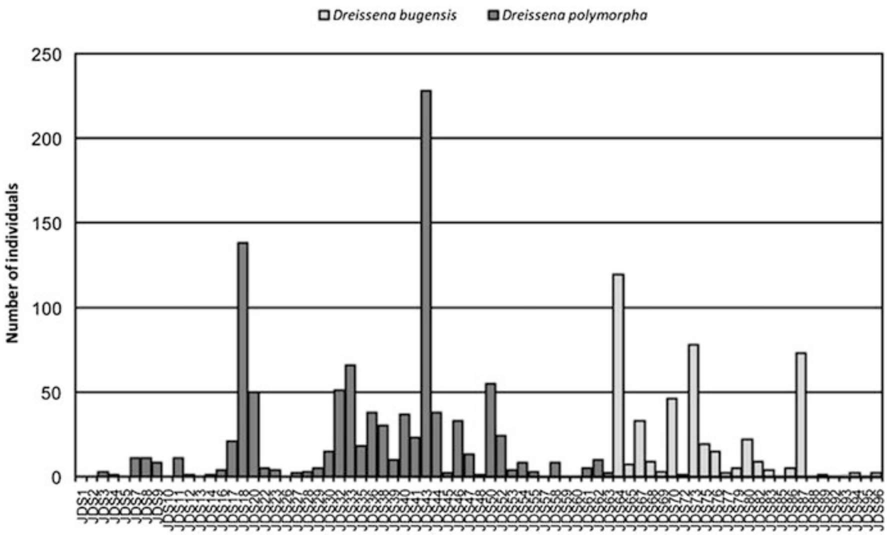

Fig. 8 The distribution of two species of *Dreissena* along the Danube River (JDS2 dataset)

Corbicula fluminea was found to be the most frequent. It occurs in 93% of the sites followed by the crustaceans *Chelicorophium curvispinum* (90%) and *Dikerogammarus villosus* (69%).

Regarding Ponto-Caspian species, it is hard to precisely determine their natural distribution range along the Danube River. This is mostly because of the lack of confident historical data for considerable number of species, but also due to the fact that borders of distribution areas are often not narrow lines, but zones with gradual reduction of frequency and abundance and finally disappearance of particular taxa. Thus, Ponto-Caspian species could be considered as nonindigenous for the Upper Danube and upper part of the Middle Danube in general, but for the lower part of the Middle and upper part of the Lower Danube, there is still a lot of confusion whether some species are indigenous or not. There are typical examples, such as highly invasive Ponto-Caspian amphipods (*Chelicorophium curvispinum*, *Dikerogammarus haemobaphes* and *D. villosus*), zebra mussel (*D. polymorpha*), Ponto-Caspian gobiids (*Neogobius fluviatilis*, *N. gymnotrachelus*, *N. kessleri* and *N. melanostomus*), etc. The presented uncertainty complicates assessment of invasive pressure of certain areas on the edge of Ponto-Caspian region, most precisely in the Iron Gate stretch.

Fauna of Ponto-Caspian area, Asia, Australia and North America are influencing very strongly the macrozoobenthic community of the Danube. The Danube is a part of the Southern Invasive Corridor (Black Sea-Danube-Main/Danube Channel-Main-Rhine-North Sea waterway), one of the four European most important routes for invasive species [16]. The river is exposed to an intensive colonization by IAS and further spreading in both (north-west and south-east) directions throughout the

Danube Basin. With few exceptions the neozoa of the Danube belong mostly to Crustacea and Mollusca.

The bottom fauna of the Upper and Middle Reach of the Danube is dominated by Ponto-Caspian neozoa. Their relative abundance averages between 60% and 80%, and they represent up to 40% of the total number of taxa. At the most sampling sites, especially species of Mollusca, Annelida and Crustacea (Amphipoda and Isopoda) are much more numerous – and the majority of them belong to the neozoa.

It should be pointed out that benthic assemblages are clearly dominated by nonindigenous, invasive or cosmopolitan elements that had been stabilized/naturalized in the Danube Basin for a long time (in the case of many taxa). It is, thus, questionable how to look at those species – as "harmful invaders" or simply as part of the recent fauna. This dilemma is valid for all communities, not only for benthic assemblages.

Regarding the fish data the significant pressure of biological invasions has been confirmed by JDS2 fish survey results as well [39, 41, 42]. During JDS2 some of non-native fish species were found to be abundant and widely distributed – gibel (or Prussian) carp *Carassius gibelio* and both bullhead (*Ameiurus melas* and *A. nebulosus*) species, as well as all Ponto-Caspian goby *Neogobius* spp. – whereas those posing the still high risk of invasiveness due to their life features, e.g. topmouth gudgeon *Pseudorasbora parva*, largemouth bass *Micropterus salmoides* and Amur sleeper *Perccottus glenii*, were yet dispersed narrowly when compared to those in the first group.

Generally, according to JDS2 dataset [39] 57 species are considered native at least in parts of the Danube catchment, and nine remain as entirely non-native (*Ameiurus melas*, *A. nebulosus*, *Anguilla anguilla*, *Ctenopharyngodon idella*, *Gasterosteus aculeatus*, *Hypophthalmichthys molitrix*, *Lepomis gibbosus*, *Oncorhynchus mykiss*, *Perccottus glenii*). As data for reference fish communities in tributaries are usually scarce, their sites are not considered here. The higher number of non-native fish upstream of Iron Gate 2 revealed by JDS2 dataset is due to an occurrence of five goby species being considered non-native there.

It should be underlined that invasive algal species were found in the Danube River (*Didymosphenia geminata* (Lyngb.)) [29, 43], as well as potentially invasive Cyanoprokaryota species *Cylindrospermopsis raciborskii* [44].

Besides the already mentioned neophyte species recorded during JDS2, *Paspalum paspaloides* was found as additional invasive taxa along the Iron Gate Danube stretch [45]. *Cyperus strigosus* could be considered as potential invaders for the Danube River, as well [45].

The high pressure of biological invasions along the main tributaries of the middle Danube stretch is documented by the works of several authors. The invasive alien taxa of the Sava River and main tributaries were reported by [32, 35, 46–50]. During the investigation of the Sava River, four allochthonous and cryptogenic species of macroinvertebrates have been registered at three localities: *Corbicula fluminea*, *C. fluminalis* (Bivalvia), *Lithoglyphus naticoides* (Gastropoda, cryptogenic taxa) and *Limnomysis benedeni* Mysidacea [13]. According to the data presented for the Sava River, allochthonous species made 26.05% of the overall number of the recorded species. Abundant and frequent presence of nonindigenous macroinvertebrate taxa along 206 km of the Sava River was also reported by [32, 47].

The International Tisza Survey [30] confirmed that the Tisa River is also under the considerable influence of neozoa. Thus, crustaceans *Dikerogammarus haemobaphes*, *D. villosus*, *Obesogammarus obesus*, *Chelicorophium curvispinum* and *Jaera istri* were found with a high frequency of occurrence and relative abundance in the lower Tisa. *S. woodiana* and *D. polymorpha* were also found to be frequent along the lower Tisa, but with lower relative abundance [30], while *Corbicula* spp. were not recorded. In addition, high pressure of biological invasions within the Serbian stretch of this river was observed. The allochthonous species were represented by the proportion of 32.59% in the total number of all macrozoobenthos species detected [13].

The situation is even worse considering neozoa in the Velika Morava River in regard to a number of detected species. Reported nonindigenous taxa (five) and introduced species made up to 18.67% of the total macroinvertebrate community [13]. Recent investigation on the Velika Morava River indicated the frequent and abundant presence of *C. fluminea* on the Velika Morava River, as well [51].

Several interesting concepts for the evaluation of the influence of IAS have been tested for the Danube River and its main tributaries. Thus, the assessment of biological contamination based on JDS2 fish datasets for the Serbian stretch of the Danube River by using the Fish Invasiveness Screening Kit (FISK) and Invasive Fish Risk Assessment (IFRA) Protocol [52] and SBC index [17] confirmed that the Danube is under the considerable influence of the alien fish species [41]. Further, the same work [41] pointed to the high level of biological contamination on the three main tributaries within the Serbian stretch – the Tisa, Sava and Velika Morava River, underlining the worst situation on the Sava River.

Further, Panov et al. [27] assessed the biological contamination rate (BCR, records of alien species per 10 years, for period 1997–2007); biological contamination level (BCL, number of established alien species since 1900); integrated biological contamination index (IBC), estimated for macroinvertebrates (after [8]): biopollution level index (BPL) (see [26]); and integrated biopollution risk index (IBPR, estimated for macroinvertebrates) on the selected assessment units within main European invasive corridors. Danube River Basin was one of the target areas of the assessment. All listed parameters showed that IAS are an important pressure for the Danube River, as well as for the investigated tributaries (the Sava and Tisa rivers).

4 Discussion

Based on the results presented above, the pressure caused by biological invasions on ecosystems of the Danube River and its main tributaries is obvious. This is confirmed by recent reporting of new invaders. Szekeres et al. [53] reported mass occurrence of freshwater bryozoan species *Pectinatella magnifica* in the Danube side arm Ráckevei-Soroksári Duna, downstream Budapest during 2011.

Despite the considerable progress in understanding biological invasions within the last 20 years, we are still facing some general problems. The open questions are:

- Are all nonindigenous taxa occurring in aquatic habitats with high abundance harmful for ecosystem functionality by the default?
- Are we able to accurately assess the influence of the each alien taxon (or majority of the taxa) to native communities?
- Can we provide effective risk assessment tool for IAS?
- Can we properly manage biological invasions?

The response to all of these questions is in our opinion negative. Thus, a lot of additional work is needed to properly address the issue of IAS in the Danube region.

Besides providing the comparable datasets for larger areas, for proper addressing the IAS issue, some conceptual problems should be resolved, as well. One of the opened topics is how to deal with already naturalized species. At which point can we stop considering a species as alien for the certain recipient area? There are lots of examples – e.g. common carp (*Cyprinus carpio*) is introduced species for the Danube River, but it, for sure, became a part of the native fish community. Introduced fish species that naturalized centuries or even millennia ago (e.g. carp) are to be considered native and omitted from risk assessments [54].

The other conceptual problem is how to treat the cryptogenic species. In many cases, cryptogenic taxa are found frequently and with high abundance. The use of those species, with unsecure knowledge about the status for the recipient area, could lead to the wrong assessment of the pressure of IAS.

The importance of the problem of biological invasions has been recognized on the European level as well. The European Commission published a communication "Towards an EU Strategy on Invasive Species" in December 2008. A detailed paper on this issue was published by [55] containing the assessment of the impact of IAS along the EU member states. According to general views, invasive alien species (IAS) are species whose introduction and/or spread, outside their natural past or present distribution, threatens biological diversity. They may cause serious damage not only to ecosystems but also to crops and livestock, disrupting the local ecology, impacting on human health and producing serious economic effects. Reduction of spread of IAS was underlined as specific action for preservation of biodiversity and landscapes in Action Plan for the European Union Strategy for the Danube Region [56]. The discussion paper published by the EC [57] deals with the development of an EU Framework for Invasive Alien Species. IAS were explicitly mentioned as the problem we have to face within the Danube River Basin District Management Plan [58], Tisa River Basin Management Plan [59] and Sava River Basin Management Plan [60]. Recently, a regulation of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species has been adopted [61] with an idea to provide effective, common platform for dealing with aquatic invasions, which is probably the most important action up to know on the EU level to deal with IAS.

In response to the threats posed by nonindigenous species to the aquatic environment, various systems (codes of practice) have been developed with the aim to identify and assess potential risks of the existing and potential future non-native aquatic species [27, 52, 62–66]. The procedures proposed therein could be considered to be applied as a tool for the assessment of impacts of IAS within the Danube River Basin.

An interesting concept for the assessment of biopollution has been proposed by Olenin et al. [26]. The method has been tested for Upper, Middle and Lower Danube, as well as for the Tisa and Sava rivers (lower stretches within the Serbian sector), and it was evaluated as effective, easy to use and applicable to the whole stretch of the Danube River [27].

The papers of Copp et al. [52, 66] and Panov et al. [27] discussed the methods for classification of nonindigenous species as potentially invasive or non-invasive. First, the system has been developed for fish: the Fish Invasiveness Scoring Kit, FISK [52]. The FISK is an adaptation of the procedure proposed for weed – the Weed Risk Assessment, WRA [67]. Later, the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK) [68] has been proposed as a tool for identifying potentially invasive invertebrates for freshwater ecosystems. FI-ISK was developed to be able to classify the nonindigenous taxa into low-, medium- and high-risk categories, by using confidence ranking (certainty/uncertainty) by the assessor to each response in order to determine appropriate score thresholds between the categories. The FISK and FI-ISK represent useful and viable tools for decision-makers for assessment and classification of freshwater invertebrates according to their potential invasiveness.

Further, based on the data on European invasive corridors which included the data from AISSIC database, the development and testing of a risk assessment tool (RAT) have been performed [27].

The negative impacts identified by the assessment include [27]:

- Impacts on Europe’s native species, habitats and ecosystem functions, which include terrestrial, freshwater and marine ecosystems, with IAS documented as a threat to many species and habitats threatened at global or European level
- Impacts on the biodiversity of Europe’s islands, including the EU overseas entities, which often underpins local livelihoods and economies
- Impacts on almost all ecosystem services that underpin human wellbeing, biological production systems and recreational/tourism amenity (e.g. food and water provisioning; regulation of water, fire and flood regimes; erosion control)
- Socio-economic effects on affected individuals and communities through harm to human health (e.g. disease vectors, parasites, allergies, asthma) and/or to local livelihoods
- Economic impacts on biological production and other sectors at European level

Out of the above-mentioned impacts, those being most relevant for the Danube River Basin should be clearly defined.

Finally, it should be emphasized that behaviour, reproduction and life strategy of IAS taxa are important issues to be clarified in the whole Danube Basin for the better understanding of their distribution processes, population dynamics and successful survival ability in their new aquatic area.

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Phytoplankton of the River Danube: Composition, Seasonality and Long-Term Dynamics

Martin T. Dokulil

Abstract Investigations on river phytoplankton in the Danube are summarised and placed into a historic perspective. Phytoplankton species composition always has been dominated by diatoms, particularly centric taxa. Longitudinal, seasonal and long-term dynamics are described and their implications are discussed. Factors responsible for the wax and wane of phytoplankton growth in the middle section of the river Danube are analysed and discussed. Survival, growth and production of phytoplankton in the Danube and in large rivers in general are then incorporated and integrated into the existing fundamental concepts of riverine ecosystems.

Keywords Danube, Interaction, Large rivers, Plankton, Seasonality

Contents

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1 Introduction

Investigations on river phytoplankton in the Danube have a long history. First qualitative studies in the years 1898 and 1899 indicated a similar species composition as nowadays [1, 2]. Diatom species (Bacillariopyceae), particularly *Aulacoseira granulata,* dominated the assemblage. Even such delicate species as *Atheya zachariasii* appeared in the river [3]. Quantitatively the authors observed considerable variation in space and time depending on environmental conditions. Both authors discussed already the applicability of the term '*potamoplankton*' introduced by Zacharias [4] for river plankton. Because of the variable and very low quantities of the plankton in the river Danube, investigations concentrated in the following years more on the Danube backwaters.

Halász [5] in Hungary and Schallgruber [6] in Austria resumed investigations in the river Danube. Schallgruber's annual quantitative data clearly indicated the dominance of centric diatoms. Therefore, he concluded that the Danube's plankton should consequently be called '*Cyclotella* plankton'. Based on his findings that algal species in the river were healthy and alive, he insisted to preserve the term 'potamoplankton' for such biocoenoses similar to suggestions by Wawrik [7]. Wherever flow is reduced or where eutrophication becomes significant, cyanobacteria and green algae became more important than diatoms, sometimes even forming surface blooms [8]. The monograph '*Limnologie der Donau'* compiled by Liepolt [9] provided a first synopsis of results obtained until then on the river Danube. In this monograph, Szemes [10] assembled the Danubian flora systematically.

Investigations expanded through the activities of the International Association for Danube Research (IAD, [11]). Comprehensive overviews were published among others by Weber [12] and Kinzelbach [13]. Major steps forward in the protection of the water quality in the Danube were the 'Bucharest Declaration' in 1985 and the International Commission for the Protection of the Danube River (ICPDR) established in 1998 which soon expanded its activities into the whole Danube River Basin initiating major projects and surveys.

The EU Water Framework Directive (WFD) and the progressing effects of climate change have meanwhile created new challenges. Evaluation techniques for potamoplankton had to be developed for the assessment of the ecological status of rivers (e.g. [14–18]), partly based on the functional algal group concept of Reynolds et al. [19].

The aim of this chapter is to summarise the widely dispersed information on the species composition, quality and quantity of the potamoplankton in the river Danube. Detailed analysis of the wax and wane of potamoplankton in the middle river section is provided, and results are brought into context with present concepts of riverine ecosystems.

2 Material and Methods

Data analysed originate from a multitude of reports, journals, publications and electronic material. Besides regional investigations (e.g. [20–23]) and results pertinent to specific stretches of the river (e.g. [24, 25]), longitudinal surveys of the Danube provided important information. These surveys have been summarised by Wachs [11], Table 3, described by Kusel-Fetzmann et al. [26] and were updated in Table **1**. Additional information on water quality for most of the Danube was provided by Weber [12] summarising data collected during 1988–1993 in fulfilment of the Bucharest Danube Declaration adding the determinant chlorophyll-*a* (chl-*a*) as a surrogate parameter for phytoplankton in 1992.

The long-term database on water quality aspects in the Danube basin collected by the ICPDR Trans-National Monitoring Network (TNMN) between 1996 and 2009 provided important information (TNMN at http://www.icpdr.org/icpdr-pages/tnmn_yearbooks.htm). Zooplankton data used here were extracted from Zsuga [32].

To convert phytoplankton cell numbers to chl-*a* or biomass equivalents, JDS2 data were systematically correlated and a graphical regression nomogram developed (Fig. 1). Results are not statistically different from relations published earlier for cells versus biomass (data in [10]) or cells versus chlorophyll-*a* [34].

Calculation of theoretical (potential) biomass increments were based on carbon content and daily primary production (PP) rates. Cell carbon concentrations were calculated from both chl-*a* and fresh-weight (FW) biomass. Conversion of chl-*a* into carbon assumed a ratio of 25:1. A carbon content of 50% ash-free dry weight (AFDW) was used for biomass conversion, assuming AFDW to be 25% of FW biomass. Theoretical biomass increments were then estimated from carbon biomass adding daily carbon uptake rates (mg C m^{-3} d^{-1}) per travel time assuming 'constant' PP rates between sites. This assumption was justified by the mean travel time of 0.5 days from one site to the next (range 0.1–4.0). Travel time was calculated from discharge and flow velocity at each site. The difference between carbon biomass observed and the potential carbon biomass calculated was assumed to represent total loss which was then differentiated into grazing loss, equivalent to 15% per day (Herzig, personal communication) and other losses.

Table 1 Danube surveys (expeditions)

Year, month	From	To	Trans., org.	Reference
1960, 9–10	Vienna	Sulina	Amur, IAD	Benda et al. [27], Wawrik [7]
1961, 9	Vienna	Origin	Car, IAD	Liepolt [9]
1978, 8–9			Ship, IAEO	Kiss [28]
1988, 3	Sulina	Vienna	Amur, IAD	Weber [29]
1990, 4	Ismail	Linz	Ship, UFD	Stoyneva and Draganov [30]
1998, 5–6	Regensburg	Mohács	Burgund, Min.	Krauß-Kalweit [31]
2001, 8–9	Regensburg	Sulina	Argus, ICPDR	http://www.icpdr.org/
2007, 8–9	Regensburg	Sulina	Argus, ICPDR	http://www.icpdr.org/

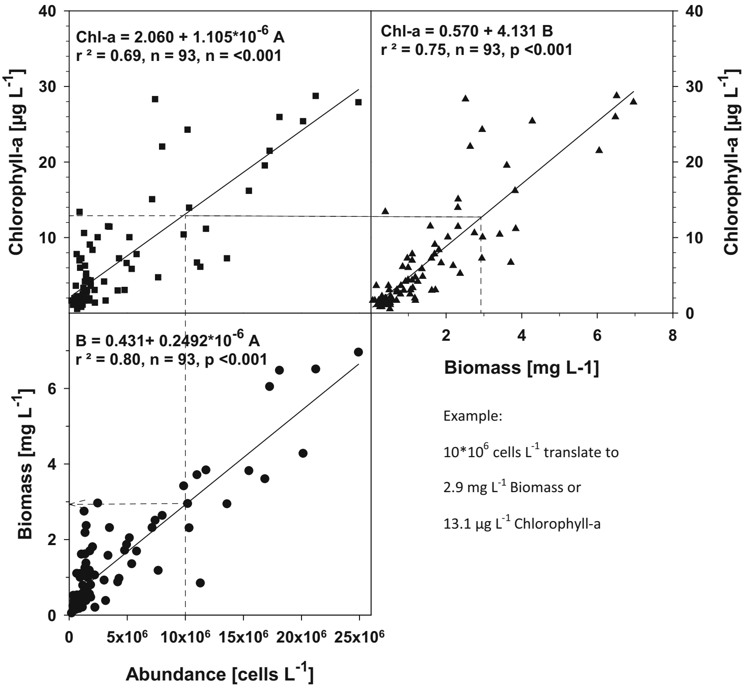

Fig. 1 Nomogram to calculate phytoplankton FW biomass and/or chlorophyll-a from cell numbers per litre based on correlating 93 results from samples obtained during JDS2 [33]

3 Results

Numerous algal taxa lists have been published. A synopsis on plankton organisms was provided by Kusel-Fetzmann et al. [26]. Details for the section Bratislava to Budapest including tributaries can be found in Makovinská [35]. More recent lists have been compiled by Nemeth et al. [36] and by Dokulil and Kaiblinger [33], reprinted here in Annex (see also [37, 38]).

Species composition of the phytoplankton has always been dominated by diatoms (Bacillariophyceae) and co-dominated by green algae (mainly Chlorococcales) during summer or in particular river stretches [7, 10, 30, 33, 36, 39, 40]. The majority of the dominant diatoms were centric taxa, such as *Aulacoseira, Stephanodiscus* or *Cyclotella* among several others (e.g. [41]). These small centric diatoms often bloom even during winter [42]. Canalization, construction of hydropower dams, impoundments and eutrophication increased phytoplankton biomass and changed species composition in the past [25, 43–46]. Prior to 1994, chlorophyll concentrations often exceeded 100 µg l^{-1} in the German river stretch due to the impounded character of this river section.

The enhanced phytoplankton production resulted in oxygen oversaturation of up to 186% [22].

A large number of published investigations report a widely varying number of algal taxa depending on season, river stretch and discharge among other factors.

During JDS2 [33] the number of phytoplankton taxa varied from 46 in the Sulina arm to 101 in the tributary Iskar. The average was 75 taxa for all 96 samples from the river and major tributaries. Bacillariophyceae (diatoms) clearly dominated the biomass at all stations in the Danube (average 59%, range 35–76). Higher contributions of green algae (Chlorophyta) were observed in the German stretch (37–63%). For the major part of the river, green algal contribution averaged 25% (range 0–64%). The small flagellated species of the Cryptophycean group appeared at all stations in the river (mean 16%, range 0–47%) with higher importance in the upper reach (Austria, Slovakia, northern part of Hungary and in the Iron Gate section). Cyanoprokaryota (Cyanobacteria) were unimportant in the river. In contrast, some of the tributaries carried large amounts, especially the Arges which contained 80% cyanobacteria exclusively species from one genus, the colonial, potential toxic *Microcystis*. Green algae dominated the river Timok (87%) and were an important component in most of the tributaries, particularly Sio, Hron and Ipoly. Cryptophyta were of minor importance in these streams except in the Tisza where the group contributed 36%.

The early investigations reported potamoplankton abundance primarily as cells per litre. Schallgruber [6] stated maximum numbers of 2.7×10^6 cells l^{-1} for the 1940s. This number increased to 5.5×10^6 cells l^{-1} by the end of the 1970s [39] and rose further to 26×10^6 by Nausch [41] which is a tenfold increase in about 45 years. These numbers translate to fresh-weight biomass of 1.1, 1.8 and 7 mg l^{-1} or chlorophyll-*a* concentrations of 5, 8 and 31 μg l^{-1}, respectively (Fig. 1).

Longitudinal surveys since 1961 consistently have indicated low to moderate chlorophyll-*a* concentrations in the upper reach from about Ulm to the Gabcikovo impoundment east of Bratislava, increasing, peaking and declining values in the middle reach, while low or only marginally increasing concentrations of chl-*a* were characteristic for the lower stretch (Fig. 2, top panel).

The expedition in 1960 [27] reported cell numbers which peaked at 27×10^6 cells l^{-1} in Budapest at km 1,647 [7]. This is equivalent to about 32 μg chl-*a* l^{-1} when converted using the relations in Fig. 1. Concentrations persisted at about this level downstream until km 1,488 but dropped to 4.2×10^6 cells l^{-1} (=6.7 μg chl-*a* l^{-1}) near the confluence with the Drava remaining low further downstream (Fig. 2, top panel, IAD 1961). The expedition in 1988 observed much higher concentrations of chl-*a* [47]. Values reached 85–136 μg chl-*a* l^{-1} between km 1,731 and 1,475, almost the same region as 17 years before (Fig. 2, top panel). Ten years later in 1998, maximum chl-*a* concentrations of 55–65 μg l^{-1} were attained between km 1,659 upstream of Esztergom and km 1,481 at the Drava confluent [31]. The survey in 2001 detected chl-*a* values as high as in 1988 (>100 μg l^{-1}) and in about the same stretch. In contrast, the observations in 2007 (JDS2) indicated a considerable reduction in the maximum concentration attained (25 μg l^{-1}) and a decline towards the section upstream of the Drava confluent (Fig. 2, top panel, JDS-2007).

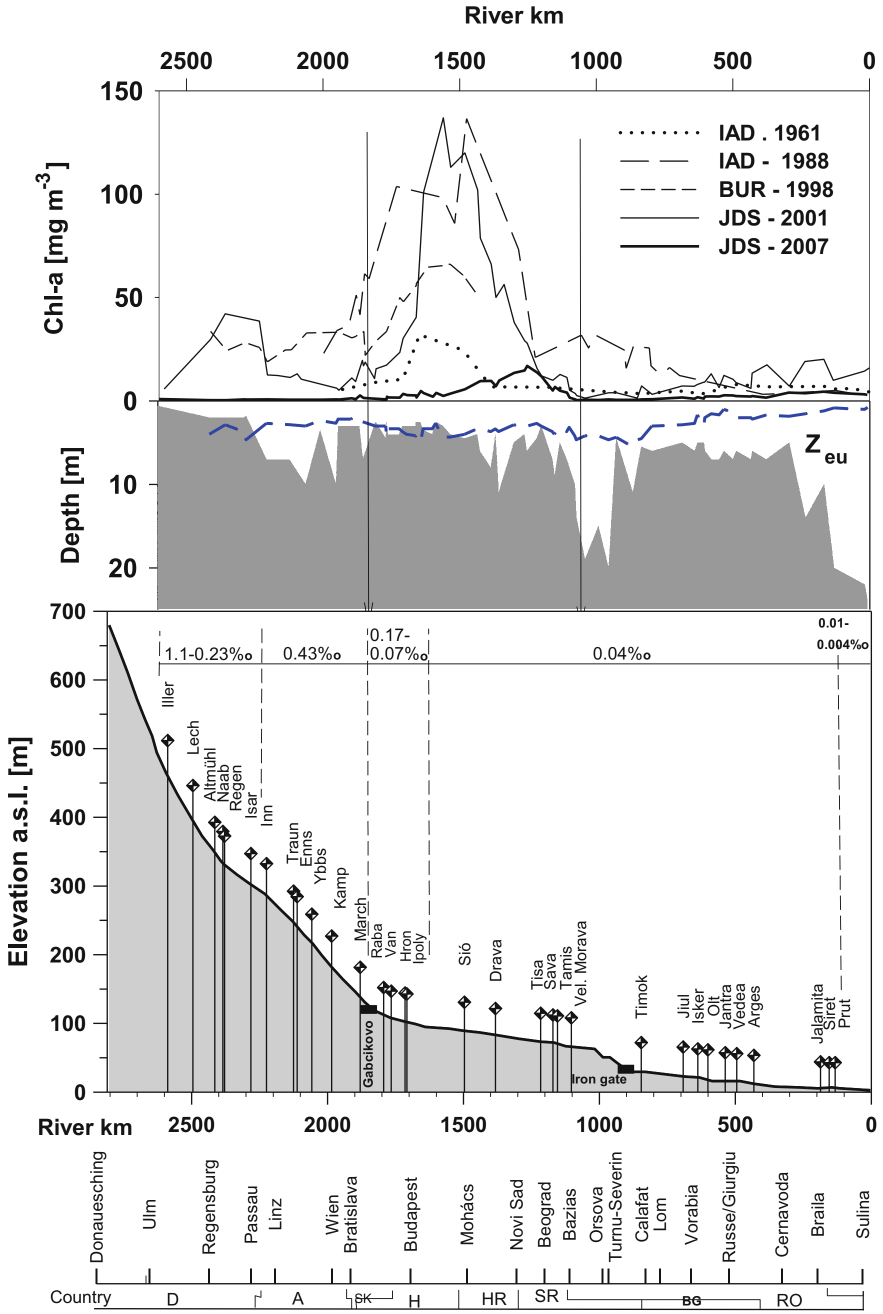

Fig. 2 *Top panel*: Concentration of chlorophyll-*a* along the river corridor for five surveys. Data for IAD 1960 [27] from Wawrik [7], for IAD 1988 [29] from Aponasenko et al. [47], for BUR 1998 from Krauß-Kalweit [31], for JDS1 and 2 from http://www.icpdr.org/. Centre panel: River depth from JDS2 Factsheets [48] and euphotic depth (z_{eu}) from Dokulil and Kaiblinger [33]. *Bottom panel*: River Danube elevation diagram with mean slopes, tributaries, main cities and countries. Modified from Lászlóffy [49]

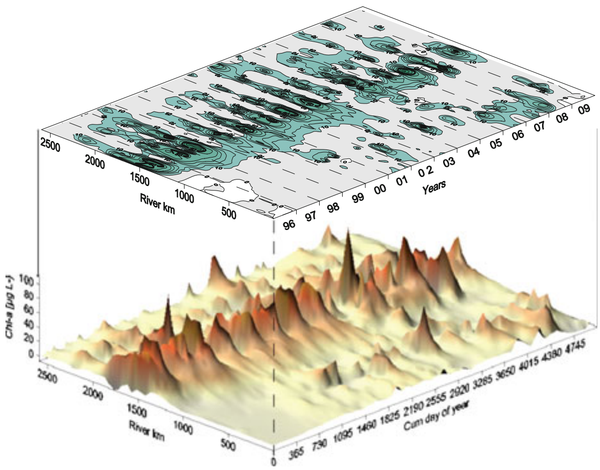

Fig. 3 Three-dimensional time-space diagram of chlorophyll-*a* concentrations in the Danube, river km 2,500–0 for the years 1996–2009 and isoplot projection. All data are from the TNMN yearbooks available at http://www.icpdr.org/icpdr-pages/tnmn_yearbooks.htm

Algal growth in the middle section of the river Danube was a reoccurring phenomenon as the long-term data from the TNMN [50] indicate (Fig. 3). A detailed analysis showed that the maximum concentration of chlorophyll-a varied interannually both in location and time (Table 1). Peaks occurred as early as March (1996) or as late as October (1997 and 2006) and varied between km 1,560 and 1,287. In the years 2005 to 2008, the peak shifted annually downstream from km 1,560 to 1,287. When comparing single surveys (Fig. 2), critical interpretation is necessary. Although both JDS investigations in 2001 and 2007 were carried out in September, the observed differences in chl-*a* levels should not be seen as an improvement in water quality. The first survey in 2001 observed high values only because a second chl-*a* maximum occurred in that year (see Table 1). The September values from JDS2 are in good agreement with the NTMN observations in magnitude and downstream shift (comp. Fig. 2 and Table 1). The absolute maximum, however, had occurred in April already. These results also shed light on the timing of such surveys. The unpredictable timing of maximum growth of phytoplankton makes decisions impossible when such investigation shall be performed.

The controlling factors for the wax and wane of potamoplankton biomass in the middle section of the river Danube can be deduced from Figs. 2 and 4. Reduced flow velocity, decreasing load of suspended solids and hence better underwater illumination (Fig. 2) were responsible for enhanced photosynthesis and growth leading to the rapid biomass increase below Dunaföldvar at river km 1,560 (Fig. 4a). Biomass peaked upstream of the Tisza confluent. Values rapidly declined thereafter which has been associated in the past with zooplankton grazing (JDS1, [51]). A closer look on the phytoplankton-zooplankton interactions in 2007, however, reveals that the small numbers of rotifers and crustaceans present had only a marginal impact on the decline of phytoplankton biomass (Fig. 4a). Rotifers dominated zooplankton abundance (>60%) as long as phytoplankton biomass rose peaking at 100% upstream of the confluence with the Tisza. Further

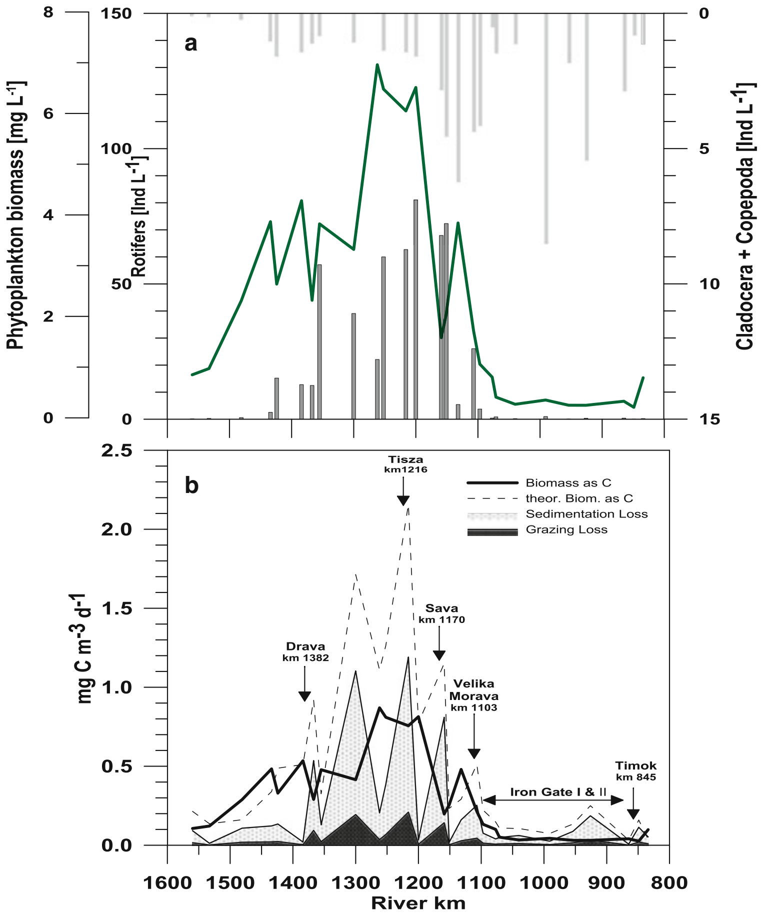

Fig. 4 Analysis of the mid-river section from km 1,600 to km 800. (**a**) Phytoplankton biomass as mg l^{-1} (*continuous line*), rotifers (*black bars*) and crustacean (Cladocera + Copepoda, *grey bars*) as Ind. l^{-1}. (**b**) Biomass as carbon, theoretical attainable carbon biomass, 'sedimentation' loss and grazing loss (see 'Legend'). The position of the main tributaries and the Iron Gate impoundments are indicated. Phytoplankton data from Dokulil and Kaiblinger [33]; zooplankton data from Zsuga [32]. For details on calculations, see 'Methods' and refer to the text

downstream small number of crustaceans gain relative importance and finally dominated zooplankton in the Iron Gate impoundment.

To get a more detailed insight into the processes affecting the observed biomass changes, loss rates were calculated from plankton biomass and primary production rates between sites (Fig. 4b). The calculated biomass increase agreed pretty well with the observed increment for the first 176 km. Losses are small and grazing was negligible. After the Drava has entered the river, both values diverged largely. Although 0.3 mg C m^{-3} phytoplankton biomass was added by the river Drava, almost 70% of the theoretical biomass was lost by grazing, dilution and

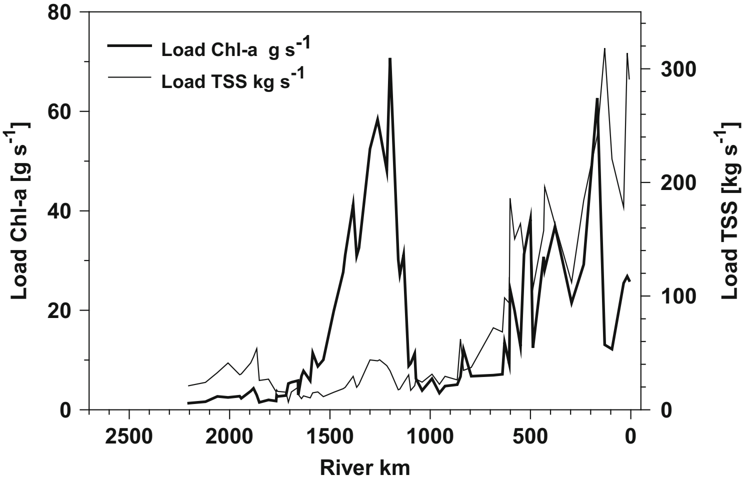

Fig. 5 Loading of chlorophyll-*a* (Chl-*a*) and total suspended solids (TSS) calculated from discharge and concentrations data in Dokulil and Kaiblinger [33] for the river Danube between river km 2,200 and 0

sedimentation (Fig. 4b). Biomass recovered slightly at Dalj 12 km further downstream almost corresponding to the calculated value. It remains unclear what has caused the 75% losses at Backa Palanka, river km 1,300. Possibly it was sedimentation loss due to greater river depth upstream. Biomass increased further until about Novi Sad but then rapidly declined through the discharges from the Tisza and Sava rivers leading to high loss rates by dilution. During average discharge, each river thinned the Danube water by about 27%. Biomass recovered then again until Grocka, km 1,132, but then further declined due to high sedimentation loss rates because of the deepening of the river (comp. Fig. 2, centre panel) and the input from the Velika Morava. Further downstream, both actual and potential biomasses remain low because of low carbon uptake rates and sedimentation in the impounded section of Iron Gate II which acts like a lake.

Loads calculated from discharge measurements for total suspended solids (TSS) and chl-*a* for the Danube exemplify conditions typical for middle river reaches (Fig. 5). Chlorophyll loading remained low as long as TSS load was above 15–20 kg s^{-1}. As the load of suspended solids decreased below this threshold because of dilution and sedimentation chlorophyll-a loading began to increase from about river km 1,719 (Esztergom) onwards peaking downstream of the river Tisa at river km 1,200. Thereafter chl-*a* load rapidly declined to a minimum in the Iron Gate Reservoir at km 954 due to dilution by lateral inflow from large tributaries carrying low potamoplankton biomass, reduced flow velocity due to damming and deepening of the river. In the lower reach below km 865, the loads of TSS and Chl-*a* then tend to increase together (Fig. 4). Analysis of the 25 samples in the lower reach yielded the equation log Chl-$a = -0.272 + (0.715 \times \log \text{TSS})$, $r^2 = 0.53$, $p < 0.001$. Although chlorophyll-*a* load increased considerably, primary production remained at low level due to unfavourable underwater light conditions caused by the increasing turbidity.

4 Discussion

Although the existence of phytoplankton in rivers has been recognised soon after it was discovered in the sea and in lakes, it has never received the same level of attention [52]. This fact is even more surprising when considering the robust assemblages of potamoplankton assembled in Reynolds and Descy [52], Table 1. In this list 59% of the taxa are diatoms of which 76% are centric. In fact, the potamoplankton of larger rivers is dominated by small or filamentous centric diatoms (comp Table 2 in [53]). Reasons for the strong selectivity for these genera is attributed to the simultaneous selective bias of several morphological and physiological adaptations to survive in the rapidly fluctuating light field of a turbid, kinetic system [52, 54–56]. Water residence time was identified as largely responsible for the selection of size structure and taxonomic composition in a comparative study of temperate rivers by Chételat et al. [57].

Increase and maintenance of autotrophic plankton assemblages critically depend on photosynthetic activity, circulation depth versus euphotic zone and the daily balance of production and respiration [58–60].

Predictive models are now available to simulate potamoplankton composition and biomass from source to mouth using discharge, river morphology, water temperature, available light and nutrient inputs as forcing variables (e.g. [61]).

Fundamental concepts of riverine ecosystems have been developed and formulated during the last three decades. Most prominent is the flood pulse concept (FPC) developed by Junk et al. [62] derived mainly from tropical rivers and expanded into a 'flow' pulse concept by Tockner et al. [63] to better comply with less predictable floods in temperate rivers. These concepts accentuated allochthonous nutrient sources emphasising a tendency of large rivers to be dominated by heterotrophic processes (R) as primary production (P) becomes limited by light penetration and/or increased water depth [64]. Autotrophy ($P/R > 1$) in these theories can only be attained when benthic primary producers dominate in mid-river reaches of higher stream order.

The role of in-stream primary production was underestimated in these concepts. Rivers dominated by phytoplankton such as eutrophied rivers may be predominantly autotrophic, exporting new autochthonous organic matter. Accordingly, the riverine productivity model (RPM) emphasising the role of autochthonous production [65] better describes large deep rivers [53]. Incorporation into the riverine ecosystem synthesis (RES), a heuristic, integrated model proposed by Thorp et al. [66] provides a framework for understanding longitudinal and lateral dimensions of river networks.

Table 2 Monthly highest chlorophyll-*a* values as μg l^{-1} for the years 1996–2008 and all river km investigated

Month													Max		River
Year	1	2	3	4	5	6	7	8	9	10	11	12	Chl-*a*	Month	Km
1996	6	50	**123**	30	68	59	74	115	12	25	7	27	**123**	3	1,560
1997	5	37	35	78	69	83	74	104	114	**146**	51	20	**146**	10	1,435
1998	10	45	87	93	102	**118**	88	89	59	9	19	6	**118**	6	1,435
1999	6	12	34	41	30	30	74	**111**	56	10	18	56	**111**	8	1,560
2000	8	8	11	32	39	90	**110**	59	104	31	54	25	**110**	7	1,436
2001	14	28	43	29	**125**	88	73	108	107	21	21	18	**125**	5	1,560
2002	6	16	49	72	121	**123**	113	80	22	16	8	6	**123**	6	1,436
2003	4	18	68	60	116	76	**143**	74	26	45	73	7	**143**	7	1,436
2004	17	32	84	60	57	36	**70**	**99**	31	11	8	22	**99**	8	1,560
2005	15	41	45	23	50	**156**	53	108	78	61	73	51	**156**	6	1,560
2006	13	33	19	82	30	38	42	59	64	**104**	19	7	**104**	10	1,435
2007	35	21	19	**110**	96	91	96	68	29	18	13	5	**110**	4	1,427
2008	5	19	18	72	**92**	92	43	36	18	14	6	7	**92**	5	1,287
2009	12	17	36	17	61	73	16	63	**122**	105	8	5	**122**	9	1,435

The absolute maximum is indicated in bold, summarised in the max chl-*a* column together with the month and river km of its appearance. All data were extracted and analysed from the TNMN yearbooks

Conclusions
The conceptual framework of rivers being potentially autotrophic has fundamental implications for the river Danube. As pollution and turbidity from the catchment decline in the river, nutrient concentrations become gradually more significant. In combination with improved underwater light intensities, nutrients will enhance algal primary production particularly in river sections where current speed is reduced or during periods of low discharge. Due to the complex hydrological situation and the large catchment of the river Danube, the timing and extent of maximum phytoplankton development is difficult to predict and varies interannually. As a consequence, any monitoring schedule must react flexible to specific hydrological and meteorological situations. In particular it will be relevant for water quality evaluation within the EU WFD.

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Annex List of phytoplankton taxa identified during JDS2

Cyanoprokaryota	*Lyngbya* cf. *limnetica*	*Gymnodinium helveticum*
Chroococcales	*Phormidium* cf. *mucicola*	*Gymnodinium uberimum*
Aphanocapsa holsatica	*Planktothrix* sp.	*Peridinium* sp.
Aphanocapsa incerta	*Planktothrix rubescens*	*Peridinium* sp. *small*
Chroococcus limneticus	*Pseudanabaena catenata*	**Euglenophyta**
Merismopedia punctata	**Cryptophyta**	*Euglena oxyuris*
Microcystis aeruginosa	*Cryptomonas ovata*	*Euglena acus*
Microcystis flos-aquae	*Cryptomonas erosa*	*Euglena proxima*
Microcystis firma	*Cryptomonas rostratiformis*	*Euglena viridis*
Microstis incerta	*Cryptomonas lens*	*Lepocinclis fusiformis*
Planktomyces bekeffii	*Cryptomonas marssonii*	*Strombomonas fluviatilis*
Synechococcus ambiguus	*Plagioselmis* (*Rhodomonas*) *lacustris*	*Phacus orbicularis*
Nostocales	**Xanthophyta**	*Phacus agilis* var. *agilis*
Anabaena solitaria	*Dichotomococcus curvatus*	*Phacus pyrum* var. *Pyrum*
f. planktonica	*Goniochloris mutica*	**Heterokontophyta**
Anabaena spiroides	*Pseudostaurastrum hastatum*	**Chrysophyceae**
Anabaena sp.	*Pseudotetraedron neglectum*	*Chrysococcus* spp.
Aphanizomenon elenkinii	**Dinophyta**	*Dinobryon sociale*
Aphanizomenon issatschenkoi	*Ceratium hirundinella furcoides*	*Kephyrion* sp.
Cylindrospermopsis raciborskii	*Gymnodinium* sp. *small*	*Mallomonas acaroides* und sp.

(continued)

Annex (continued)

Bacillariophyceae	*Achnanthes clevei*	*Cymbella affinis*
Centrales	*Achnanthes conspicua*	*Cymbella amphicephala*
Acanthoceras zachariasii	*Achnanthes exigua*	*Cymbella caespitosa*
Actinocyclus normanii morphotyp *subsalsus*	*Achnanthes hungarica*	*Cymbella cistula*
Aulacoseira alpigena	*Achnanthes laevis*	*Cymbella helvetica*
Aulacoseira ambigua	*Achnanthes lanceolata*	*Cymbella lanceolata*
Aulacoseira granulata	*Achnanthes lanceolata*	*Cymbella microcephala*
Aulacoseira islandica var. *helvetica*	ssp. *dubia*	*Cymbella minuta*
Aulacoseira subarctica	*Achnanthes lanceolata*	*Cymbella prostrata*
Cyclostephanos dubius	ssp. *frequentissima*	*Cymbella silesiaca*
Cyclostephanos invisitatus	*Achnanthes lanceolata*	*Cymbella sinuata*
Cyclotella atomus	ssp. *frequentissima*	*Cymbella tumida*
Cyclotella cyclopuncta	var. *rostratiformis*	*Denticula tenuis*
Cyclotella glomerata	*Achnanthes minutissima*	*Diatoma ehrenbergii*
Cyclotella krammeri	*Achnanthes ploenensis*	*Diatoma mesodon*
Cyclotella meneghiniana	*Amphora inariensis*	*Diatoma moniliformis*
Cyclotella ocellata	*Amphora libyca*	*Diatoma vulgaris*
Cyclotella pseudostelligera	*Amphora montana*	*Diatoma vulgaris,* morphotype *capitulata*
Cyclotella radiosa	*Amphora ovalis*	*Diploneis ovalis*
Cyclotella schumannii	*Amphora pediculus*	*Diploneis* sp.
Melosira varians	*Amphora* sp.	*Epithemia* sp.
Skeletonema potamos	*Amphora veneta*	*Fragilaria arcus*
Stephanodiscus cf. *binderanus*	*Asterionella formosa*	*Fragilaria brevistriata*
Stephanodiscus hantzschii	*Bacillaria paradoxa*	*Fragilaria capucina*
Stephanodiscus medius	*Caloneis amphisbaena*	*Fragilaria capucina*
Stephanodiscus minutulus	*Caloneis bacillum*	var. *gracilis*
Stephanodiscus neoastraea	*Caloneis silicula*	*Fragilaria capucina*
Stephanodiscus parvus	*Caloneis* sp.	var. *rumpens*
Thalassiosira sp.	*Cocconeis pediculus*	*Fragilaria capucina*
Thalassiosira visurgis	*Cocconeis placentula*	var. *vaucheriae*
Thalassiosira weissflogi	*Craticula accomoda*	*Fragilaria construens*
Pennales	*Cymatopleura elliptica*	*Fragilaria crotonensis*
Achnanthes biasolettiana	*Cymatopleura solea*	*Fragilaria fasciculata*

(continued)

Annex (continued)

Fragilaria leptostauron	*Navicula contenta*	*Navicula slesvicensis*
Fragilaria parasitica	*Navicula costulata*	*Navicula splendicula*
var. *parasitica*	*Navicula cryptocephala*	*Navicula* ssp.
Fragilaria parasitica var. *subconstricta*	*Navicula cryptotenella*	*Navicula subhamulata*
Fragilaria pinnata	*Navicula cuspidata*	*Navicula subminuscula*
Fragilaria ulna var. *acus*	*Navicula decussis*	*Navicula tenelloides*
Fragilaria ulna var. *ulna*	*Navicula erifuga*	*Navicula tripunctata*
Frustulia vulgaris	*Navicula gallica*	*Navicula trivialis*
Gomphonema angustum	var. *perpusilla*	*Navicula veneta*
Gomphonema augur	*Navicula gastrum*	*Navicula viridula*
Gomphonema gracile	*Navicula goeppertiana*	var. *rostellata*
Gomphonema micropus	*Navicula gregaria*	*Navicula viridula* var. *viridula*
Gomphonema minutum	*Navicula integra*	*Neidium dubium*
Gomphonema olivaceum var. *olivaceum*	*Navicula lanceolata*	*Neidium* sp.
Gomphonema parvulum	*Navicula menisculus*	*Nitzschia acicularis*
Gomphonema sp.	var. *grunowii*	*Nitzschia amphibia*
Gomphonema tergestinum	*Navicula menisculus*	*Nitzschia angustata*
Gomphonema truncatum	var. *menisculus*	*Nitzschia angustatula*
Gyrosigma acuminatum	*Navicula minima*	*Nitzschia calida*
Gyrosigma attenuatum	*Navicula minuscula*	*Nitzschia capitellata*
Gyrosigma scalproides	*Navicula oblonga*	*Nitzschia clausii*
Hantzschia amphioxys	*Navicula placentula*	*Nitzschia constricta*
Meridion circulare	*Navicula protracta*	*Nitzschia debilis*
Navicula atomus var. *atomus*	*Navicula pupula*	*Nitzschia dissipata*
Navicula atomus var. *permitis*	*Navicula pygmaea*	var. *dissipata*
Navicula bacillum	*Navicula radiosa*	*Nitzschia dubia*
Navicula capitata	*Navicula recens*	*Nitzschia fonticola*
Navicula capitata	*Navicula reichardtiana*	*Nitzschia fruticosa*
var. *lueneburgensis*	*Navicula reinhardtii*	*Nitzschia graciliformis*
Navicula capitatoradiata	*Navicula rhynchocephala*	*Nitzschia graciliformis*
Navicula cari	*Navicula salinarum*	*Nitzschia gracilis*
Navicula confervacea	*Navicula schroeterii*	*Nitzschia heufleriana*
chain forming	*Navicula seminulum*	*Nitzschia hungarica*

(continued)

Annex (continued)

Nitzschia inconspicua	*Pteromonas aculeata*	*Scenedesmus dispar*
Nitzschia intermedia	**Chlorococcales**	*Scenedesmus ecornis*
Nitzschia levidensis	*Actinastrum hantzschii*	*Scenedesmus intermedius*
Nitzschia linearis	*Ankyra lanceolata*	*Scenedesmus obtusus*
Nitzschia linearis var. *subtilis*	*Ankistrodesmus gracilis*	*Scenedesmus opoliensis*
Nitzschia microcephala	*Chlorococcum* spp.	*Scenedesmus protuberans*
Nitzschia palea var. *palea*	*Chodatella quadriseta*	*Scenedesmus quadricauda large*
Nitzschia paleacea	*Closteriopsis limneticum*	*Scenedesmus quadricauda small*
Nitzschia recta	*Coelastrum astroideum*	*Scenedesmus quadrispina*
Nitzschia sigmoidea	*Coelastrum microporum*	*Scenedesmus spinosus*
Nitzschia sinuata var. *delognei*	*Coenococcus planktonicus*	*Scenedsmus acuminatus*
Nitzschia sociabilis	*Crucigenia tetrapedia*	*Secenedesmus acutus* var. *globosus*
Nitzschia spp.	*Crucigeniella apiculata*	*Secenedesmus ecornis disciformis*
Nitzschia sublinearis	*Crucigeniella rectangularis*	*Schroederia setigera*
Nitzschia tryblionella	*Dictyosphaerium ehrenbergianum*	*Tetradesmus major*
Nitzschia tubicola	*Kirchneriella lunaris*	*Tetraedron caudatum*
Pinnularia borealis	*Lagerheimia genevensis*	*Tetraedron minimum*
Pinnularia major	*Lagerheimia longiseta*	*Tetraselmis cordiformis*
Pleurosira laevis f. laevis	*Micractinium pusillum*	*Tetrastrum staurogeniaeforme*
Rhoicosphenia abbreviata	*Monoraphidium contortum*	*Tetrastrum* sp.
Surirella angusta	*Monoraphidium griffithii*	**Ulotrichales**
Surirella brebissonii	*Monoraphidium markovae*	*Elakatothrix* sp.
Surirella crumena	*Monoraphidium minutum*	*Gloeotila* sp.
Surirella linearis var. *helvetica*	*Oocystis lacustris*	*Koliella longiseta*
Surirella minuta	*Oocystis marssonii*	**Zygnematales**
Surirella sp.	*Pediastrum boryanum*	*Spirogyra* sp.
Tabellaria flocculosa	*Pediastrum duplex* var. *duplex*	**Desmidiales**
Chlorophyta	*Pediastrum duplex* var. *gracillimum*	*Closterium acutum* var. *linea*
Volvocales	*Pediastrum simplex* var. *simplex*	*Closterium acutum*
Chlamydomonas braunii	*Pediastrum simplex* var. *echinulatum*	*Closterium moniliferum*
Chlamydomonas monadina	*Pediastrum tetras*	*Cosmarium* sp.
Chlamydomonas sp. (elongated)	*Scenedesmus acuminatus elongatus*	*Staurastrum cingulum*
Chlamydomonas sp. (ovoid)	*Scenedesmus armatus*	*Staurastrum paradoxum*
Pandorina morum	*Scenedesmus brevispina*	
Phacotus sp.	*Scenedesmus denticulatus*	

Gaps and Uncertainties in the Ecological Status Assessment in the Danube River Basin District

Franz Wagner

Abstract The EU Water Framework Directive demands the good ecological status in all surface waters within the time frame 2015–2027. The status is monitored by the member states using national sampling and assessment methods designed after the requirements of the WFD and adjusted in an international intercalibration process. In the implementation process, still gaps and uncertainties exist. For solving the open issues, more data and research is necessary; often countries could use approaches developed by other EU member states.

Keywords Assessment, Danube, Ecological status, Methods, Monitoring, Sampling

Contents

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1 Challenge Ecological Status

Since the year 2000 the Water Framework Directive [1] (WFD – Directive 2000/60/EC) commits European Union member states to achieve good qualitative and quantitative status of all water bodies within the time frame 2015–2027. For surface waters the qualitative aspect demands the good ecological and chemical status.

What is the significance of the ecological status? It is an estimation of the quality level of the ecological functionality of the aquatic ecosystem, including the total environment and the extensive network of biota. Ecosystems are extremely complex and an evaluation of their total entity is impossible. Thus, selected organismic groups are serving as indicators that describe the key functionality: fish, aquatic plants (macrophytes and phytobenthos), phytoplankton and benthic invertebrates.

The use of biological indicators for the assessment of ecological quality has a long tradition that started with Lauterborn [2] decades before the WFD was issued. However, most of the early systems concentrated on evaluation of organic pressure and few biological elements, mainly benthic invertebrates and phytobenthos. The WFD is a substantial step forward using the complex analysis of pressures and impacts and the assessment of the status of water bodies. There are normative definitions for the methods for classification of the ecological status, but member states are free to design their own national methodology for sampling and assessment [3]. To ensure international comparability and adjustment of the various methods to a common level, an intercalibration process was foreseen.

The WFD-compliant methods for the assessment of ecological status are type specific. For each type of surface water, reference conditions are defined, and the assessment methods measure the deviation of the actual status from the reference status on the scale of selected parameters. Thus, the challenge for developing the new methods was to differentiate between types; to define reference conditions by using either pristine sites, historical data or a modelling approach; and to find appropriate indices that describe the reaction of the biological quality elements to the relevant pressures.

The European Commission provided general rules for these processes [4], and the detailed implementation on the national level has to be reported to the European Commission.

National River Basin Management Plans (RBMP) contain the information about all the steps in the implementation process of the WFD. At the level of the Danube River Basin (DRB), the ICPDR produced the Danube River Basin Management Plan which is a roof report covering the entire catchment area at the basin-wide scale [5]. Details of this report concerning the monitoring and assessment of the chemical and ecological status were dealt with by the Monitoring and Assessment Expert Group (MA EG) of the ICPDR. This expert group also analysed the gaps and uncertainties within the monitoring programmes in place. Findings of this analysis (status as of 2012) are summarised in this chapter.

Key questions addressed in the gap analysis are as follows: Where are the deficits in the implementation of the WFD requirements? How can they be overcome? Are there still problems with methods, data and the assessment of water body status? Special attention is given to the assessment of large rivers with the Danube being the second largest and longest river in Europe. Large rivers are especially challenging for reasons of difficulties in sampling and assessing the reference conditions.

2 Sampling Methods

Requirements for sampling methods are given in Annex V of the WFD. In some member states WFD-compliant sampling methods are not available for some of the quality elements (Table 1).

With few exceptions, in the EU countries sampling methods for all quality elements are in place. As the requirements of the WFD result in similar sampling methods all over Europe, the existing sampling methods can eventually be adopted to the special needs in the non-EU countries which still have a need for the development of sampling methods for about 25% of the quality elements.

For large rivers and especially for the Danube, satisfactory sampling methods for the evaluation of the ecological status do not exist for some quality elements. The reason for this is that representative and quantitative sampling for some quality elements requires taking samples from deep areas which is technically difficult and expensive. This is especially the case for fish and macrozoobenthos. To which extent the deep areas have to be sampled remains still unclear.

3 Assessment Methods

Requirements for assessment methods are given in Annex V of the WFD. An overview of the availability of WFD-compliant assessment methods is shown in Table 2.

For each country the assessment is linked to the national sampling methods, because reference conditions for the metrics or indices used depend on efficiency and design of sampling. For example, for macrozoobenthos, the abundance will increase with decreasing mesh size used for sampling. However, for the assessment of the status, the relation of the actual measurement to the reference value is used. Thus the strict definition and adherence of the reference values is more important than the technical details of the sampling method (such as mesh size) as long as the sampling method is efficient in recording the variables of the ecological community required by Annex V of the WFD.

Only in few EU countries, assessment methods do not exist for all quality elements, and for some countries specific quality elements are not relevant (e.g. in some countries even the large rivers do not sustain an autochthonous plankton community).

For large rivers like the Danube, the reference conditions often are not known due to the fact that they are anthropogenically utilised and therefore

Table 1 Sampling methods in the DRB countries

	Do WFD-compliant methods exist?							Does WFD-compliant method for large rivers exist?						
	Phyto plankton	Macrophytes and phytobenthos	Macro zoobenthos	Fish	Hydro morphology	General phys.–chem. conditions	Specific pollutants	Phytoplankton	Macrophytes and phytobenthos	Macrozoobenthos	Fish	Hydromorphology	General phys.–chem. conditions	Specific pollutants
AT	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
BA	Y/nc	Y/nc	Y/nc	N	N	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	N	N	Y/nc	Y/nc
BG	N	Y	Y	Y	N	Y	Y	N	N	N	N	N	Y	Y
CZ	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
DE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
HR	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y
HU	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
MD	Y	Y/nc	Y/nc	N	Y/nc	Y	Y	Y	Y/nc	Y/nc	N	Y/nc	Y	Y
ME	Y/nc	Y/nc	Y/nc	N	N	Y/nc	N	Y/nc	Y/nc	Y/nc	N	N	Y/nc	N
RO	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
RS	Y	Y/nc	Y	Y/nc	Y	Y	Y/nc	Y	Y/nc	Y/nc	Y/nc	Y/nc	Y	Y
SI	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
SK	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y/nc	Y	Y	Y
UA	Y	N	N	N	N	Y	Y	Y	N	N	N	N	Y	Y

Y WFD-compliant method available, *Y/nc* method available but not compliant, *N* no method available at the moment (status as of 2012)

Table 2 Assessment methods in the DRB countries

	Do WFD-compliant methods exist?							Does WFD-compliant method for large rivers exist?						
	Phyto plankton	Macrophytes and phytobenthos	Macro zoobenthos	Fish	Hydro morphology	General phys.–chem. conditions	Specific pollutants	Phyto plankton	Macrophytes and phytobenthos	Macrozoobenthos	Fish	Hydromorphology	General phys.–chem. conditions	Specific pollutants
AT	Y/nc	Y	Y	Y	Y	Y	Y	Y/nc	Y	N	Y	Y	Y	Y
BA	Y/nc	Y/nc	Y/nc	N	N	Y/nc	Y/nc	N	N	N	N	N	N	N
BG	N	Y	Y	Y/nc	N	Y/nc	Y/nc	N	N	N	N	N	Y/nc	Y/nc
CZ	Y	Y	Y	Y	Y/nc	Y	Y	Y	Y	Y	Y	Y/nc	Y	Y
DE	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y
HR	N	Y	Y	Y	N	Y	Y	N	Y	Y	Y	N	Y	Y
HU	Y	Y	Y	Y/nc	Y	Y	Y	Y	Y	Y	Y/nc	Y	Y	Y
MD	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc
ME	Y/nc	Y/nc	Y/nc	N	N	Y/nc	Y/nc	Y/nc	Y/nc	Y/nc	N	N	Y/nc	N
RO	Y	N/Y	Y	Y	Y	Y	Y	Y	N/Y	Y	Y	Y	Y	Y
RS	Y	Y	Y	Y/nc	N	Y	Y/nc	Y	Y	Y	Y/nc	Y/nc	Y	Y/nc
SI	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y
SK	Y/nc	Y	Y	Y	Y	Y	Y	Y/nc	Y	Y	Y/nc	Y	Y	Y
UA	Y/nc	N	N	N	N	Y/nc	Y/nc	Y/nc	N	N	N	N	Y/nc	Y/nc

Y WFD-compliant method available, *Y/nc* method available but not compliant, *N* no method available at the moment hydromorphologically modified since centuries. Thus in most countries the assessment methods are based on theoretical reference values that are stated by historical data or a modelling approach.

4 Monitoring Programmes

The proper installation and alignment of the monitoring system is an essential prerequisite of data availability, data quality and data quantity. General guidelines for establishing monitoring programmes are given in the WFD in Annex V. For some EU member states within the Danube River Basin, the following problems still exist:

- The number and the best location of sampling sites within a water body are unclear or too low for a reasonable assessment of the ecological status of the water body. Official requirements for setting the monitoring system (e.g. fixed in a guideline) do often not exist.
- The overall number of sampling sites is too low for an assessment of the ecological status that covers the whole area of the state.
- Data on hydromorphological elements are missing.
- Intercalibration is still missing for some biological methods for individual quality elements. This is a general problem for all EU member states.
- Impossibility of statistical correlations between BQEs and physical and chemical supporting elements because of monitoring data collected at different time periods in the year.
- Lack of taxonomic expertise for the application of complex assessment methods. This is especially the case for macrozoobenthos and phytobenthos.

In the EU countries of the Danube River Basin, only for about 10% of the quality element unclarities exist concerning number and positioning of sampling sites within the water body, while in the non-EU Danube countries, the number and location of monitoring sites is still not clear for more than 40% of the quality elements (Fig. 1).

Fig. 1 Question: is the necessary number and location of the monitoring sites within a water body clear?

Information exchange between the member states is a crucial factor for filling the information gaps in the RBMP caused by problems with the national monitoring systems. Requirements of the WFD result in the application of similar methods all over Europe. This means that the methods existing in EU member states can be used or adapted to the special needs in another country where such method is still not available.

Gap analysis revealed that in many cases higher sampling frequencies would be necessary in national monitoring programmes. The major reason of low monitoring frequencies is financial constraints. It has to be however pointed out that the ecological assessment according to the WFD requires an investigation of environmental variables in relation to their reference conditions. Therefore, it is not always necessary to investigate all seasons and all possible spatial variability. In the event that the reference condition refers to a special time (e.g. the abundance of macrozoobenthos in spring), samples can be also taken only during this time. Applying the adaptive monitoring frequency, it has to be made sure that the conditions for the used variables are robust in time and space.

5 Intercalibration

The intercalibration was included in the implementation process of the WFD to guarantee a similar assessment of comparable ecological status situations with different national assessment methods. At the same time this exercise serves as a quality assurance and control procedure for the national assessment systems. There are numerous reasons of data variability including differences in national typologies, difficulties in defining reference conditions and difficulties in selecting suitable indices that correlate with the applied national assessment methods.

However the intercalibration should serve as a comparison at a coarse level taking into account a considerable variability. Thus in general the data variability is not a major problem as long as the intercalibration process is seen as rough match and adjustments are made carefully and with sense of proportion.

At present two phases of the intercalibration process have been accomplished (for details see Commission Decisions [6, 7]). The rivers of the Danube River Basin have been covered in the Eastern Continental Geographical Intercalibration Group, and the intercalibration is completed for the quality elements, macroinvertebrates, phytobenthos and macrophytes. The quality element fish was intercalibrated for the entire Europe (without separation into types) with the results included in the Commission Decision 2013.

In large rivers the situation is more complicated. Sampling is difficult, and assessment systems are often missing or unsatisfactory due to missing information concerning reference conditions, dominance of alien species and lack of monitoring data. In the Commission Decision 2013, only results for phytobenthos are included; the intercalibration for macrophytes, phytoplankton, fish and benthic invertebrates is expected to be completed until 2016. Another open issue is the intercalibration of heavily modified water bodies.

6 The Role of Alien Species

In all DRB countries neobiota are a substantial problem for the assessment of the ecological status. On the one hand, they are replacing the native species which is not automatically altering the ecosystem quality substantially, but the effect of this is difficult to evaluate. On the other hand, neobiota are colonising habitats with anthropogenic origin (like flood protection fortifications), making the relation to natural reference conditions impossible. Designing a programme of measures addressing alien species is a very problematic issue which cannot be solved before addressing the question of how to deal with the invasive alien species in the assessment of the ecological status. This question is still being under discussion all over Europe. In reaction to this issue, the Monitoring and Assessment Expert Group of the ICPDR agreed on the joint position that invasive alien species should not be considered en bloc as having a negative impact without further analysis and prepared a list of species with more detailed information. For more information, see Paunović et al. [8].

7 From Monitoring Data to Ecological Status

Data from WFD monitoring programmes are obtained from sampling sites that should be selected to be representative for the whole water body. Until present the criteria of representativeness were not compared or harmonised – intercalibration focuses on the comparison of sampling methods applied at the sampling sites.

In most Danube countries not enough monitoring data is available for an ecological status assessment of all water bodies. Part of the total number of water bodies can be assessed by a grouping procedure that is explicitly allowed by the WFD: A group of water bodies with comparable conditions concerning typology and reference conditions, but also pressures, can be assessed by sampling sites in a representative number of water bodies. The results from these water bodies are then transferred to the whole group.

As often the available data and the grouping procedure are not sufficient for the assessment of all national water bodies, many countries use a confidence concept (Fig. 2) similar to that used in the DRBMP [5]. The basis of this concept is:

- High confidence: assessment by monitoring data or reasonable grouping
- Medium confidence: assessment with insufficient data or grouping

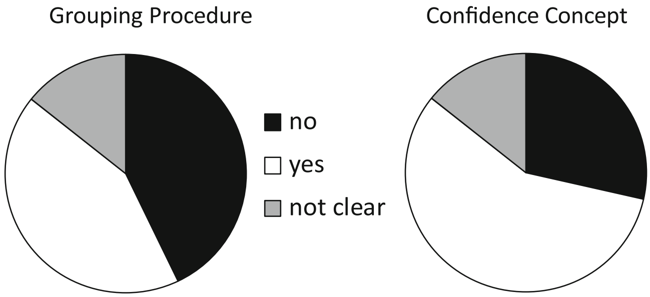

Fig. 2 Use of grouping procedure and confidence concept for the assessment of all water bodies in the Danube River Basin. No: grouping procedures are not used in the national monitoring system, yes: grouping procedures are an integral part of the national monitoring system, not clear: use of grouping procedures in the national monitoring system is still under discussion

- Low confidence: assessment without data, mostly by transferring risk to status (e.g. no risk = good status with low confidence, risk = moderate status with low confidence)

8 Conclusions

The implementation of the WFD clearly promoted progress in the monitoring and assessment of aquatic ecosystems and will continue to be a driving force in future research and development. Nevertheless, the procedure of monitoring and assessment is not in the final stage but will be a process of permanent adaptation and further advancement. Not only gaps concerning sampling and assessment have to be eliminated, but also intercalibration has to be completed, and further challenges (e.g. in the fields of neobiota, climate change and upcoming pressures) will have to be taken into account.

Countries are facing similar problems and gaps; thus, solutions (e.g. typology, methodological approaches) may be acquired together or adopted from existing methods.

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Microbiological Water Quality of the Danube River: Status Quo and Future Perspectives

Alexander K.T. Kirschner, Gerhard Kavka, Georg H. Reischer, Regina Sommer, A. Paul Blaschke, Margaret Stevenson, Julia Vierheilig, Robert L. Mach, and Andreas H. Farnleitner

Abstract Fecal microbial pollution is a major problem throughout the Danube River Basin, posing a threat to various types of water use, including drinking water

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production from river bank filtrates, water supply for agricultural and industrial use, and the role of the river as a recreational space. Fecal microbial pollution is introduced into the river by point sources, such as discharges of treated or untreated sewage from human sources or livestock, and by nonpoint sources, such as urban and agricultural runoff. In addition, fecal input from wildlife may be of importance in specific regions. Despite huge efforts to improve wastewater management in the past decade, in many sections, the river and its tributaries exhibit very high levels of fecal microbial pollution. To assess microbiological water quality, indicators of fecal pollution are used as surrogates for the potential presence of intestinal pathogens. However, the standard indicators cannot provide any reliable information regarding the origin of fecal pollution, nor can their concentration levels be directly related to human health risks for many types of exposure and situations.

The aim of this book chapter is to summarize the historical developments in microbiological water quality research and to reflect the most recent publicly available data on the fecal microbial pollution status of the Danube River. Moreover, the first results on fecal microbial source tracking by molecular biology methods are presented along with their applicability in river water quality monitoring, including the monitoring of riparian wells and alluvial groundwater resources. Finally, a discussion of the general state of water quality and public health is presented concerning (i) the current situation and potential limitations of the Water Framework Directive regarding the microbiological quality elements, (ii) further improvements regarding sampling and monitoring strategies, and (iii) the recently introduced concept of "integrated framework of fecal pollution monitoring and management" and expected further methodological developments in the context of the Danube watershed. Rapid progress in research and development is currently being made in the area of fecal microbial source tracking, pathogen detection, and health risk assessment, and these innovations are also likely to complement basic fecal pollution monitoring programs for river systems such as the Danube in the near future.

Keywords Fecal pollution, Microbial source tracking, Microbiological water quality, Review, Sustainable water management

Contents

1 Introduction

Microbes are fundamental in aquatic ecosystems and occupy – due to their dual role – a special position among biological quality elements. On the one hand, autochthonous microbes (including bacteria, viruses/phages, and protozoa) act as ecological components of system functioning and represent the most abundant group of organisms, being mainly responsible for the decomposition of organic matter, remineralization of inorganic nutrients, and energy and organic matter transfer to higher trophic levels (microbial loop [1]). In so doing, they predominantly contribute to the so-called "self-purification" process and thus characterize the saprobic status and ecological integrity of rivers. Moreover, if river water is used for drinking water production, the composition of organic matter and the capacity of the autochthonous microbes to degrade organic matter are crucial for the biostability of the end product. On the other hand, predominantly allochthonous microbes, which are introduced into rivers from external sources, can be important pollutants with relevance to human and animal health. Within the allochthonous microbes, those that are spread via the fecal-oral infection pathway are the most significant group. For the comprehensive characterization of river water quality, both components have to be considered, but to date only the aspect of pollution microbiology, due to its critical importance in public health, has been included in international regulations for water quality, such as the EU Bathing Water Directive [2] and the Drinking Water Directive [3]. The focus of this paper is restricted to the microbiological fecal pollution component, while the microbial ecological component is only discussed in the historical background section. Nevertheless, we want to reemphasize the importance of this topic, as understanding of microbial processes is the basis for understanding the whole system and the microbiological pollution patterns in rivers in particular. There is still a high demand for research in this area, and the debate on how microbial ecological methods can be integrated to define microbes as biological quality elements of rivers has not even started.

The Danube River has a total length of 2,870 km; its basin covers an area of 801,500 km^2 with approximately 81 million inhabitants in 19 countries [4] contributing to a large extent to the water pollution of the Danube. In addition to chemical contamination, fecal microbial pollution is a major problem throughout the Danube River Basin, posing a threat to various types of water use [5], including drinking water production from river bank filtrates [6], the supply of water for agricultural and industrial use, and the role of the river as a recreational space. Approximately ten million people along the course of the Danube River receive treated drinking water from river bank filtration (www.iawd.at), and the International Association of Water Supply Companies in the Danube River (IAWD) issued a declaration as early as in 1992, "to improve and safeguard the water quality of the Danube and its tributaries and [. . .] encouraging all measures and efforts aimed at avoiding and eliminating the pollution of, and threat to, the status of raw water in the interest of drinking water supply" [6]. Fecal microbial pollution is introduced into the river by point sources, such as discharges of treated or untreated sewage from human sources or livestock, and by nonpoint sources, such as urban and agricultural runoff. In addition, fecal input from wildlife may be important in regions where the main river is highly interconnected with floodplains. Despite huge efforts to improve wastewater management in the past decade, in many sections, the river and its tributaries still receive incompletely treated sewage, leading to serious deterioration of water quality [7].

Fecal microbial pollution of water sources relevant to human health is related to the occurrence of pathogenic microorganisms that are spread via the fecal-oral route. They may originate from infected humans (anthroponotic) or from animals (zoonotic) and are shed via their feces into the water source and subsequently taken up via ingestion. Because not all fecal-associated pathogens can be detected from a potentially contaminated water source, microbiological indicators of fecal pollution are used as surrogates for the potential presence of intestinal pathogens. Fecal indicator bacteria such as *Escherichia coli* (*E. coli*) and intestinal enterococci occur almost ubiquitously in high concentrations in human and animal fecal material and are valuable indicators for fecal pollution detection. These microbiological indicators are quantitatively determined via standard culture-based methods. Recovering quantitative information on fecal pollution is the basis for microbiological water quality monitoring. However, in the case of pollution problems, information on the origin of contamination is also needed for effective target-oriented management strategies. Furthermore, information on the expected health risk in relation to the respective type of usage (recreation, swimming, irrigation, drinking, aquaculture) is increasingly required for water safety management. However, the standard indicators, *E. coli* and intestinal enterococci, cannot provide any reliable information regarding the origin of fecal pollution (e.g., human vs. animal), nor can their concentration levels be directly related to human health risks for many exposition types and situations. Rapid progress in research and development is currently being made in the area of fecal microbial source tracking, pathogen detection, and health risk assessment, and these innovations are also likely to complement basic fecal pollution monitoring programs for river systems in the near future (see Sect. 5).

The aim of this book chapter is to summarize the historical developments in microbiological water quality research and to relay the most recent publicly available data on the fecal microbial pollution status of the Danube River. Recent data sets are mainly derived from two scientific reports and one publication in an international scientific journal and include data from two whole-river surveys

(Joint Danube Survey 2001, 2007) and from the International Commission for the Protection of the Danube River (ICPDR) Transnational Monitoring Network. On a national basis, the Danube riparian states may have ample additional data sets on fecal microbial pollution concerning their section and its major tributaries. In addition, the IAWD supports microbiological monitoring activities at Danube sites where water is used for drinking water production. However, these data are not easily accessible because the data are not available in international scientific databases and could thus not be incorporated in the manuscript. As a further focus of this chapter, the first results on fecal microbial source tracking by molecular biological methods will be presented, and their applicability in river water quality monitoring – including the monitoring of riparian wells and alluvial groundwater resources – is discussed. The final Conclusion and Outlook section covers a discussion of the general state of microbial water quality and public health, the current situation and potential limitations of the Water Framework Directive regarding the microbiological quality elements, further improvements regarding sampling and monitoring strategies, and, finally, the recently introduced concept of "integrated framework of fecal pollution monitoring and management" and expected methodical developments in the context of the Danube watershed.

2 Historical Overview of Microbiological Research on the Danube River

This brief overview summarizes the historical development of the microbiological water quality research on the Danube River and important tributaries. This general overview is focused on relevant scientific publications and reports related to the field of pollution microbiology, primarily addressing allochthonous microorganisms such as intestinal indicator bacteria (e.g., *E. coli*, intestinal enterococci) and pathogenic microorganisms from external sources (e.g., sewage, runoff). Several publications in the field of microbial ecology, mainly concerning autochthonous microbial communities, referring to the assessment of water quality, are included as well. In addition, innovative studies involving first applications of molecular biological investigation techniques are cited. Transnational project studies in the Danube basin are emphasized. Early monitoring results from the Danube River were frequently published in German.

The first papers addressing microbiological water quality research of the Danube River were published around the turn of the nineteenth century. For example, Heider [8] already showed that the sewage from Vienna, after its discharge into the Danube River, flowed along the right bank of the stream, preserving its own bacterial characteristics and not mixing perfectly with the river water for more than 24 miles (44.5 km). Brezina [9] performed the first comparative investigations and found 1,900 culturable bacteria per ml in the Danube River upstream of Vienna and

110,000 culturable bacteria per ml downstream at the mouth of the Danube Canal in Vienna due to wastewater impact.

In the subsequent four decades, microbiological studies on the Danube River were scarce. Halophilic or salt-tolerant bacteria were investigated in the Danube Delta [10]. Joos [11] tested the presence of bacteria of the typhoid-paratyphoid group in Danube water and sewage. Investigations on the influence of pollution on bacterial nitrogen transformations were accomplished by Stundl [12].

The 1950s were the beginning of systematic investigation programs in the Danube River Basin [13]. Threats by microbiological pollution to human health via various types of water use were already observed [14].

In the 1960s, microbiological monitoring and research was extended to all riparian countries [13, 15–21]. In the book chapter “Die Mikrobiologie der Donau,” microbiological data from the riparian countries were summarized for the first time, e.g., total bacterial count, colony count, coliforms, *E. coli*, and enterococci (Mucha [22], in: Limnologie der Donau, edited by Liepolt 1967, in German with English summaries). The author noted the importance of the application of comparable and standardized methods and sent proposals to the national labs – with moderate success – as he commented himself. Microbiological research in the Danube River Basin received essential contributions from the International Association for Danube Research (IAD), an expert group in Microbiology/Hygienics and scientific platform for microbiologists working on Large River Ecosystems [22], which is still in operation today (www.iad.gs).

In the 1970s, research on pathogenic microorganisms (e.g., *Salmonella* spp.) was intensified (e.g., [16, 23–25]). A main finding was that *Salmonella* spp. occur frequently in wastewater and cannot be eliminated by biological sewage treatment plants. Therefore, *Salmonella* spp. can be easily isolated in polluted rivers downstream of wastewater discharges. Kohl [26] forced the bacteriological investigation of sediment and periphyton to improve the microbiological assessment of water bodies. An essential advance was the publication of a classification system for the heterotrophic plate count (colony count) and fecal indicator (fecal coliforms) parameters by Kohl [16].

In the 1980s, many investigations focused on the determination of the microbiological pollution of the Danube River (e.g., [27–31]). Several authors applied bacterial numbers and biomass as well as biochemical activity parameters, such as phosphatase activity, for the characterization of the microbiological water quality ([32–37]). In 1985 an international monitoring program was established based on the Bucharest Declaration, containing transboundary cross sections on the Danube River [7]. A highlight was the international Danube expedition in 1988, organized by the IAD, from Vienna resp. Bratislava to the Danube delta [38–43]. The data from the expedition indicated unacceptable fecal pollution levels in the Danube downstream of the cities Silistra, Nikopol, Vidin, Visegrad, Gabcikovo, and Bratislava because of sewage discharges [41]. Investigations of the German and Austrian Danube, due to technical reasons, could not be performed in this ambitious research program. Kasimir [42] investigated the total bacterial count, biomass, percentage of free living and attached bacterial cells, percentage of free dividing cells, and bacterial secondary production along the Danube River. Methodical comparisons of the direct count parameter from Austrian and Czechoslovakian labs demonstrated differences of approximately one order of magnitude, emphasizing again the requirements of standardized methods to obtain comparable results.

In the end of the last century, the focus was on microbiological long-term water quality alterations [44–48]. For example, in the Austrian section of the Danube River, bacteriological monitoring has been performed since 1957. The collected data suggested an improvement of bacteriological water quality between 1957 and 1997 [49]. Downstream of Vienna, the bacteriological data indicated the need for further action. The Transnational Monitoring Network (TNMN) was officially launched 1996 to support the implementation of the Danube River Protection Convention in the field of monitoring and assessment (www.icpdr.org). Popp et al. [50] published a classification scheme for the assessment of bacteriological water quality of running waters. Several papers also addressed natural microbial communities and their associated activities (microbial ecology), especially regarding water quality issues [51–57]. In 1998, the research boat MS Burgund traveled the great European waterway of the Rhine, Main, and Danube Rivers from Mainz on the Rhine to the Hungarian-Croatian-Serbian border, passing the German, Austrian, and Hungarian stretch of the Danube. The goal of this research trip was a joint evaluation and comparison of water quality, including microbiological parameters [58].

In the last decade (beginning in 2001), many innovative microbiological research activities have been undertaken in the Danube River Basin. Microbial community analysis, biotransformation processes, and enzymatic activities received special attention [59–62]. Farnleitner et al. [63, 64] presented enzymatic techniques for the rapid detection of *E. coli* in polluted river water. Schade et al. [65] discussed the wastewater UV disinfection method to improve water quality. Kolarevic et al. [66], Hosam et al. [67], and Ajeagah et al. [68] investigated the sanitary risks and aquatic ecosystem hazards in the Danube sections of Serbia, Hungary, and Romania. In its Annual Report for 2009/2010, the International Association of Water Supply Companies in the Danube River Catchment (IAWD) presented microbiological data from the current monitoring sites along the Danube River [6]. Monitoring focuses on the abstraction points for drinking water production at the respective Danube River bank filtration sites (bank filtrate).

During two whole-river surveys (Joint Danube Survey, JDS 2001, 2007), organized by the International Commission for the Protection of Danube River (ICPDR), samples were taken for the first time along the whole stretch of the Danube River from Germany to the Black Sea with uniform methods [5, 69, 70]. A third JDS was performed in 2013, but data from this survey was not integrated into the book chapter. The variation of fecal pollution in the longitudinal profile of the Danube and its main tributaries was determined by bacteriological standard parameters (details are presented in the next section). The first microbiological water quality map of the whole Danube River was created, illustrating the degree of fecal pollution at more than 75 Danube sampling stations and 21 tributaries [69]. A five-level classification system for microbiological fecal pollution to harmonize with other available classification systems according to the EU Water Framework Directive (EU-WFD; [2]) was developed by Kavka et al. [71] and applied in the Danube Survey 2007 [5, 70]. Longitudinal changes in the genetic and morphological population structure of the natural bacterial community of the Danube River, a fundamental part of ecosystem functioning and integrity, were studied during JDS1 (in 2001) and JDS2 (in 2007) [62, 72, 73]. The observed development of the bacterial compartment along the Danube River generally supported the river continuum concept [62]. Furthermore, DNA material recovered during the JDS 2001 and JDS 2007 water sampling activities was used to evaluate the applicability of DNA-based microbial source tracking approaches along the whole Danube River stretch to foster target-oriented management in the catchment [70, 74]. An overview on the applicability of microbial source tracking is presented in the next sections.

3 Fecal Microbial Pollution of the Danube River: A Snapshot Analysis

3.1 Methods Background

Cultivation-based methods are still the gold standard for the assessment of microbiological water quality. Within the current version of the EU Bathing Water Directive [2], which needed to be implemented by all European Union member states by 2008, the determination of *E. coli* and intestinal enterococci is compulsory. Previously, total coliforms, fecal coliforms, and fecal streptococci had to be investigated as fecal indicators, as well as salmonellae and enteroviruses as indicator pathogens [75]. Total coliforms were excluded in the new directive because several findings indicated that a significant portion of these bacterial species can multiply in the aquatic environment and are thus not suitable as fecal indicators ([76] and citations therein). *E. coli* is now used instead of fecal coliforms, as this species makes up the majority of fecal coliforms found in water and is a better indicator of fecal pollution. Similar arguments apply to the switch from fecal streptococci to intestinal enterococci. Finally, the mandatory investigation of salmonellae and enteroviruses has been omitted because (i) their detection is quite time consuming and (ii) the concentration of *E. coli* and intestinal enterococci shows a good correlation to epidemiological data of bathing water-associated diseases [77].

The data set presented in this article compiles the most recent available data from international scientific publications and public reports on microbiological water quality of the Danube River and its most important tributaries. This set includes

Table 1 Microbiology-based classification system of water quality according to fecal pollution (Modified after [5]; with permission from Elsevier)

Classification of fecal pollution		Class				
		I	II	III	IV	V
Parameter	Fecal pollution	Little	Moderate	Critical	Strong	Excessive
Escherichia coli EC	In 100 ml water	≤100	>100–1,000	>1,000–10,000	>10,000–100,000	>100,000
Intestinal Enterococci ENT	In 100 ml water	≤40	>40–400	>400–4,000	>4,000–40,000	>40,000
Total coliforms TC	In 100 ml water	≤500	>500–10,000	>10,000–100,000	>100,000–1,000,000	>1,000,000

information from two whole-river surveys (JDS1 and JDS2) and data from the ICPDR Transnational Monitoring Network (TNMN 2001–2005; http://www.icpdr.org/main/activities-projects/tnmn-transnational-monitoring-network) that were extracted from Kavka and Poetsch [69] and Kirschner et al. [5, 70].

Because the different data sets come from different years under different bathing water regulations, the assessment of microbiological water quality along the Danube River is based on a variety of parameters (fecal indicators) that were either assessed according to international standards [78–81] or other appropriate methods that were validated during the investigations with standard methods (Colilert 18, Idexx, Germany). Details can be found in the references mentioned above [5, 69, 70].

To translate concentrations of fecal indicators into levels of fecal microbial pollution, a five-level classification system [71] that integrates the guidelines for bathing water quality [2, 75] with the European Water Framework Directive (EU-WFD) [82] was applied. In this system, five classes of fecal pollution were defined such that classes I and II are below and quality classes III, IV, and V exceed the fecal pollution limit values for good bathing water quality (Table 1).

3.2 *Joint Danube Surveys*

During two whole-river surveys, samples from 96 (JDS 2007) and 98 (JDS 2001) sampling stations were taken, along a stretch of 2,600 km. In 2007, the selected sampling sites included 75 Danube River sites and 21 tributaries and branches; in 2001, they included 76 river sites and 22 tributaries/branches (Fig. 1). At all Danube and large tributary sampling stations, water samples were collected directly from the cruise ship in the middle of the river. Samples were taken with sterile 1 L glass

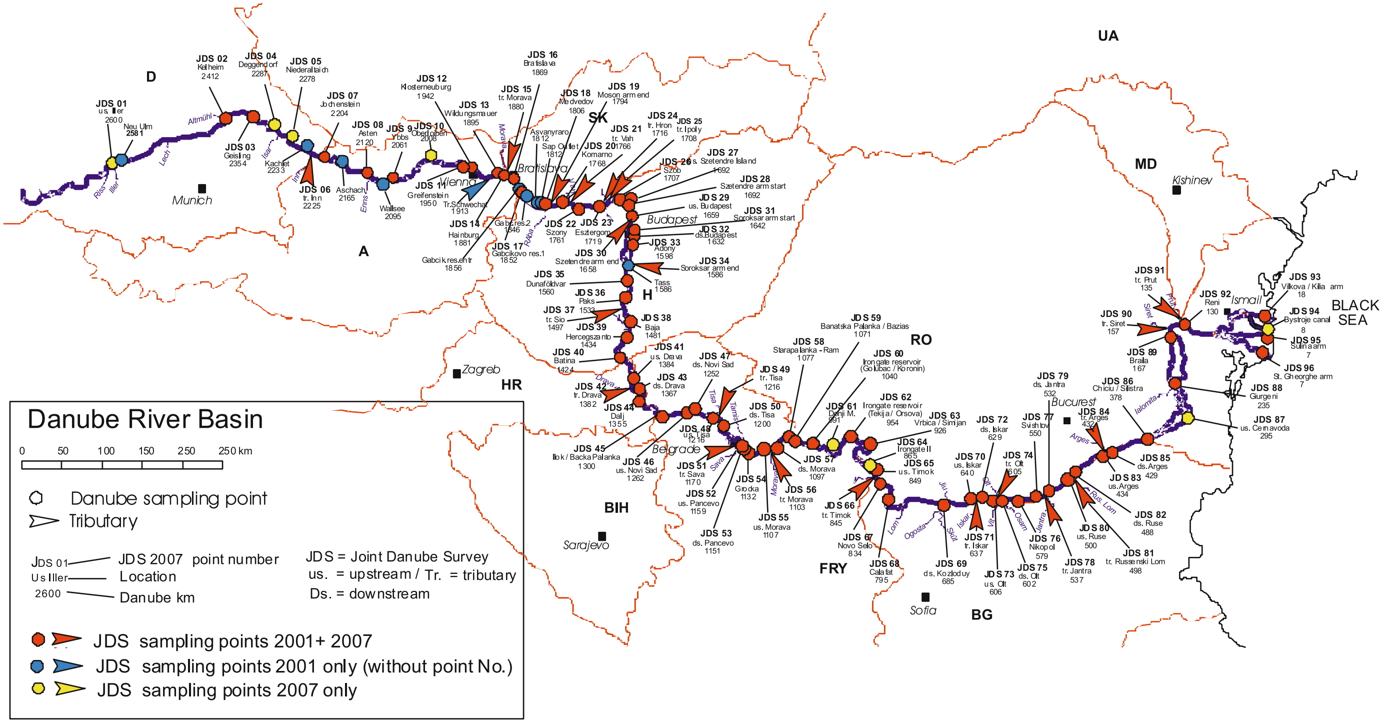

Fig. 1 Sampling sites in the Danube River Basin selected during the Joint Danube Survey 2001 and 2007. *Red circles* indicate sampling stations common to JDS 2001 and JDS 2007, *blue circles* indicate sampling stations unique to JDS 2001, and *yellow circles* indicate sampling stations unique to JDS 2007 (Modified after [5]; with permission from Elsevier)

flasks fixed to a sampling rod at a water depth of approximately 30 cm [81]. For smaller tributaries and branches, samples were taken in the same manner from small boats. All samples were immediately processed on board.

3.3 Summarized Data from the Joint Danube Surveys

Fecal pollution varies significantly along the course of the Danube and is to a large extent determined by the influence of the large urban areas of Vienna, Bratislava, Budapest, Belgrade, and Bucharest. The highest fecal pollution levels, however, are observed in specific tributaries and branches, related to the cities Györ (Mosoni Danube), Budapest (Rackeve-Soroksar Arm), Ruse (Rusenski Lom), and Arges (Bucharest).

According to levels of fecal indicator concentrations, six sections of fecal pollution along the Danube River can be delineated (Fig. 2). The first section has little to moderate pollution and ranges from the headwaters of the Danube in Germany to rkm (river kilometer) 1,942 (upstream of Vienna, Austria). Due to the influence of the urban areas of Vienna and Bratislava (Slovakia), the second section starts with a significant increase in fecal indicator concentrations to critical levels of fecal pollution, followed by a decreasing trend down to low levels until upstream of Budapest, Hungary (rkm 1,659). Because Budapest did not possess a state-of-the-art wastewater treatment plant until 2010, a dramatic increase of fecal indicator concentrations to strong pollution levels is observed at the beginning of section III. Critical levels of fecal pollution remain dominating in this section until downstream of Belgrade (Serbia). After the merging of Velika Morava (rkm 1,107), fecal pollution levels in the fourth section decreased markedly down to low levels until Orsova (Romania, rkm: 954) due to the absence of large cities and abundant agriculture in this section. Additionally, the deep Iron Gate reservoirs most likely enable sedimentation of particles and associated fecal indicators. The fifth section, ranging from rkm 954 to Cernavoda (Romania, rkm: 290), is characterized by a steady increase in fecal pollution from low to critical levels after merging with the excessively polluted water from the Arges (collecting untreated sewage via the Dambovita river from Romania's capital Bucharest). In the last section, a slight decrease in fecal indicator concentrations to moderate levels is observed.

The highest fecal pollution levels (strong-excessive) were observed in the Arges tributary and the Rusenski Lom (Bulgaria), Rackeve-Soroksar Arm, and Mosoni Danube (Slovakia, receiving wastewater from Györ, Hungary) branches. Other tributaries with pollution levels exceeding guideline values for bathing water quality are the Schwechat (Austria), Morava (Austria, Czech Republic, Slovakia), Vah (Slovakia), Ipoly (Hungary), Drava (Croatia, Hungary), Velika Morava (Serbia), Siret (Romania), and Prut (Romania, Moldova) Rivers. Other tributaries show moderate to low fecal pollution (Fig. 2).

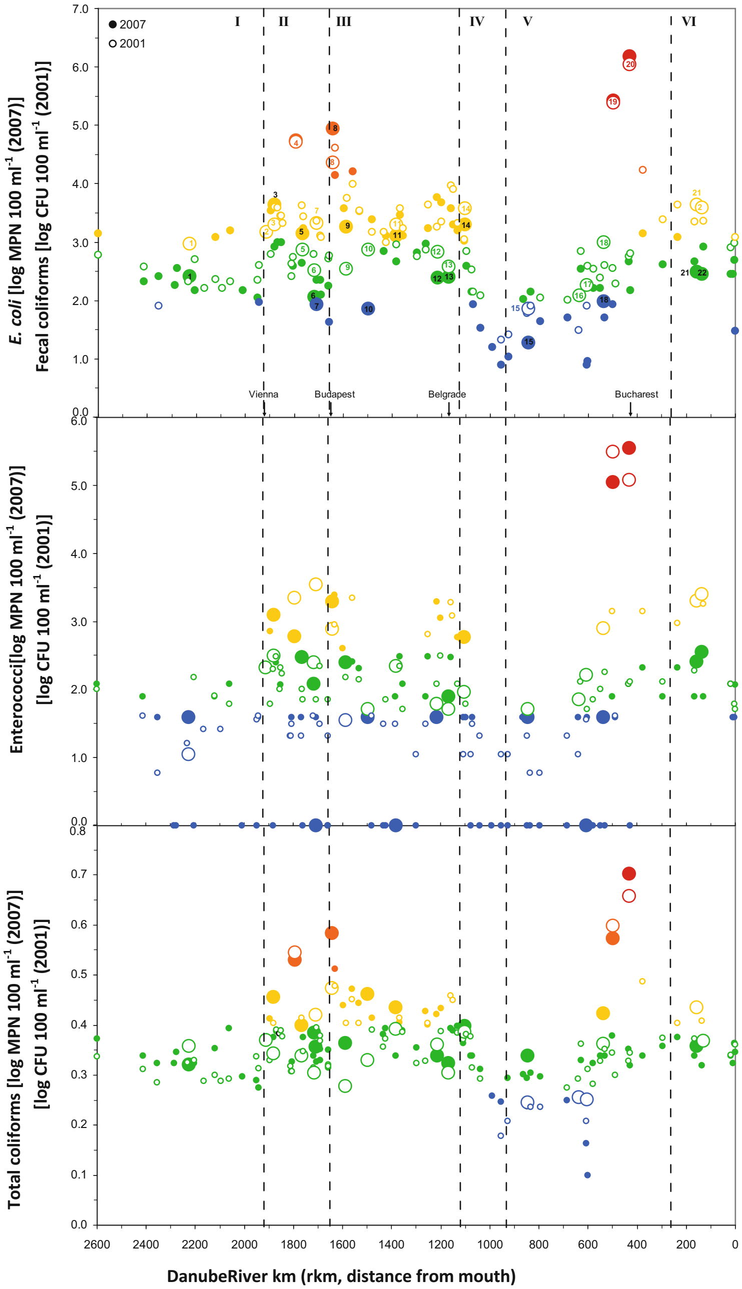

Fig. 2 Longitudinal development of fecal microbiological pollution in the Danube River (*small circles*) and its major tributaries (*large circles*) during JDS 2001 (*open symbols*) and JDS 2007 (*closed symbols*). Colors were chosen according to the microbiology-based pollution classification system in Table 1: *blue*, little; *green*, moderate; *yellow*, critical; *orange*, strong; and *red*, excessive pollution. *Upper panel*, *E. coli*; *mid panel*, *Enterococci*; *lower panel*, total coliforms; tributaries/

3.4 Data from the Transnational Monitoring Network

Fifteen representative stations on the Danube River and one tributary (Arges River) that coincided with JDS sampling points and where continuous data sets from 2001 to 2005 for the middle of the river were available were chosen from the TNMN database. At each station, 20–120 measurements for each fecal indicator were available. Different methods were used in the different countries for the determination of fecal coliform (FC), enterococci, and total coliform concentrations. Figure 3 shows the variability of the fecal indicator concentrations over the 5-year period with concentrations ranging over two to four orders of magnitude. Despite the rather high variability of the TNMN data and despite the coarser spatial resolution, the data clearly reflect the pattern of fecal pollution developed from the two Joint Danube Surveys (Fig. 2). The average *E. coli*/FC concentrations of both surveys measured at the 16 common sampling points significantly correlated with the median TNMN concentrations ($\rho = 0.624$; $p < 0.01$). Because of the high number of zero values of enterococci measured during JDS 2007, no significant correlation to the TNMN data was obtained (Fig. 3). A weak but statistically insignificant correlation between the JDS and TNMN enterococci data was achieved when zero values were excluded from the data set ($\rho = 0.453$; $p = 0.07$). For total coliforms, a very high correspondence of the data sets was observed; only the data from the Arges tributary exceeded the maximal TNMN concentrations by approximately 1.5 orders of magnitude (Fig. 3). Median values from the two data sets were highly intercorrelated ($\rho = 0.800$; $p < 0.001$).

3.5 Short Summary of Fecal Pollution Levels in the Danube River Sections

A clear picture of the longitudinal development of fecal microbial pollution could be drawn from the data set used in this study. Six sections of fecal pollution, which were mainly determined by the influence of the large capitals of the riparian countries, were delineated. Similarly, tributaries and branches receiving wastewater from large cities were hot spots of fecal pollution. In addition to the influence of municipal wastewater, the continuous increase of fecal indicator concentrations in section V (Romania, Bulgaria) likely indicates significant input from agricultural sources in this rural region. Of the identified sections, section I (Germany, Austria) and significant parts of section IV (Serbia, Romania) and V (Romania, Bulgaria) showed little to moderate fecal pollution.

Fig. 2 (continued) branches, 1; Inn, 2; Schwechat, 3; Morava, 4; Moson Danube, 5; Vah, 6; Hron, 7; Ipoly, 8; Rackeve-Soroksar Arm start, 9; Rackeve-Soroksar Arm end, 10; Sio, 11; Drava, 12; Tisza, 13; Sava, 14; Velika Morava, 15; Timok, 16; Iskar, 17; Olt, 18; Jantra, 19; Russenski Lom, 20; Arges, 21; Siret, 22; Prut (Taken from [5]; with permission from Elsevier)

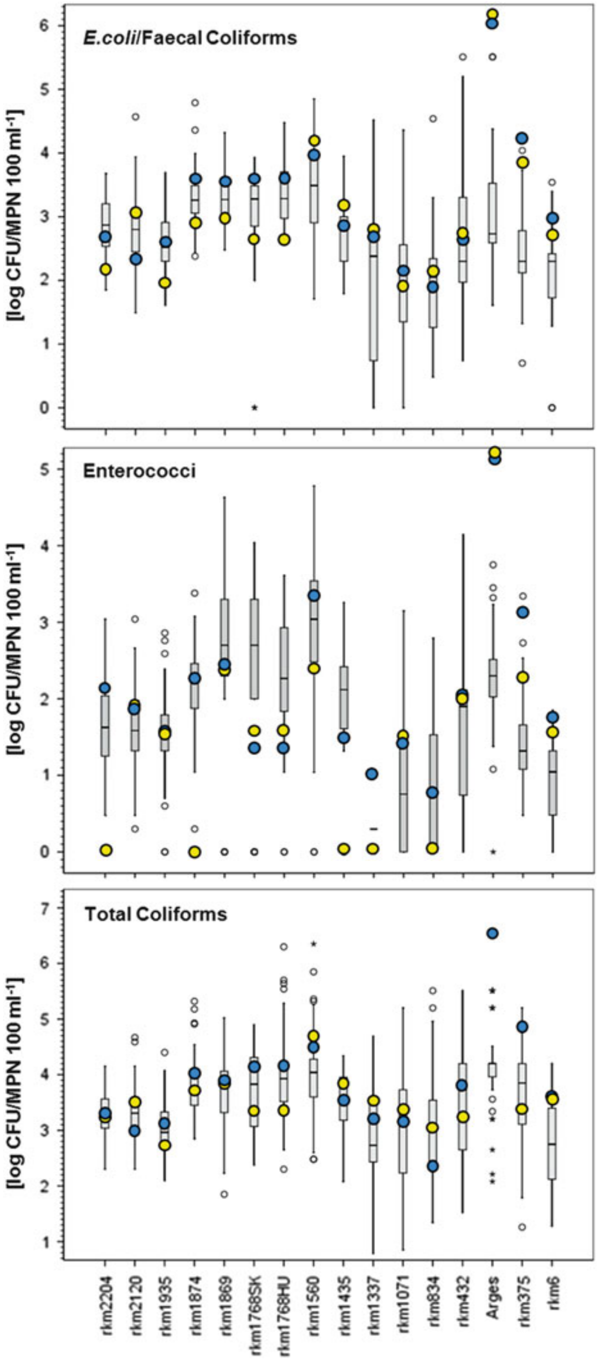

Fig. 3 Box-Whisker plots of the TNMN sampling data recorded during 2001 and 2005. For each fecal indicator class, between 20 and 120 sampling points were measured at each sampling point. Different methods were used in the different countries for the determination of fecal coliform, enterococci, and total coliform concentrations. For comparison, the data obtained during the two Joint Danube Surveys were added as *blue* (JDS 2001) and *yellow circles* (JDS 2007). *rkm* river kilometer of the Danube (Modified after [5]; with permission from Elsevier)

4 Genetic Fecal Marker Detection and Fecal Microbial Source Tracking

4.1 Methods Background

Knowledge on the origin of fecal pollution in the Danube River and its tributaries is of high interest as it allows for targeted protection and for the evaluation of the effectiveness of environmental management practices. Furthermore it supports water safety assessment and health risk management regarding recreational activities, bathing, irrigation, and drinking water usage. As demonstrated above, the extent of fecal microbial pollution can be determined via standard bacterial fecal indicators. However, *E. coli* or enterococci do not easily allow fecal source differentiation as they occur – per definition – in humans and homoeothermic animals (i.e., giving a measure of the amount of total fecal pollution).

Microbial source tracking (MST) or the determination of fecal pollution sources using host-associated genetic fecal markers [83, 84] has become increasingly popular. One of the most frequently used methods is based on the quantitative polymerase chain reaction (qPCR) detection of host-associated Bacteroidetes populations [85]. Bacteroidetes are one of the dominating bacterial groups in human and animal fecal excreta representing up to 30% of the biomass in feces (in contrast *E. coli* or enterococci constitute less than 1‰). Furthermore some microbial cell lines of Bacteroidetes show remarkable host adaptations, i.e., they are strongly associated with a specific type of pollution source (human, ruminant, etc.). Hence, Bacteroidetes represent ideal candidate targets for MST. The detection method of choice is direct molecular detection as most of these Bacteroidetes populations cannot be detected by standard microbiological cultivation procedures (Fig. 4). MST is a very young and rapidly evolving discipline, and no standardized procedure – comparable to the detection of standard fecal bacteria – exists as of yet. Most detection approaches have also been developed based on the background conditions at the respective watersheds of interest and need to be evaluated for application to other regions.

4.2 Testing Molecular MST at the Danube River Tributaries for Human Fecal Impact

Preserved DNA, recovered during the JDS 2001 and JDS 2007 water sampling activities, was used to evaluate the principle applicability of the Bacteroidetes-based MST approach along the whole Danube River stretch [70, 74]. Emphasis was placed on the tributaries as an exaggerated contamination range – from very low to excessive fecal pollution – was expected. The human-associated fecal marker, BacH, was detected in 82% of the investigated tributary samples for the JDS 2007. The marker equivalent concentrations (ME) ranged from 1.4×10^2 ME L^{-1}

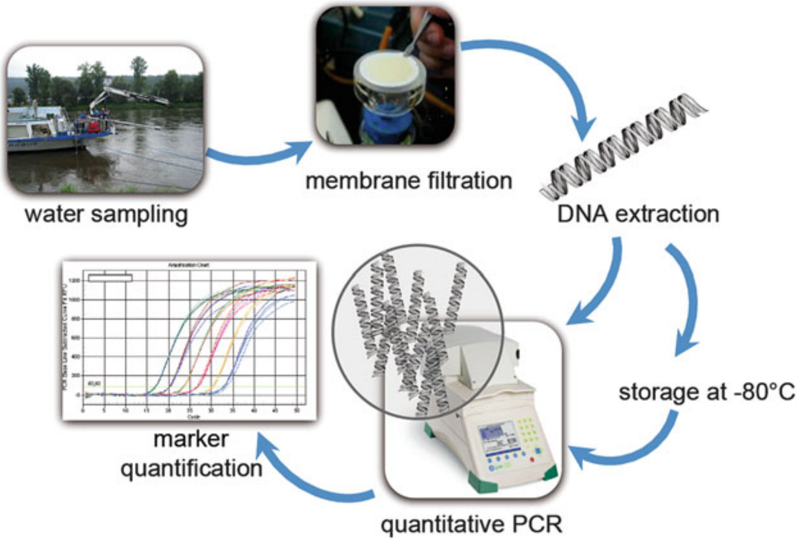

Fig. 4 Procedure for the quantification of genetic fecal markers (e.g., BacH) for library-independent microbial source tracking (MST). The working steps include sampling, filtration of the water samples via 0.2 μm polycarbonate membranes, extraction of DNA from microbial cells retained on the filter, and subsequent quantification of the respective genetic marker concentrations by quantitative polymerase chain reaction (qPCR) detection. DNA extracts can be stored at −80°C for several months until qPCR analysis is performed. A detailed description of the method can be found in [86, 87]

to 5.8×10^7 ME L^{-1} [70]. Statistical analysis revealed a high association between the human BacH marker and the *E. coli* enumerations (Fig. 5), strongly pointing to the importance of fecal pollution from sewage effluents at the investigated tributaries for the situation during the JDS 2007 [70]. Investigations regarding the JDS 2001 [74] revealed a very similar picture. Excluding the Timok River, a very high correlation between BacH and fecal coliforms for the rest of the investigated Danube River tributaries was evident ($r = 0.93$). The Timok River was heavily polluted with emissions from mining industries (e.g., high concentrations on heavy metals and organic pollutions), which might have strongly affected the performance characteristics of the investigated microbiological parameters [74].

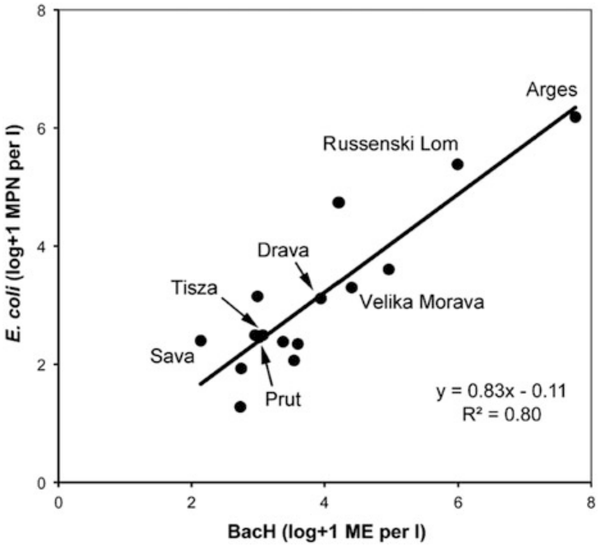

Fig. 5 Regression analysis between the logarithms of the *E. coli* (a measure for total fecal pollution) and the BacH marker (associated with human/sewage fecal pollution) concentrations from the Danube River tributaries during the JDS 2007 [70]. The statistical analysis revealed that 80% of the *E. coli* variations could be predicted by the human-associated BacH marker concentrations. This correlation is a strong indication that a dominant part of the fecal pollution, as measured by *E. coli*, could be traced back to sewage impact. The BacH marker was determined according to Reischer et al. [87]. Abbreviations: *MPN* most probable numbers, *ME* marker equivalents, a $\log_{10}$+1 transformation was applied

4.3 Testing the Genetic Fecal Marker Approach for Alluvial Well Water Resource Monitoring

Very recently, the applicability of the genetic Bacteroidetes-based MST approach for water quality monitoring from alluvial groundwater fed by Danube River bank filtration was tested. Alluvial groundwater is of essential importance for the drinking water supply throughout the Danube River Basin (see Sect. 1). Thus, MST methods that are applicable at the river surface and corresponding alluvial groundwater locations would clearly add a significant benefit for the possibility of integrated water resource monitoring. From 2010 to 2012, five test wells at a Danube River backwater area southeast of Vienna (PGAW1 to PGAW5) were comparatively analyzed for cultivation-based standard fecal indicators, host-associated genetic fecal markers, genetic markers for total fecal pollution, and bacterial direct count numbers (Table 2, Fig. 6). Basic microbial characterization of the wells by bacterial direct counts indicated that wells generally had median cell counts below 100,000 cells per ml. However, PGAW4 and PGAW5 were less protected during periods of surface water influence compared to the others (as shown by the increased maximum bacterial count values). This observation corresponds with

Table 2 Cultivation-based detection of standard fecal indicators (*E. coli*, enterococci, *C. perfringens*) and genetic fecal indicators associated with human (BacHum) and ruminant (BacR) fecal pollution in the PGAW1 to PGAW5 groundwater wells from 2010 to 2012. The results are given as number of positive samples per the total number investigated. The last column shows the total sums per investigated parameter (for details about the methods, see Fig. 6)

	PGAW1	PGAW2	PGAW3	PGAW4	PGAW5	Σ
E. coli (CFU L^{-1})	0/19	0/18	0/19	0/17	0/18	0/91
Enterococci (CFU L^{-1})	0/19	0/18	0/19	1/17	0/18	1/91
C. perfringens (CFU L^{-1})	0/19	0/18	0/19	0/17	0/18	0/91
BacHum (ME L^{-1})	0/19	1/19	1/19	1/17	3/18	6/91
BacR (ME L^{-1})	0/19	0/18	0/19	0/17	1/18	1/91

the proximity of these wells to the branches of the Danube River backwaters, the water levels of which can markedly increase during flood events at the Danube River. Analysis for fecal pollution vulnerability of the wells by standard indicators revealed no signs of fecal pollution, although the volume of investigation was 1 L. Except for one positive detection (enterococci), all samples ($n = 91$) were negative for *E. coli*, enterococci, and *Clostridium perfringens* spores (i.e., no colony forming units detectable per 1 L of analyzed well water, Table 2). Detection of human-associated (BacHum) and ruminant-associated (BacR) fecal markers revealed a similar picture compared to the standard fecal indicators (Table 2), although a somewhat higher positive detection rate was discernible. BacHum and the BacR were detected in 6 and in 1 case(s), respectively, of the ninety-one 1 L samples analyzed. Five of the seven positives were found at the more vulnerable wells PGAW4 and PGAW5. Furthermore, 50% of the human marker positives were from a single sampling date coinciding with a pronounced high water event at the Danube River (discharge peak approximately 7,000 m^3). From this pilot study, it may be concluded that MST approaches also work in alluvial well water locations, and a combination with fecal standard parameters can provide very valuable information. The MST approach tended to show a higher sensitivity for fecal pollution detection in the wells compared to fecal standards and indicated the possibility of minute human fecal influence at the more vulnerable wells. However, the actual detection levels were extremely low, ranging from 5.3×10^1 ME L^{-1} to 6.1×10^2 ME L^{-1} ($n = 7$).

In sharp contrast to the host-associated fecal markers, detection of genetic markers for total fecal pollution had no fecal indication value at all. The markers could be detected at all times at high concentrations, strongly suggesting their ubiquitous and natural occurrence (Fig. 6). This observation is in line with recent findings from other habitats that the proposed Bacteroidetes-based genetic markers for total fecal pollution are not specific for fecal pollution and also detect non-intestinal natural bacterial communities in water resources [90, 93].

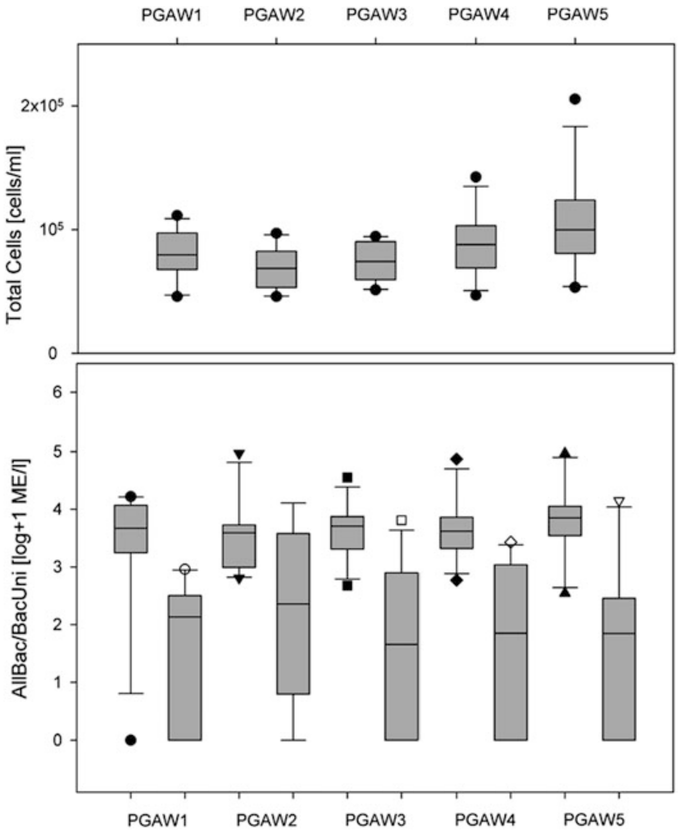

Fig. 6 Results from a pilot study testing the applicability of Bacteroidetes-based genetic fecal marker detection in alluvial water wells (PGAW1–PGAW5) fed by Danube River bank filtration southeast of Vienna during 2010–2012 (*lower panel*, A). To increase detection sensitivity, 1 L of well water was investigated per parameter. The proposed genetic markers for total fecal pollution, AllBac (*closed symbols*) and BacUni (*open symbols*), were detected as described previously [86, 88–90]. To sensitively detect artificial contamination events potentially introduced during sampling or sample processing, rigorous controls were designed. Controls for sampling, filtrations, DNA extractions, PCR reagents, and PCR runs were performed. The threshold of marker detection was approximately 20 marker equivalents per filter. Standard fecal indicators were measured according to ISO standards (ISO 2000; ISO 2001, [78, 91]). For comparison, total bacterial direct count (*upper panel*) was performed as described in Kirschner and Velimirov [92]

5 Outlook and Conclusions

5.1 *Public Health Aspects*

Appropriate microbiological quality of water and water resources is considered of utmost importance for public health by the World Health Organization [94, 95]. Contrary to common public opinion, unacceptable health burdens due to unsafe water and sanitation are not restricted to developing countries – although massive epidemics are most easily recognized in these regions – and still occur in developed regions such as Europe. For example, Valent et al. [96] demonstrated that a large proportion of deaths and disability adjusted life years (DALYs) in European children are attributable to inadequate water and sanitation. Furthermore, the EFSA [97] reported 17 waterborne outbreaks involving 10,912 cases for eight member states of the European Union (Denmark, Finland, Ireland, the Netherlands, Poland, Slovakia, Spain, and Sweden) in 2007 alone. A study by Frost et al. [98] using surplus sera from Hungarian women revealed that those whose drinking water came from water supplies delivered from surface or surface-influenced water resources had significantly increased antigen titers from *Cryptosporidium* infections (a fecal-associated waterborne parasite) compared to women whose water was supplied from confined groundwater aquifers. *Cryptosporidium* and *Giardia* spp. parasites were regularly detected in the Danube River water at Budapest, although river bank filtration at these sites was found to be effective in removing these pathogens [99]. Although these investigations cannot be considered sufficient, the results indicate remarkably well that the impact of microbiologically polluted water can be measured and leaves its diagnosable footprint in the European population.

5.2 The Danube River: Fecal Microbiological Pollution Status and the Water Framework Directive

As seen in the present overview, several sections of the Danube River and its tributaries are currently critically affected by fecal microbiological pollution. Clearly, recreational activities such as swimming or drinking water production based on near-to-nature principles – as described in the Danube, Meuse, and Rhine Memorandum by the IAWR and the IAWD [6] –are unsafe at these locations. The data set used in this report does not contain data from the past 5 years (2008–2012), and does not reflect recent developments with respect to the implementation of the central wastewater treatment plant in Budapest (fully established in 2010) or the initiation of the wastewater treatment plant in Bucharest (established in 2011) as well as other measures and efforts taken to improve water quality. Nevertheless, data indicate the strong need to closely follow the status and the future developments on the microbial water quality alongside the Danube River.

One might assume that the good ecological health of aquatic systems would support the long-term health of surrounding human and animal populations. However, the current legal situation does not support this assumption. The Water Framework Directive (EU-WFD) currently defers to the EU Drinking Water Directive (EU-DWD) and EU Bathing Water Directive (EU-BWD) and does not directly define microbiological quality targets for aquatic systems [2]. Hence, general monitoring programs governed by the EU-WFD do not include any microbiological parameters, although they are considered a priority in the EU-DWD and EU-BWD.

In contrast, chemical targets, including problematic micro-pollutants, are directly considered. The EU-WFD would benefit from including basic targets for fecal microbial pollution, which would promote equal treatment of chemical and microbiological hazards and support the harmonization of water quality monitoring programs in rivers.

5.3 Sampling Design and Methodological Aspects

In addition to these policy issues, current methodological constraints and future possibilities also have to be discussed. One important aspect concerns the sampling design of the monitoring programs. During both JDSs, samples were only taken from the middle of the river and not from its edges. Sampling location can have a highly significant influence. In contrast to impoundments of hydropower stations, where complete mixing may occur and river bottom clogging processes may have significant influence on the biota [100], in stretches devoid of meanders and hydropower plants, horizontal mixing of water masses from tributaries entering the Danube can be a very slow process, stretching out for dozens of kilometers [62]. Along such a stretch, however, intense vertical mixing may take place, and water of the boundary layers may be effectively cleaned by benthic filter feeders such as bivalves or amphipods, which represent the most important functional feeding groups at the bottom of the Danube River [62, 101]. By these processes, polluted water masses (tributaries or sewage inflows) may only partly enter the midstream if at all, and only taking midstream samples may not reflect the true pollution status of the Danube. Representative sampling at such a large river as the Danube should thus always include samples from the midstream and both boundary layers. Extrapolating the given results to the respective riverbank locations may thus result in a significant underestimation of the actual microbiological pollution.

The fecal indication capacity for bacterial standard indicators, especially for *E. coli*, has been increasingly questioned by many scientists recently. Naturalized *E. coli* populations, being not of immediate fecal origin, have been detected in the environment, such as in soil or algae mats (for an overview see [102]). This issue is far from being solved, and it is currently not clear in which environments and situations fecal monitoring programs might become methodologically limited. In contrast to this criticism, a recent detailed investigation on *E. coli* and enterococci in Austrian alpine water resources revealed excellent fecal indication capacity for both indicators [103].

The detection of microbiological fecal pollution by two biologically differing indicators, namely, *E. coli* and enterococci, yielded comparable fecal pollution patterns alongside the investigated sections of the Danube River for both JDS. Furthermore, a high correlation between *E. coli* and the human-associated genetic Bacteroidetes marker (BacH) was observed for the investigated Danube tributaries. Thus, a significant indication bias seems unlikely for the presented data set. However, basic research should be conducted into the fecal indication capacity of bacterial standard indicators for fecal microbial pollution monitoring at the Danube River watershed and its tributaries to provide a sound scientific basis for routine monitoring.

5.4 A Framework for Future Fecal Microbiological Pollution Analysis and Management

New methods and strategies for the analysis of microbiological water quality are emerging rapidly. This progress is mainly based on the continuously increasing importance of molecular biology diagnostics combined with improved data analysis and modeling. In Fig. 7 a framework for integrated fecal microbial pollution analysis and management in rivers is presented, visualizing the challenges but also the possibilities in the future [107]. The framework shows the different levels of data requirements, methods for its generation, and how it can be used for management activities. The basis of the approach is the detection of fecal pollution, as routinely performed using laboratory-based methods and monitoring programs [107]. At particularly sensitive points, such as at official bathing places or water abstraction sites for drinking water production, more rapid information on the actual microbiological water quality is required. Field monitoring methods will thus gain importance in the future. Near-real-time prediction of microbial water quality may be realized by modeling and/or measuring online surrogate variables or by automated and rapid detection of microbial parameters directly in the field. Both areas are currently a topic of intensive developments, and innovative technologies will likely be available in the future [107]. In cases where fecal pollution levels are above certain levels of acceptance, fecal hazard characterization or the

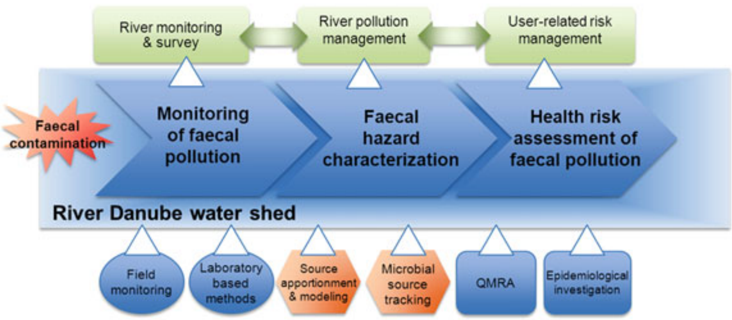

Fig. 7 The conceptual framework for fecal pollution analysis of water resources adapted for river systems (Modified after [104–106]). Three interacting levels characterize the backbone of the concept with relevance to the following issues: (i) is there any problem with fecal pollution, (ii) if yes, who is responsible for it, and (iii) what is the actual health risk in relation to the fecal source (s) contributing to the observed pollution? Note that various methods are available at each level. The suggested framework was also referred to as a “bottom-up approach” because it starts at the most general level (i.e., level of general fecal pollution monitoring) and becomes more specific as it proceeds to the right end of the diagram

determination of the sources of pollution becomes important [107]. Knowing the actual source(s) of pollution allows for target-oriented management and the evaluation of options available to solve the problem. Source apportionment, transport, and load modeling, based on sound hydrological principles and information [108], are required to support adequate predictions in the future. Thus, interdisciplinary collaboration between microbiologists and hydrologists is needed. Molecular fecal source tracking (MST) will largely support fecal microbial source characterization (see also chapter 4). MST is a very young discipline and far from being standardized. However, in the near future, it can be expected, that robust methods will be available, allowing the simultaneous quantification and host-specific discrimination of fecal pollution sources. Very intensive research activities throughout the world reflect the need and the progress that is made regarding this area [83]. Information about the expected health risk in relation to the extent of fecal microbial pollution and the respective type of usage (recreation, swimming, irrigation, drinking, aquaculture) is required for water safety management [107]. For example, to achieve the required safety levels for consumers of drinking water, a clear understanding of the extent of water treatment and disinfection is required regarding the actual level of microbiological pollution in the river water. Knowing the source of pollution helps to search for the representative fecal-associated pathogens (reference pathogens) and to foster the selection of adequate infection and disease risk models [107]. State-of-the-art primary and secondary biological wastewater treatment is a first fundamental barrier to significantly reduce fecal microbial pollution loads. However, wastewater treatment plants (WWTP) are not designed to quantitatively remove pathogens, and effluents of WWTP have to be considered infectious sources [109]. Quantitative microbial risk assessment (QMRA) can help to evaluate whether a further disinfection step (tertiary treatment) can help to reach the water quality targets at specific locations (e.g., WWTP upstream of a bathing area). As for the area of MST, QMRA is a young discipline as well and similarly requires the close cooperation between microbiologists, hydrologists, and modelers. Health risk assessment is considered an essential element in sustainable water and environmental management of the future, although data availability may limit the level of precision achievable. The suggested framework for integrated fecal microbial pollution analysis and management in rivers provides a basis where traditional pollution monitoring and further novel investigation targets can be integrated to use the strength of each individual approach in a sustainable way.

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Hydromorphology of the Danube

Ulrich Schwarz

Abstract Hydromorphology describes the physical and hydrological characteristics of rivers and its habitats including the underlying processes from which they result. Hydromorphology is a supplementary but mandatory element of WFD ecological assessment, and hydromorphological alterations were recognized as one of the most important river management issues across Europe. Hydromorphological assessments try to integrate and provide information on how far the conditions derive from pristine conditions (so-called hydromorphological reference conditions). The ICPDR Joint Danube Survey (JDS) 2 in 2007 delivered results on hydromorphological alterations for the navigable Danube River (from Kelheim (rkm 2,416) to the Danube Delta) for the very first time. A five-class assessment similar – but not equal – to the WFD ecological status classes was implemented according to European standards and methodological approaches for large rivers using the three main categories (1. channel; 2.banks; 3. floodplains).

Keywords Banks, Channel, Danube, Floodplains, Hydromorphology, River morphology

Contents

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1 Introduction

Detailed and sectoral information on the geology, geomorphology, river cross sections and longitudinal profiles, sediments (e.g. grain size distribution and channel degradation), hydrology (discharge regime, amplitude and magnitude of floods) or floodplains and its vegetation are widely spread and analysed on catchment, river section and river reach scales, describing also the "river history" and the impacts of hydropower dams and river regulation for navigation and flood protection [1]. Therefore, hydromorphology considered as cross discipline of fluvial morphology/(river) hydrology, ecology and river engineering is one of the most challenging "river disciplines". It gained more awareness in the last decade of the twentieth century after solving the significant river pollution by sewage water in Western Europe from 1970 to 1990. From 1990 many EU countries developed hydromorphological methods and inventories.

The WFD adopted in 2000 includes hydromorphology as a supplementary but mandatory aspect of fulfilling the requirements for achieving the good ecological status in 2015. The results of the river basin analysis according to WFD Article 5, which was carried out in 2005, and of the river basin management plans published from 2009 strongly indicate the hydromorphological alterations across Europe (the European Environmental Agency [2] summarized that 50% of all European rivers are subject of considerable hydromorphological alterations). Solving this problem however requires a strong dialogue with other sectors such as hydropower development (Renewable Energy Directive), waterway transport (EU TEN Trans-European Transportation Networks), flood protection (EU Floods Directive) as well as the biodiversity and nature protection (Flora Fauna Habitat Directive).

To accommodate an increasing demand on harmonized methods, the CEN published in 2004 [3] and 2010 [4] important framework standards for hydromorphological assessments. In the case of the Danube and in large rivers in general, the applications are scarce and specific (e.g. [5]). The results of the hydromorphological assessments within the WFD should be used to supplement the ecological status assessment of water bodies and to indicate the "heavily modified water bodies". In currently strongly polluted water bodies not reaching the good ecological status, hydromorphological improvements can significantly improve the habitat conditions. Hydromorphologically intact river reaches can be seen as resilience hot spots (retreating area for many species) to be used in concepts aiming to reach the good status and to find best positions in the river continuum for restoration measures (so-called Strahlwirkungskonzept, [6] or [7]).

Hydromorphological alterations are recognized by ICPDR as one of the four basin-wide significant water management issues. The most significant alterations were categorized into longitudinal continuity interruptions (dams, weirs), lateral connectivity interruptions (loss of floodplains, bank reinforcements) and hydrological alterations (water abstraction (residual water) and hydropeaking).

The main impacts of hydromorphological alterations on the riverine habitats will cause the decline of species biodiversity, the decline of species abundance, altered population composition and hindrance of species migration and the corresponding decline of naturally reproducing fish populations (e.g. sturgeon).

2 Approach and Assessment

The lack of harmonized standard methods for the assessment of hydromorphological features on the Danube made it necessary to develop a methodology based on CEN ((EN146142004) for the assessment of hydromorphological features of rivers [3]) which could be applied for large rivers (compare [5, 8–10]). This method was used for the second Joint Danube Survey and consisted of a longitudinal overview survey evaluating the hydromorphological situation of the river and water bodies and of a detailed site survey which is needed for the interpretation of biological result at a particular sampling site.

The description and evaluation of hydromorphological characteristics for large rivers are strongly depending on various background data such as historical, topographical and navigation maps, satellite images, hydrologic and morphometric data as well as land use data (also for the determination of reference conditions) [10]. Hydromorphological assessment carried out during the second Joint Danube Survey was the first time that hydromorphological parameters were surveyed systematically by a uniform method for the entire navigable longitudinal Danube stretch over 2,415 rkm.

The hydromorphological parameters are supportive to biological quality elements for the assessment of the ecological status, primarily to the physical habitat description of fish, macrozoobenthos and macrophytes. Another issue for hydromorphology is to assess the capability of connected floodplains and natural channels to act as nutrient sinks, their resilience function after accidents with hazardous substances and their retention potential for flood protection.

The survey in general led to a better understanding of whether the river habitats are impacted by hydropower, navigation and flood protection. Based on the hydromorphological risk assessment, the “Programmes of Measures” as required by the WFD will be designed. To achieve the objectives of the WFD, it will also be necessary to set technical measures such as restoring continuity for migratory species or improving habitat conditions. Those stretches with still intact hydromorphological features threatened by navigation and hydropower projects should be protected or planning has to follow strict guidelines (compare [11]).

For the continuous longitudinal survey, a huge amount of already existing information and data were used to make a division of the Danube into homogenous about 50 km long stretches and to prepare the necessary data for the evaluation such as the general plan form and sinuosity, the main river engineering structures, longitudinal and lateral continuum interruptions as well as the floodplain with adjacent land use. The survey was used to update, approve and validate the preliminary results, especially those for the river banks. The five-class evaluation for channel, banks and floodplains was the base for the total evaluation using the mean values for the three categories [12].

3 Methods and Basic Variables

Preliminary subdivision to the river stretches was based on the river typology, water bodies, morphological characteristics and main hydrological alterations. The biological continuity interruptions were excluded from the assessment itself.

Channels were assessed using the following criteria: degree of morphological and flow condition alterations (based on hydrological alterations, navigation map, historical maps and plan form validated by field survey) and taking into account the type-specific reference conditions. Five classes were used for the assessment:

Class 1: Channel nearly natural
Class 2: Channel slightly modified
Class 3: Channel moderately modified
Class 4: Channel severely modified
Class 5: Channel totally modified

Banks (integration of left and right banks) were assessed by evaluating bank dynamics and modifications (based on navigation map, validated by field survey) taking into account the type-specific reference conditions. Five classes were used for the assessment:

Class 1: Banks nearly natural
Class 2: Bank reinforcements in small sections
Class 3: Bank reinforcements in large sections
Class 4: Continuous bank reinforcements
Class 5: Totally modified banks

[1] Not identical with WFD “ecological potential”.

Floodplains (integration of left and right floodplain) were assessed based on the ecological quality classes ("ecological potential"[1]) according to the DPRP Wetland study 1999 [13] considering the floodplain width (relation between active and morphological floodplain) and land use. Five classes were used for the assessment:

Class 1: Floodplain with very high ecological value
Class 2: Floodplain with high ecological value
Class 3: Floodplain with moderate ecological value
Class 4: Floodplain with low ecological value
Class 5: Floodplain totally modified

3.1 Overall Assessment

Five-class assessment (arithmetic mean) of channels, banks and floodplains with intervals of 1 for classes 2–4 and 0,5 for class 1 (reference conditions) and class 5.

3.2 Assessment Class Boundaries

1,0–1,4 = Class 1 reference conditions (blue)
1,5–2,4 = Class 2 (green)
2,5–3,4 = Class 3 (yellow)
3,5–4,4 = Class 4 (orange)
4,5–5,0 = Class 5 (red)

4 Results

During JDS2 66 homogenous stretches along the Danube River including the three delta branches (2,610 rkm) were delineated. The mean length of an evaluation stretch was some 40 km, (varying between 15–135 km). In general, the length of homogenous river segments increased from the upper to the Lower Danube.

4.1 Channel

Most of the hydropower plants in Germany and Austria fell into class 4 (severely modified). Totally modified, canalized and impounded Danube stretches can be found along the city stretches such as Vienna as well as in the Gabcikovo tailrace canal. Due to the compromise to assess longer river stretches, not all impoundments

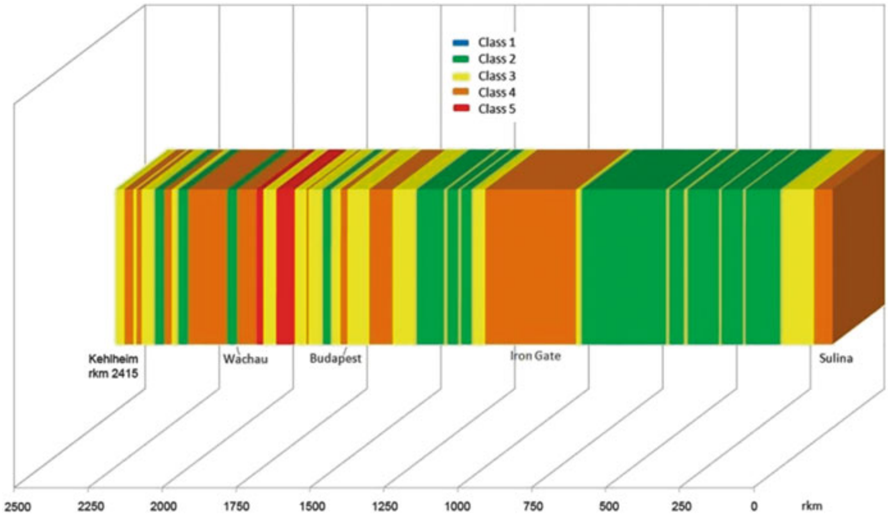

Fig. 1 Channel assessment as longitudinal colour-ribbon visualization

were reflected in detail so far. The total length of impoundments including backwater and transition sections would cover 1/3 of the river course. Barely moderate conditions can be found over the long free-flowing stretches in Hungary mostly due to the strongly reduced length of the river by meander cutoff since the eighteenth century, bed degradation (dredging) and navigation reasons (low-water regulation works). Still good conditions can be found in some breakthrough/gorges reaches such as Wachau (Austria) and Danube Bend (Hungary) and in the lowland stretches along the Croatian-Serbian border (without influence of the Iron Gate backwater). The largest stretches in good conditions were found along the Romanian-Bulgarian Danube. Still meandering reaches are very rare and most of the meanders were cut even within the last decades as such for the Sft. Gheorghe branch in the Danube Delta. None of the stretches can be assessed as class 1, due to river regulations for navigation and flood protection as well as due to the altered sediment balance (dams in the upper and middle course of the Danube and many tributaries) (Fig. 1).

4.2 Banks

The river banks are in particular enforced in Austria and Germany. Further downstream, the banks of the Danube are totally reinforced only in the area of towns. In the Hungarian reach, the banks are enforced in large sections (class 3). Along the entire Lower Danube, the bank reinforcement covers only few percent of the total river course, but local erosion protection activities increase currently the length of reinforced banks (Fig. 2).

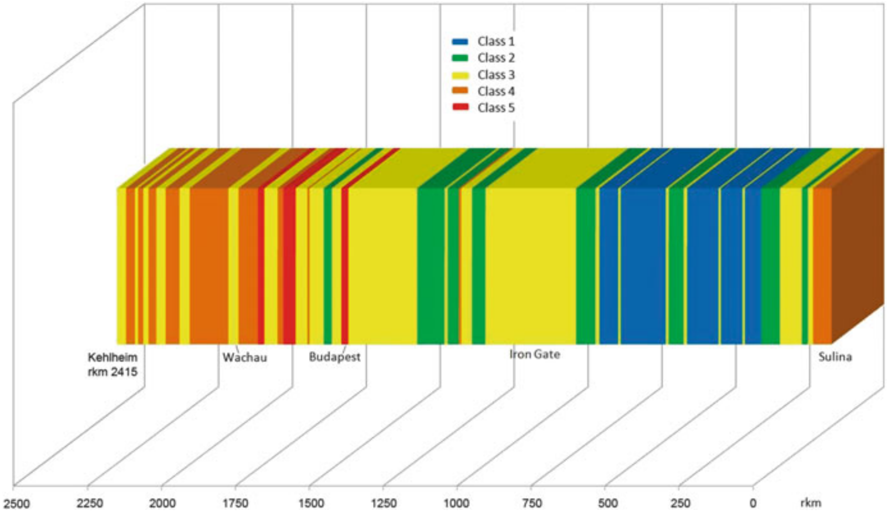

Fig. 2 Bank assessment

4.3 Floodplains

Most of the floodplains of the Danube (about 70% in total [14]) are disconnected by flood protection dikes, especially in areas where they spread over 10–20 km width along the Hungarian Danube south of Budapest and along the entire Romanian-Bulgarian stretch and towards the Danube Delta.

Only few reaches along the Danube have nearly intact or still remaining floodplains (21% in total according to Fig. 3). The largest existing continuous active floodplain areas along the Danube are as follows:

- Danube National Park (Austria): 10,000 ha
- Danube-Drava National Park (Hungary): 28,000 ha (Danube part only)
- Kopački Rit and Gornje Podunavlje Nature Parks (Croatia/Serbia): ~40,000 ha
- Floodplain forests of the Serbian Danube upstream of Tisza confluence: ~20,000 ha
- Small Braila Island protected area (Romania): ~20,000 ha
- Danube Delta (Romania, Ukraine): ~500,000 ha

In addition the remaining near-natural islands of the Lower Danube (Romania and Bulgaria) provide valuable and unique floodplain habitats as well.

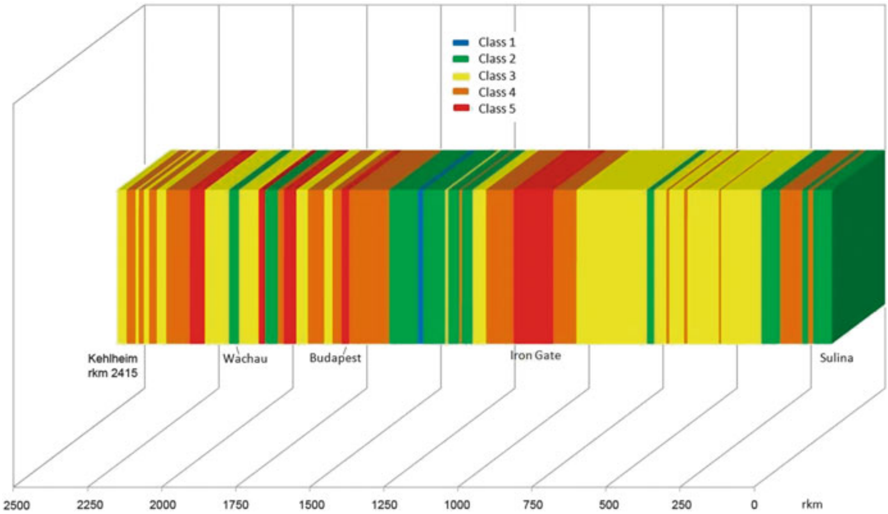

Fig. 3 Floodplain assessment

4.4 Overall Hydromorphological Assessment

One third of the Danube from Kelheim to the Black Sea can be characterized as strongly altered (classes 5 and 4) and another third as moderately altered (class 3). On the other hand, at least one third of the Danube belongs to the second, good hydromorphological class (see Fig. 4), which is a significant portion in comparison to other large rivers in central Europe. A more detailed analysis of the upper (Kelheim-Bratislava), middle (Bratislava-Iron Gate) and Lower Danube indicates that the upper reaches in Germany and Austria are those being most affected by significant hydromorphological changes. There are only small free-flowing stretches in that area such as Straubing-Vilshofen (Bavaria), Wachau Valley (see Fig. 4) or the Danube downstream of Vienna (Austria). On the other hand, the middle and lower courses of the Danube are interrupted and affected by the three large hydropower plants (the Gabcikovo Dam in Slovakia and the two Iron Gate Dams along the Serbian-Romanian border).

As for the "best available sites", only very short stretches (not visualized in this overall assessment) can be found within the highest class (class 1) for the assessment groups "banks" (along some steep banks of the Croatian-Serbian, Bulgarian and Romanian Danube) and "floodplains" (along the protected sites of Kopački Rit and the Gornje Podunavlje in Croatia/Serbia and on the right bank along the Small Braila Island in Romania). The channel itself is largely modified for navigation and only few kilometres remain along the island sections of the Romanian-Bulgarian Danube and in the major side channels along the less used delta branches where the highest class would be reached. The protection of those remaining "intact" stretches is essential. The necessary restoration activities along the Danube were already set,

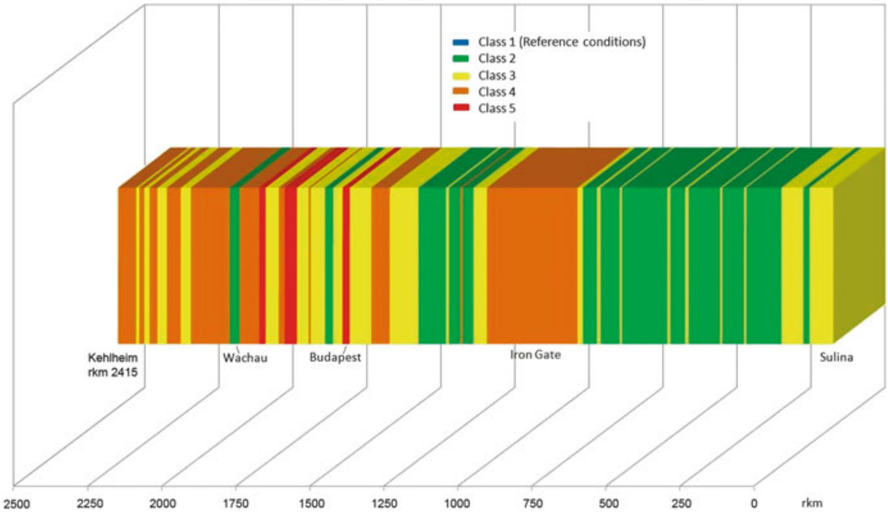

Fig. 4 Overall total hydromorphological assessment in five classes

e.g. at the Bavarian Danube (upstream Straubing) or the Austrian Danube (two fish passes in Melk and Vienna, restoration of the Danube National Park downstream of Vienna), to improve the ecological situation. Nevertheless these improvements cannot change significantly the overall situation along the Danube as they have importance mostly at the local and regional scale.

4.5 Results of the First Danube River Basin District Management Plan (DRBDMP) 2009

According to DRBDMP 2009 [15], 18 dams can be found on the entire navigable Danube reach from Kelheim to the Black Sea. Only at two dams fish migration facilities (bypasses at Melk and Wien-Freudenau) have been constructed and are in function. The backwater caused by the impoundments depends on the height of the dam and on the slope of the river course as well as on the discharge conditions (much longer backwaters during low-water conditions). The longest backwater (about 250 km) has the Iron Gate, while the shortest backwater reaches (about 5 km) can be found in Germany and Austria. In total DRBMP 2009 refers to 78 barriers including smaller weirs on the Upper Danube. The total official length of impoundments was estimated to be 1,111 rkm (including the non-navigable Upper Danube). Significant water abstraction along the navigable Danube can be found only at the Gabcikovo Dam. Hydropeaking was not defined for the Danube (not reaching the assessment mark of >1 m of daily water level oscillation); however, irregular water changes or slight daily peaking can be observed in the

Austrian Danube reach downstream of Enns confluence and downstream of the big dams (Gabcikovo and Iron Gate). Fifty-six percent of the entire Danube reach was designated as heavily modified water bodies.

5 Conclusions

The overall hydromorphological assessment indicates that the hydromorphological situation of the Danube varies from source to the delta. The hydromorphological conditions in the Lower Danube are much better than in the upper reach. In the DRBDMP 2009 [15], various measures were proposed improving the hydromorphological situation to reach the environmental goals for the period until 2015 and 2021, respectively (next full water management cycle). The first focus was set to the reduction of river continuity interruptions for migratory species. The construction of fish passes also for larger dams along the Upper Danube (in total five dams) is planned for 2015. A prioritization approach sensitive for migratory fish species and their habitats will support the further planning. Further activities are needed to improve the sediment transport through the chain of dams. As regards the lateral connectivity, few existing areas are planned to be reconnected until 2015 (in total about 45,000 ha [15] mostly within the active floodplain in protected areas such as national parks). For the Lower Danube, huge areas are under consideration to be reconnected by Romania. Finally the future infrastructure planning concerning the Danube should be based on the principles agreed in the "Joint Statement on the guiding principles for the development of inland navigation and environmental protection in the DRB" which defines environmental standards for inland waterway infrastructure projects [11].

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Danube River Sediment Transport and Morphodynamics

Helmut Habersack, Elisabeth Jäger, and Christoph Hauer

Abstract Hydromorphological alterations of large rivers are evident and have to be related to multiple anthropogenic pressures. The presented results of an integrated study concerning the actual status of the hydromorphology of the Danube River Basin show that in particular, the sediment regime features a heavily disturbed system at various scales. Combined impacts of flood protection, navigation and hydropower measures applied over a long period of time have been identified based on the river-scaling concept (RSC) for being responsible for these specific alterations (lack of bed load and suspended load in the remaining free-flowing sections). Moreover, long sections of the Danube River have been narrowed, channelized, disconnected from floodplains and morphologically degraded over the last 200 years. This has caused increased bottom shear stresses, increased sediment transport capacities and in addition a lack of lateral self-forming processes and corresponding reduced morphodynamics in the non-impounded sections. As a consequence of both longitudinal and lateral disturbances of the sediment supply and additional impacts of the channelization, the remaining free-flowing sections are subject to various forms of river bed degradation. Such degradation or river bed incision leads to a loss of instream structures in general, with a disappearance of gravel bars at the Upper Danube, and changes of sandbars at the Lower Danube. Hence, for river systems and large river basins, it has to be stated that the preservation and restoration of morphodynamics is one of the most relevant issues for river engineering and ecology. This has to be considered especially for the implementation of legal directives and/or future river basin management plans.

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Contents

1 Introduction

Undisturbed hydromorphology in large river systems is rare and characterized by a dynamic equilibrium between hydraulic (e.g. flow dynamics) and morphological parameters (e.g. sediment supply [1]). Hence, anthropogenic influences have considerable effects on especially large river systems due to summarizing anthropogenic impacts of the entire (large) catchment areas, ending up in multiple and severe hydromorphological alterations. Therefore, not only for large rivers is the issue of hydromorphological alterations a potential area of conflict between environmental protection and other uses of the river, such as, e.g. inland navigation or flood protection. For that reason, hydromorphological alterations, as one of the main ecological pressures, have been identified as a significant issue for water management, especially according to the European Water Framework Directive (WFD). Since the implementation of the WFD in 2000, all European waters have to be managed by a river basin approach. Especially for large rivers, international commissions are responsible for, e.g. the protection of the Rhine and the Sava River Basins and moreover for coordinating national actions within this framework. For the Danube this responsibility and the accompanying requirements have been realized by the Danube countries and the International Commission for the Protection of the Danube River (ICPDR) through the so-called first Danube River Basin District Management Plan (DRBMP) in 2009. In this management plan, the central hydromorphological alterations at the Danube are listed, which, e.g. highlight the necessity to distinguish between the impacts and hydromorphological consequences of longitudinal and/or lateral interruption of the river and habitat continuity [2, 3]. Alterations of the Danube morphology which already started in the fifteenth century [1] are mainly related to the engineering approaches to create a single, straightened channel accompanied by changing the depth or width of the river. The consequently strongly affected ecological quality of the Danube, but also for other larger river systems, is reflected in significant alterations of riverine habitats, subsequently leading to the decline of species biodiversity [4]. Especially the decline of species abundance, the altered population composition, the prevention of species migration routes for the aquatic/semiaquatic fauna and the corresponding decline in naturally reproducing fish populations (e.g. sturgeon) have to be mentioned [2]. In general, as key pressures for large river systems causing such a multitude of dramatic hydromorphological alterations navigation, flood protection and hydropower use have been identified in previous studies [1, 5–7]. Moreover, it is hypothesized that hydromorphological alterations of large rivers may be partially superimposed by other anthropogenic influences like urban settlements, agriculture or land use in general. Already discussed but not clearly figured out so far was the mentioned superimposition and interrelated processes of disturbed sediment regime and hydromorphology of the Danube and/or large rivers in general. It is partially evident and has already been analysed that anthropogenic interferences/barriers are frequently not only referred to a single pressure but contain multifunctional characteristics which, however, have not been identified for the entire Danube River Basin so far. Besides single aspects concerning the variety of anthropogenic pressures, a lack of integrative studies is evident for large river systems, dealing with multiple and/or superimposed pressures on sediment regime and the entire hydromorphological catchment-wide conditions.

Hence, the aim of the presented paper is to provide a scientific assessment based on the comprehensive description and analysis of anthropogenic pressures and impacts on Danube sediment regime and/or hydromorphology to address this lack of data. The assessment takes a case study approach to compile, evaluate and discuss historical as well as future impacts of the sediment regime and the morphological condition of this large European river. Besides the *DPSIR Framework* (d*riving forces*-p*ressures*-s*tate*-i*mpac*t-r*esponses*), formulated by the European Environment Agency, the *river-scaling concept (RSC)* [8] was used to identify and evaluate the different sedimentological and/or morphological issues from small channel patterns up to the entire Danube River Basin. Moreover, the presented study should highlight a way of a systematic identification of hydromorphological alterations which could be applied to other large river basins as well. This book chapter is based on Habersack et al. [9].

2 The Danube River Basin

The Danube River Basin is located in Central and South-Eastern Europe. The main river is 2,857 km long with a catchment area of 801,463 km^2 [3] including all of Hungary; most parts of Romania, Austria, Slovenia, Croatia and Slovakia; and significant parts of Bulgaria, Germany, the Czech Republic, Moldova and Ukraine. Large territories of the former Federal Republic of Yugoslavia (today of Serbia and of Montenegro), of Bosnia and of Herzegovina and small parts of Italy, Switzerland, Albania and Poland are also included in the basin [10]. The Danube

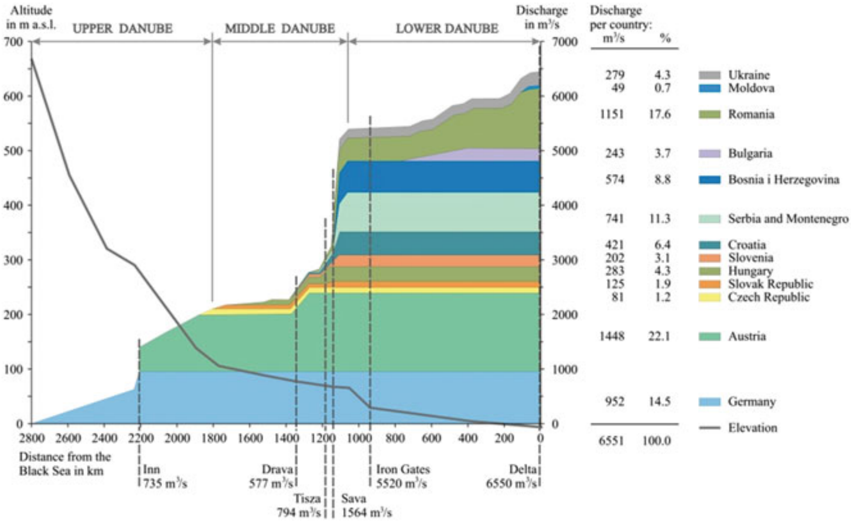

Fig. 1 Longitudinal profile of the Danube with river kilometres and the most important tributaries [11]

has a multiannual mean discharge of 6,500 m^3 s^{-1} into the Black Sea and is the 21st largest river of the world and, after the Volga River, the second largest river of Europe [10]. One third of the Danube River is mountainous, while the other 2/3 of the Danube passes hills and plains. The mean altitude of the Danube River Basin is only 475 m. Figure 1 shows the altitude, the discharge and the main tributaries of the three sub-catchments (sub-reaches) in a longitudinal profile of the Danube River. From the area of 10,508 million km^2 of total Europe, expanding between the western coast of Ireland and the Ural Mountains, the Danube catchment's share is 0.801 million km^2 (7.8%). About 783 million inhabitants are living on the continent, thereof more than 80 million people in the catchment of the Danube River. In the year 2013, 19 countries are sharing the catchment. Among them, there are 14 countries being the ICPDR Contracting Parties (with catchment areas >2,000 km^2), the biggest shares of the catchment belonging to Romania (29.6%), Hungary (11.5%), Serbia (10.1%) and Austria (10.0%).

According to various authors, the entire Danube River can be divided into four sub-catchment areas, Upper, Middle and Lower Danube and Danube Delta, as shown in Fig. 1. The Upper Danube Basin (1) reaches from the sources in the Black Forest Mountains to the Gate of Devín (also called 'Pannonian Gate'), near Bratislava, where the foothills of the Alps, the Small Carpathians and the Leitha Mountains meet. The area covers in the north the Swabian and Franconian Alb, parts of the Oberpfälzer, the Bavarian and the Bohemian Forests, the Austrian Mühl- und Waldviertel and the Bohemian-Moravian Uplands. At the south of the Danube, the Swabian- Bavarian-Austrian Alpine foothills as well as large parts of the Alps up to the water divide in the crystalline Central Alps are situated [11]. The Middle Danube Basin (2) covers a large area reaching from the Gate of Devín to the

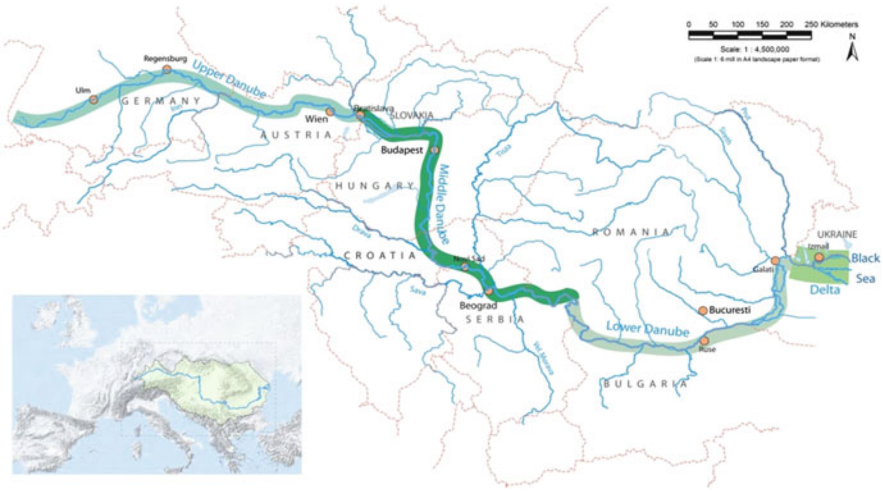

Fig. 2 Zoning of the Danube River into the Upper, Middle and Lower Danube and Danube Delta [15]

impressive gorges of the Danube at the Iron Gate (Iron Gate I and Iron Gate II), which divides the Southern Carpathian Mountains in the north and the Balkan Mountains in the south.

The Middle Danube Basin is confined by the Carpathians in the north and the east and by the Karnic Alps, the Karawankas, the Julian Alps and the Dinaric Mountains in the west and south. These mountains incorporate the Pannonian Plains and the Transylvanian Uplands [11]. The Lower Danube Basin (3) covers the Romanian-Bulgarian Danube sub-basin downstream of Cazane Gorge and the sub-basins of the Siret and Prut River. It is confined by the Carpathians in the north, by the Bessarabian Upland Plateau in the east and by the Dobrogea and Balkan Mountains in the south [11]. Finally the Danube Delta (4) has to be mentioned, which reaches from the confluence of the Prut River (Ukraine) to the mouth into the Black Sea (Ukraine) with an entire planimetric extent of 5,640 km^2 (Fig. 1), as the second largest river delta in Europe (Volga River = 23,000 km^2) (Fig. 2).

3 Methods

The driving forces in river morphology and the related instream habitats are strongly influenced by the unsteady transport of water and sediments on various scales in which the size of areas of interest and the upscaling and downscaling of possible driving forces are crucial. Thus, the applied method for characterizing anthropogenic pressures along the Danube was based on two main approaches allowing a detailed evaluation of especially the upscaling and downscaling of sedimentological and morphological issues. Both the so-called river-scaling concept *(RSC)* [8] and the *DPSIR* Framework (driving forces-pressures-state-impact-responses), formulated by the European Environment Agency, were found to be valid to address the specific aims of the presented study. Especially the *RSC* has to be used as a basis for the assessment of the ecological integrity. Within the *RSC*, the following three scales are analysed in a hierarchical way: from large scale to small scale a differentiation between processes and the corresponding sedimentological/morphological condition of the entire Danube River Basin (catchment-wide scale), the Danube River reaches (reach/sectional scale) and the Danube River site-specific characteristics (local scale; e.g. river restoration East of Vienna). First of all, for all scales, the history and present status of hydromorphological alterations have been analysed and evaluated based on historical maps, published studies, governmental reports and engineering projects of the various Danube countries ($n = 14$). All alterations were discussed in order to identify historical as well as current pressures from diverse driving forces (e.g. inland navigation, flood protection) on hydromorphology especially along the Upper, Middle and Lower Danube.

In addition to the *RSC*, the *DPSIR Framework* has been implemented for a consistent structuring of the results. The European Environment Agency formulates the definition of *DPSIR* as 'the causal framework for describing the interactions between society and the environment, dividing driving forces, pressures, states, impacts and responses' (extension of the PSR model developed by OECD). The results of this paper refer predominantly to the components: driving forces, states and impacts on various scales. Moreover, due to the aims of the presented study, this paper should provide a scientific assessment based on the description and analysis of the anthropogenic impacts by addressing the main driving forces, and thereby all pressures and impacts on the hydromorphology of the Danube River have been listed. The entire assessment takes a catchment scale approach to compile, evaluate and discuss historical as well as future impacts from the main driving forces impacting the Danube's sediment regime and/or hydromorphology for a future river basin management.

4 Results

The presentation of results is divided into two main chapters according to the aims of the presented study. First, the alterations and changes of sediment regime and sediment transport are described, which have to be seen as additional driving force on possible changes of Danube hydromorphology, which is presented under the second heading of results.

4.1 Status of the Sediment Regime

One of the basic problems concerning the entire Danube River Basin is a modified longitudinal and lateral sediment continuity and related regime. Especially during the last decades, the sediment regime of the Danube River has drastically been changed. Between 1950 and 1980, sixty-nine reservoirs, with an overall storage volume of about 7,300 million m^3, were constructed in the Danube River Basin. In total 78 barriers exist along the Danube main stem, keeping only five free-flowing sections. Moreover, in addition to these numbers, the deficit of bed load in the Danube has been strongly affected by the decline of former bed load input from the main tributaries in the Basin, where more than 700 large hydropower plants/weirs have been constructed. Therefore, the bed load transport in and output from the upper reaches of the Danube dramatically declined after 1960 as shown in Fig. 3. Exemplarily, at the Upper Danube today, the bed load input from tributaries is reduced by about 90–95%. Comparing to the historical situation, significant reductions of bed load can be documented especially for the Austrian rivers Lech, Isar and Inn. For example, the bed load in the River Lech, formerly transporting 180,000 t $year^{-1}$, decreased at the confluence with the Danube to nearly no transport, whereas the bed load in the River Inn (main tributary for the Upper Danube) decreased from 540,000 to 180,000 t $year^{-1}$.

In contrast to the reduction of bed load transport of the tributaries and consequently the reduced input into the free-flowing sections of the Danube, there is a surplus of deposited sediments in impounded sections and reservoirs. The sediment trapping efficiency varies with time and depends on several factors, e.g. the size and shape of reservoirs, water depth and occasionally vegetation. Large reservoirs intercept more than 40% of the total water discharge, and thereof, 70% are subject

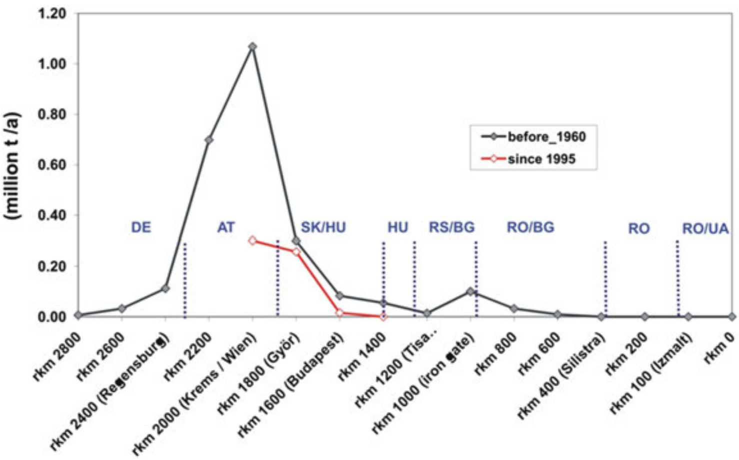

Fig. 3 Bed load transport (million t/a) within the Danube River ([12]; [13])

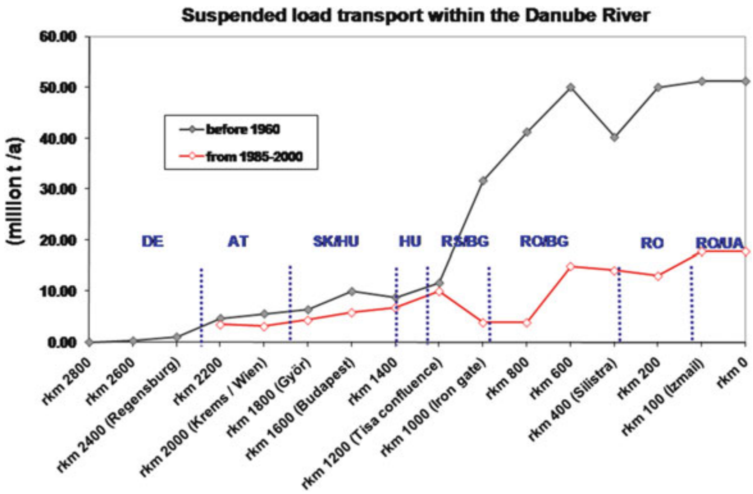

Fig. 4 Suspended load transport (million t/a) within the Danube River ([15])

to a sediment trapping efficiency of more than 50% of the entire Danube River Basin. In general, estimations indicate that about 25–30% of the sediment load to the coastal sea is trapped behind dams [14]. For the Upper Danube, the sediment trapping efficiency is about 17%. The most influencing constructions on the Lower Danube are the Iron Gate Complex, which comprises the largest dam system at the Danube. The Iron Gate dams and reservoirs influence the sediment transport significantly in two ways as they are, on the one hand, a trap for suspended sediments (Fig. 4) and, on the other hand, an important nutrient sink and deposition area of hazardous toxic matters for pollution [16].

Moreover, during the period 1972–1994, about 325 million t of sediments (10% of the entire reservoir) were retained by the Iron Gate dams, leading especially to a strong decline in suspended sediment transport along the downstream Lower Danube [14]. In addition, it has to be mentioned that also the temporal distribution of suspended load totally changed during the last decades as a consequence of the construction of reservoirs. Nowadays significant suspended transport occurs only at large flood events, in which most of the transported material is deposited along the floodplains during the falling limb. During these floodings, a strong remobilization of suspended sediments, however, occurs in the Danube reservoirs, whereas for the historical un-impounded Danube, the transport of suspended sediment was distributed over the entire year (e.g. during smaller floods). Especially upstream reaches of dams (impounded reaches) suffer from over proportional sediment surplus, as sediments accumulate due to lower flow velocities. These mostly coarser depositions often have to be extracted in order to maintain river depth for navigation as well as to limit the height of water level in case of floods [17]. Furthermore, the reduced bed slope and flow velocity and the related aggradation of sediments in the reservoirs affect natural gravel bed river habitats as they are covered with fine sediments and clog the hyporheic interstices which moreover lead to a decrease in oxygen flow into the bed substrate [18]. These alterations in bed material composition may have effects on macroinvertebrates, fish fauna (e.g. spawning habitats) and aquatic flora [11]. In addition to storage in reservoirs, the sediment accumulation processes between river training measures, e.g. groins and chevrons (especially at the Lower Danube) constructed to improve navigation, modify the sediment transport in large river systems [19].

Moreover, as second crucial aspect in limiting sediment supply, the disturbances by the lateral interruption of sediment supply have to be mentioned. Habersack [20] stated that due to the prevention of side erosion and self-forming processes at the Upper Danube (e.g. by flood protection measures or for navigational purposes), the lateral connectivity in general, the sediment exchange between side arms and the main channel and thus the lateral exchange and input of sediments (bed load) have been reduced enormously, resulting in an additional deficit of sediments within the Danube River channel. In contrast, at the Lower Danube, it has to be noted that the lateral sediment (suspended sediment) input is more or less not impacted as the river is not embanked in most parts. The river banks in Romania are almost natural (near-natural); thus, side erosion plays an important role for the sediment regime and sediment transport, respectively. Nevertheless, at the Lower Danube where lateral river bed erosion may reduce the water depth due to larger cross sections and dislocate the navigation fairway in the Danube, additional river training works as well as dredging of fords are carried out to maintain the minimum fairway depth, thereby altering the sediment regime [14].

As the third crucial driving force influencing the sediment input along the Danube, land use has to be mentioned. In general, human-induced changes of the vegetation cover in river basins cause strong geomorphic response by disturbing sediment supply, transport and deposition regimes. As an example for the Upper Danube sub-basin (reach scale), changes in Austrian's land cover (being of major significance for the whole basin) have been investigated in the period 1950–1995. The largest relative changes are for settlement areas which increased continuously by 109% between 1950 and 1995. Absolute changes are largest for woodlands with an increase of 4,004 km^2 and grassland with a decline of 4,187 km^2 [21]. From these data two options concerning changes in the sediment regime are possible: on the one hand, an increased input of fine sediments from adjacent areas into the Danube. Due to the intensification of agricultural production (enhanced soil erosion) and in areas of the Danube basin, where glaciers will retreat as a consequence of climate change, an increase of mainly fine sediments is predicted. But on the other hand, the input of sediments may also decrease as a consequence of reforestation.

Beside the longitudinal and lateral disruption of the sediment continuum, also the vertical dimension plays an important role for the sediment conditions at the Danube. Today, on the one hand, especially for the improvement of navigational conditions or flood protection (but formerly also for commercial purposes), the vertical sediment connectivity is disturbed due to regular (or even singular) dredging activities impacting/changing the bed composition. On the other hand, longitudinal impacts of dredging are evident by affecting the entire sediment regime,

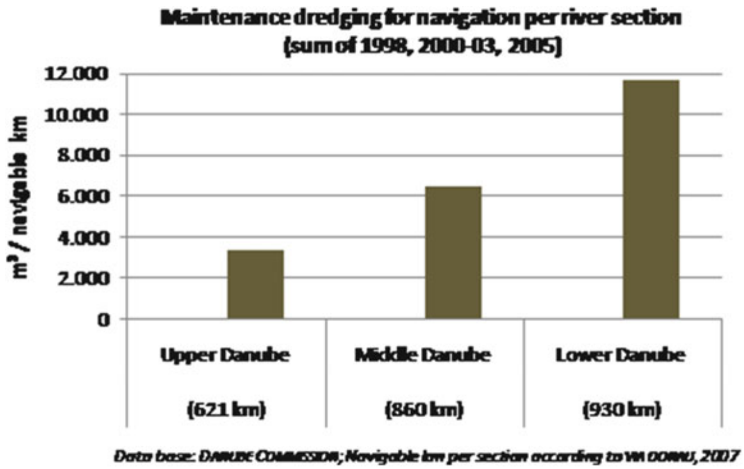

Fig. 5 Maintenance dredging for navigation per river section (sum of 1998, 2000–2003, 2005) ([15]; data base: Danube Commission; navigable km per section according to Via Donau [22])

thereby leading to river bed incision. Sectional differences of dredging volumes along the Danube are exemplified by dredged volumes for navigational purpose (Fig. 5). In contrast to the past, along the Upper Danube, however, e.g. in Austria, a defined refilling of the dredged material is performed (if possible upstream of the dredging site), meaning that there is no extraction of sediments in total (no loss of sediments). According to the studied literature, there is no evidence that such refilling measures are performed, e.g. along the Lower Danube, as well. Based on the reports of the Danube Commission, the dredged volumes of especially fine material for the improvement of the fairway are much higher at the Lower Danube compared to upper reaches (considering the difference in the sectional river length and the grain size of the material), as shown in Fig. 5. Hence, in a summarized view of the river basin sediment regime, it has to be stated that the Danube River partially features a totally disturbed sediment system due to the combined impacts of the four driving forces influencing the Danube's sediment balance: flood protection, navigation (dredging), hydropower and land use.

4.2 Status of the Hydromorphology

Within this chapter the hydromorphological alterations and man-made changes are presented which are more or less enormous. In the course of several river training measures, beginning at the fifteenth century and performed along the whole reach in the upper reach in the nineteenth century, the Danube was shortened in length and width, which especially leads to increased shear stress resulting in bed degradation (erosion). On the one hand, due to channelization and bank protection measures, the

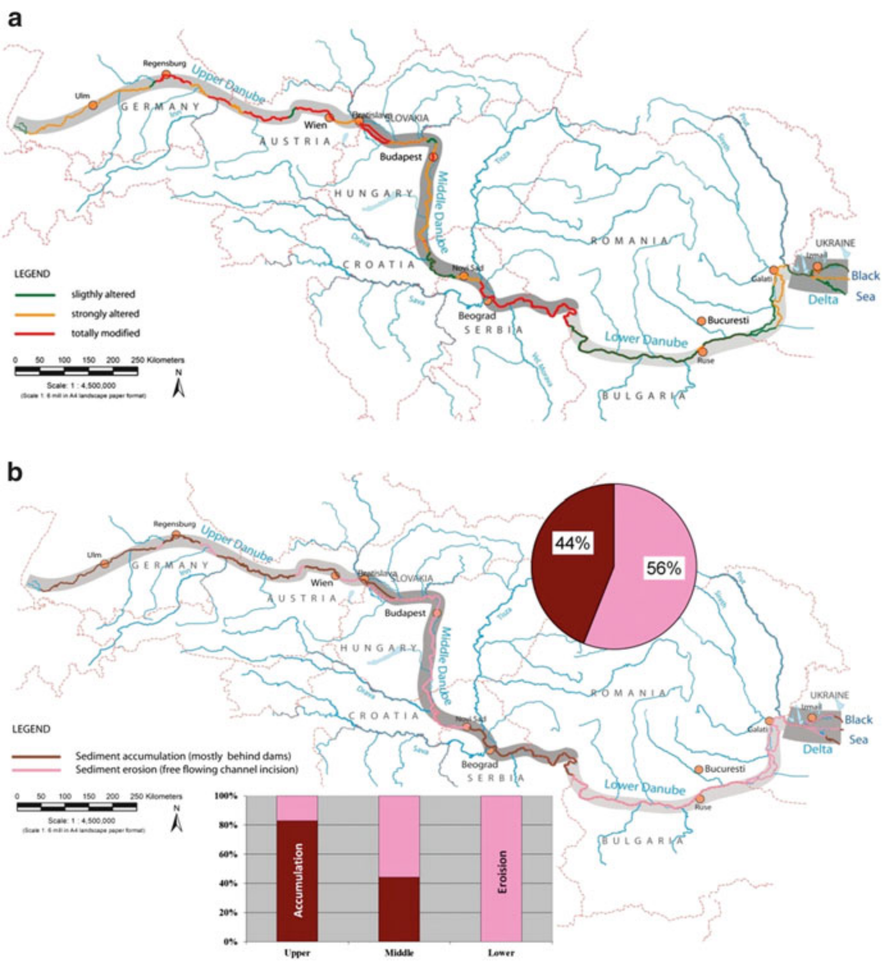

Fig. 6 (**a**) Degree of alteration of river morphology (channels and banks) (data base: Joint Danube Survey [2]), (**b**) erosion and accumulation reaches along the Danube River: maps (**a**) and (**b**) produced by Ulrich Schwarz

former morphodynamics and self-forming processes are prohibited with consequently significant ecological deficits [20]. On the other hand, deposition may occur due to side erosional processes, leading to wider river sections. This is enhancing island formation or increases at the Lower Danube, leading to bottlenecks for navigation. The current hydromorphological alterations of the channels and banks for the entire river basin are illustrated in Fig. 6. Moreover, it could be figured out that the hydromorphological conditions differ enormously between the upper and lower reaches of the Danube. The Upper Danube is mostly heavily impacted, while the Lower Danube predominantly still exhibits good hydromorphological conditions. Summarizing Fig. 6, 1/3 of the entire Danube River shows good hydromorphological conditions, while 1/3 is strongly altered.

On the sectional scale due to meander cut-offs (e.g. the Hungarian Danube was shortened from 472 to 417 km) and/or stabilization of river banks as well as due to the disruption of river continuity (e.g. disconnection of side arms), the resulting singular uniform river bed resulted in significant hydrological and hydraulic long-term impacts ([23]). Exemplarily for the Upper Danube and parts of the Middle Danube, the reduced river length (e.g. Bavarian Danube 15%, Austrian Danube 15% and Hungarian Danube 18%; compare Table 3 [5]), decreased active channel width (e.g. in Austria from over 3 km to 300 m after the channelization) and increased bed slope and average flow velocity (flow time) consequently lead to lowered water levels (for the same discharge).

Especially on the sectional scale, the hydromorphological status of the Danube has to be linked to the already described disturbances in the sediment regime. Since the Danube River lost its longitudinal sediment continuum over the last decades and the lateral sediment continuum over the last hundreds of years, different erosion and accumulation reaches have been developed. Considering the entire Danube River Basin, the erosion and accumulation reaches are presented in Fig. 6b. The sum of accumulation reaches amounts to 44% of the entire Danube River, mostly appearing at the Upper Danube and less at the Middle Danube, while the erosion reaches amounts to 56%, e.g. representing the entire Lower Danube. These different sectional patterns in erosion and deposition have severe influence on local scale river morphology and the related instream habitat quality, which is exemplarily presented for the reaches with erosional trends based on the outcomes and ongoing research at the Danube East of Vienna (at the National park 'Donau-Auen').

Former river training measures (especially the regulations at the end of the nineteenth century), but also the retention of sediments due to dams and similar interferences in the Upper Danube catchment (e.g. torrent control), have forced erosion processes along the free-flowing sections (e.g. the reach East of Vienna), meaning a permanent decrease of load supply and consequently river bed degradation. The prevented side erosion and braiding restricts the lateral input of bed load to the regulated river bed itself, where the transport capacity is enhanced by the reduced channel width and slope increase. Moreover, the river banks of the Danube are continuously embanked (bank reinforcement by ripraps); thus, bed load uptake processes occur only in form of bed erosion (vertical erosion). The process of river bed incision is highlighted by an example of the Danube River East of Vienna in Austria at the gauging stations Fischamend (left) and Wildungsmauer (right) (Fig. 7a). Despite an artificial gravel supply of up to 200,000 m^3 $year^{-1}$ downstream of the hydropower plant Freudenau, the river bed erodes by about 2 cm $year^{-1}$ along the Danube reach East of Vienna [24, 27]. The situation at some reaches along the Middle Danube is similar. For example, the erosion process at the Hungarian Danube at Dunaföldvár between 1949 and 2003 amounts to about 2.3 m (Fig. 7b).

The reach and local scale conditions at the Lower Danube, characterized as erosion reach (Fig. 6), are different in comparison to the upper reaches. The Lower

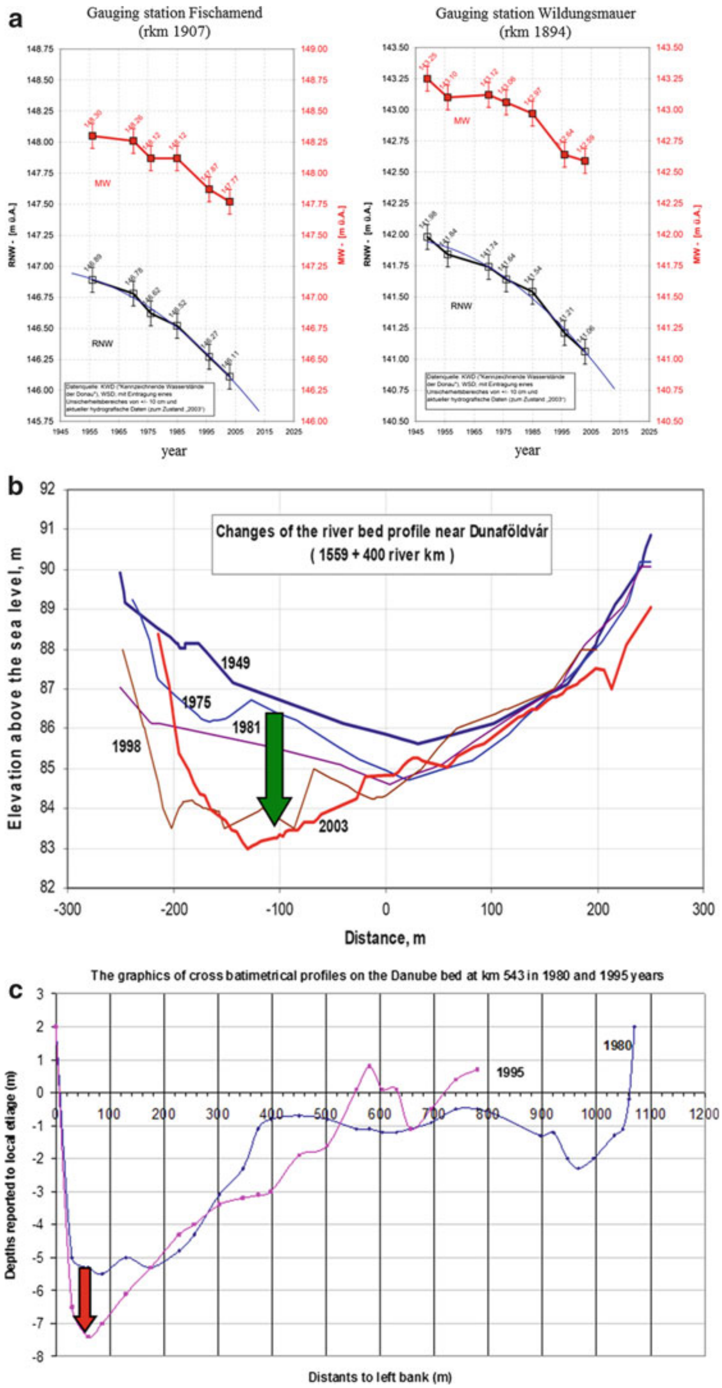

Fig. 7 (**a**) Erosion rate at the Danube East of Vienna at the gauging station Fischamend and Wildungsmauer [24], (**b**) erosion rate at the Hungarian Danube at Dunaföldvár [25], (**c**) river bed

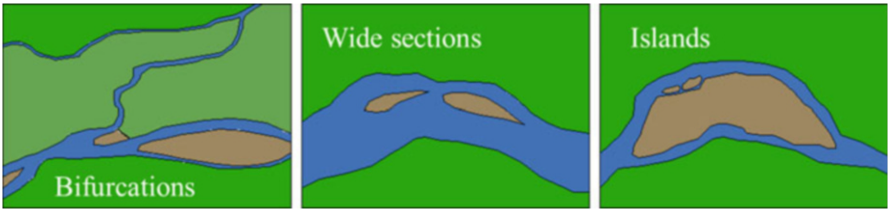

Fig. 8 Actual situation at the Lower Danube (*bottlenecks*)

Danube has to be described as a sandbed river with partially anastomosing morphology. Most of the river banks are still unprotected forming sandbars, which are ecologically very important. River bed incision is dominating. In calculating means, bed erosion has been determined which amounts to 1–3,5 cm year^{-1} between 1985 and 2005, highlighted, e.g. by a river transect at rkm 543 (Fig. 7c). The depths in the figure are corresponding to the low water level, compared to which the river bed incised in total by 2 m at that location. Similar is the situation between Corabia and Oltenita, which exhibited river bed incision along this section in the studied period between 1982 and 2000. At the present situation, mainly river bed erosion but also depositional processes (resulting in diverse morphological conditions, e.g. bifurcations, wide sections and islands) occur on the local scale of the Lower Danube together with the diverse demands of several stakeholders (e.g. navigation, ecology) which result in different bottlenecks (especially for navigation) (Fig. 8).

As there are still conflicts of interests given along the unprotected banks and islands of the Lower Danube, the already mentioned process of lateral (local) erosion has to be addressed in detail. Exemplarily, within the reach between Turnu Severin and Chiciu Calarasi, the number of islands increased from 93 in the year 1934 (with a total length of 283 km) to 135 in the year 1992 (with a total length of over 353 km) as a result of successive river bank erosion (side erosional processes) as a consequence of river bed erosion [26]. As one of the reasons for the formation of sandbars and islands, the lack of sediment input from upstream has to be mentioned (causing incision and the related bank failure).

The alterations in river morphology and the sediment regime (longitudinal/lateral) and the consequent disturbances in river morphology may be further negatively enhanced due to extensive floodplain degradation in the river basin. Along the entire Danube River, land use modifications since the nineteenth century have led to partially drastic interventions into the river system and especially to the adjacent land (floodplains). The process of wetlands and floodplain forests destruction has accelerated over the last decades [16], as the building of flood protection dike and drainage canal systems allowed intensive, industrial development but also

Fig. 7 (continued) erosion at the Lower Danube at rkm 543 (between Zimnicea and Giurgiu) measured in 1980 and 1995 [26] contributed to the overall loss of some 80% of the former Danube floodplains during the last 100 years [28]. About 80% of the original floodplain area in the Danube River Basin has been lost since the twentieth century (e.g. loss of floodplains in Hungary, 10,000 ha; in Slovakia, 4,000 ha; in Bulgaria, 72,600 ha; in Romania, 426,000 ha) leading to the loss of important functions for the entire river system (purification of water, flood storage, groundwater recharge). The total area of historical floodplain and wetlands along the Danube and some main tributaries was about 41,600 km^2; the remaining floodplain wetlands amount only to 7,845 km^2, which results in a floodplain loss of more than 3/4 of the former dimension (only ~20% of the former floodplain area is remaining) [3, 28, 29]. Of course this reduction of floodplain width leads to an increase in bed shear stress of the main channel, thus increasing river bed erosion.

In summary, the formerly morphologically undisturbed Danube River system suffers on various scales from the combined impacts of several driving forces (predominantly flood protection, navigation as well as hydropower generation) which have been identified in the presented study. In order to support the conditions for navigation and for flood protection and hydropower generation purposes, most of the Danube has been constricted, channelized into one single channel and disrupted from the adjacent floodplain areas, leading to severe morphological degradation. Hence, the non-impounded sections feature conditions as increased shear stresses, sediment transport, reduced lateral sediment exchange and input and morphodynamics. Moreover, as a consequence of the sediment supply limitation and channelization, the free-flowing sections are subject to various forms of river bed degradation and loss of instream structures.

5 Discussion

Throughout the presented study, various anthropogenic pressures on Danube sediment regime and morphology have been identified and presented at different scales. As it could be clearly figured out, concerning the aims of the paper, the impacts are not always related to one trigger factor but have to be seen as a sum of multiple pressures on the river. In addition to sediment regime and hydromorphology, the role of hydropower plants (dams and weirs) in relation to a changed hydrology and hydraulics has also to be discussed [14]. Large hydropower plants alter the hydrology and hydraulic as they increase the water level upstream of the impoundments (e.g. Gabčíkovo – in Bratislava by about 2 m between 1992 and 1996) and lower flow velocities in hydropower reservoirs. In addition to that, the flood retention capacity has been reduced significantly (e.g. the retention capacity during floods at the Lower Danube reduced from 15.6×10^9 to 4.0×10^9 m^3) resulting in increased flood wave velocities downstream (by approx. 12 h between 1950 and 2012 for the Upper Danube [30]) and with obviously negative consequences for flood protection. Moreover, the effects of intermittent hydropower generation on river hydrology and hydraulics in the form of hydro-peaking may cause huge water level changes by releasing water by pulses several times per day [31].

Besides the already mentioned impacts from land use (e.g. decrease of floodplain areas and thereby impacting hydrological and hydraulic characteristics of the Danube), drainage and irrigation are also responsible for the change (drop) in water levels [11, 14] and have to be discussed and/or considered for future river basin management. Especially the changes in adjacent forest cover alter hydrological processes significantly. With regard to diffuse sources, the change from natural systems to agricultural land use heavily increases the nutrient emissions into the river system even if nutrient management is optimized for water protection [32]. An important issue concerning future land use change/management in the Danube River Basin is surface run-off in general, which goes hand in hand with soil erosion (less infiltration contributes to surface erosion). As the compaction of soil leads to higher surface run-off in general, soil cultivation/land management influences the intensity of surface run-off.

Moreover, additional increased impacts of climate change (global warming) are expected for the Danube hydrology affecting the entire river basin, increasing those pressures which are already given. The impacts on river hydrology resulting from climate change (e.g. the reduction of floods in springtime) are manifold. Strong regional differences have to be considered. Especially for alpine catchments (main tributaries of the Upper Danube), the effects especially in terms of snow accumulation and snow melt will be strong. There is the tendency of decreased snow accumulation and earlier snow smelt caused by higher air temperature and a higher rate in liquid precipitation. This will result in more run-off during winter and less in the summer period. In areas with lower altitude, the low flow periods will be strongly affected. Here a clear increase in days of low flow (e.g. dry periods) was recorded. Moreover, an overall trend to a seasonal change in flood appearance may be possible. The number of floods in summertime will decrease in which the amounts of the seasonal shift will vary from area to area.

The expected future costs of EU policies on climate change are enormous. Exemplarily, for the Upper Danube the estimated total damage of a 100-year flood is projected to rise by about 40% of the current damage estimate (corresponds to an increase of €18.5 billion) for the high emission scenario and about 19% for the low emission scenario (control period 1961–1990; future period 2071–2100). The number of people affected in the Upper Danube is estimated to increase by 242,000 (~11%) for the high emission scenario and 135,000 (~6%) for the low emission scenario [33]. Moreover, drought periods (e.g. in 2003), related to climate change, will have significant consequences on, e.g. hydro-generation, navigation as well as water quality (e.g. increased nutrient concentrations in the Danube Delta). The extreme drought in 2003 showed a significant reduction of hydro-energy production in the range of run-off-river power stations. In Austria, it was the least production since 1955 [34]. All these aspects of climate change have to be considered and discussed in addition to the alterations of sediment regime and hydromorphology according to the aims of the WFD for the necessary river basin management at the Danube.

For future mitigation, especially along the upper and middle reaches of the Danube, river restoration combined with the planned improvement of navigation should be implemented as an integrated aim. At the Lower Danube, however, preservation of already given morphodynamics and restoration of floodplains in combination with the improvement of navigation should be the target. Based on the findings of the presented study, ways for the preservation and/or restoration/ improvement of the sediment continuum (i.e. sediment transport) along the entire Danube and its tributaries across hydropower plants and torrent control structures have to be discussed. Therefore, a catchment-wide sediment management concept should be developed under an integrated synopsis of bed morphological processes with the elaboration of measures (e.g. against river bed degradation and aggradation of reservoirs and of the inundation areas) that considers the improvement of the ecological status (EU legal requirements). Hence, the ongoing river bed degradation has to be stopped by, e.g. implementation of a sustainable stabilization of the mean bed level.

Referring to river restoration, the implementation of such measures has to be analysed and discussed according to the given river morphological processes by allowing side erosion as well as bed and side-arm development, which positively influence at the same time the heterogeneity in river morphology and the habitat diversity. Furthermore, the river morphology (type and processes) and sediment qualities (physical, chemical, biological) should be assessed prior to planning and executing any interventions. Based on the findings of the presented study (identification and listing of multiple alterations), the restoration of the longitudinal and lateral river continuum has to be seen as the basis for the sustainable improvement of the ecological status, especially at shorelines and side arms, by means of reconnection of the former side-arm system or at least connection during higher discharges, river bank restoration and the improvement of aquatic/semiaquatic habitat quantity and quality (pioneer and dynamic sites). Additionally, the remaining floodplains should be conserved and restored as natural landscapes and flood retention areas by initiating self-forming processes (morphodynamics).

The alterations described in this paper should be considered in all future projects and river basin management plans as there is the need for an integrated design of ecologically compatible measures for navigation, hydropower and flood protection (win-win situation) in order to equally regard hydraulic, morphological and ecological criteria. Moreover, the possible implementations of new measures need to have a repairing/restoring effect for hydromorphology. For example, navigation should be improved on the reach scale by developing ecologically compatible measures (preparation of an integrated design for regulation structures) adapted to the local situation (e.g. modification of existing groins where suitable, construction of new modified forms and lengths of groins with respect to distance relations, usage of innovative bed stabilization measures (e.g. granulometric bed improvement at the Upper Danube)).

Another essential point is the need for the application of an integrated planning approach and principles in order to improve the current situation from various perspectives. The establishment of interdisciplinary planning teams involving key stakeholders, including Government bodies responsible for transport, water management and environment, waterway administrations, administrations of protected areas, local authorities, nongovernmental organizations, river-related stakeholders, scientific institutions and independent (international) experts is absolutely necessary. The interdisciplinary planning teams have to define joint planning objectives, set up transparent planning processes, avoid/minimize the impacts resulting from structural/hydraulic engineering interventions, consider climate change effects, monitor the effects of implemented measures and consult existing good practice measures to improve the purposes of diverse needs (e.g. navigation versus ecology). However, most of the identified alterations, the central statements or even recommendations derived in this paper are not only valid for the Danube River Basin but also for other large river basins as well. Thus, the scientific assessment on hydromorphological disturbances along the Danube should deliver a basis for discussion, information exchange and probably a method which can be applied for other (navigable) large rivers in Europe and beyond.

6 Conclusions

The results of this paper show that the Danube River partially features a totally disturbed system (e.g. sediment balance), due to the combined impacts of flood protection, navigation (dredging, channelization, erection of groins, cutting off side arms etc.) and hydropower. The sediment continuum has been decreased to a minimum (due to torrent control, hydropower etc.), leading to a lack of bed load and suspended load in the downstream free-flowing sections. For the improvement of inland navigation, flood protection and hydropower generation, the Danube River has over long distances been narrowed, channelized, disrupted from the floodplains and morphologically degraded, thus leading in the non-impounded sections to increased shear stresses, increased sediment transport, a lack of lateral sediment transport and reduced morphodynamics. As a consequence of the limited sediment supply and channelization in the entire catchment, the free-flowing sections are subject to different forms of river bed degradation on various river scales (reach and/or local scale). Results are a loss of instream structures, especially a disappearance of gravel bars, and changes of sandbars. With the lack of morphodynamics, spawning places are disappearing, leading to a worsening of the ecological status. One of the main conclusions is that hydromorphology is not only an ecological issue but also an essential aspect for future navigation, flood protection and hydropower generation. Moreover, hydromorphological processes differ between each river section along the Danube (Upper ↔ Middle ↔ Lower Danube). In addition, cumulative effects on hydromorphology are found not only in the downstream direction but also backwards (upstream). Although a number of mitigation schemes were initiated at the Danube, e.g. in Austria, Hungary and Romania, to avoid or reduce negative effects on river environments and the continuous loss of riverine landscapes, further actions will be necessary in the future to mitigate existing impacts and prevent future ones.

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Hydrological and Biogeochemical Characterization of the Danube River System Using Isotopes

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Abstract Meeting Danube Basin monitoring and management objectives such as those implied by the EU Water Framework Directive requires a comprehensive understanding about the hydrological and biogeochemical functioning of not only the river system but also the connections between groundwater and surface water across the basin. While hydraulic and geochemical measurements can provide some of this understanding, it is often difficult to obtain knowledge about some of the more critical aspects of basin functioning or it can take decades of intensive monitoring before adequate interpretations can be made. Isotope hydrology

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approaches can often provide critical insights on surface water/groundwater interactions and biogeochemical cycling with only moderate effort and cost. Such information can help clarify local behaviors as well as overall basin responses. Approaches using "environmental" stable and radioactive isotopes (i.e., isotopes that are already in the environment and not intentionally applied) have been used to understand sources and losses of water in the Danube, the importance of groundwater discharge, basin residence times, tributary mixing, and nitrate cycling using isotope methods. We review existing studies as well as present new isotope data that reveal important spatial and temporal dynamics occurring in the Danube River, tributaries, and across the basin.

Keywords Deuterium, Isotope hydrology, Nitrogen-15, Oxygen-18, Radon-222, Rivers, Tritium

Contents

1 Introduction and Background

Stable and radioactive isotopes provide a powerful suite of tracers for understanding important hydrological and biogeochemical aspects of the Danube River system at reach to basin scales. Although the amount of isotope data available for the Danube Basin is not that extensive given the size of the basin, there are existing isotope studies and databases that reveal important conceptual and quantitative information about water and nutrient cycling within the basin and main river channel. This chapter reviews some of the key published studies on the Danube system and also presents new data that help clarify important processes relevant to the EU Water Framework Directive and other hydrological, biogeochemical, and ecological activities in the basin. We also hope that this discussion helps motivate additional use of isotopes for future studies in the Danube and other large river basins. The structure of the chapter is based on a set of major hydrological and biogeochemical characteristics rather than on isotope type because we wish to emphasize what one can obtain from isotope approaches as opposed to the methods themselves. Additional details on isotope laboratory and field methods, as well as interpretations can be found in the citations included in this chapter. As a brief overview, stable isotopes do not decay with time but can be very useful tracers to, for example, indicate sources of water or nutrients (in this case we focus on nitrate) or certain processes such as evaporation. Stable isotope values are described using what is known as "delta notation" (e.g., $\delta^{2}H$ and $\delta^{18}O$ for the isotopes of water or $\delta^{15}N$ for the nitrogen in nitrate). Delta values do not represent concentrations of isotopes but are based on ratios of the rare to common stable isotopes in a sample (e.g., $^{18}O/^{16}O$) and the same ratio in a standard. Negative values often occur because the ratio of the standard is subtracted from the ratio in the sample. Radioactive isotopes (e.g., tritium) on the other hand do decay with time which makes them useful for age dating. They can also be used to understand processes like mixing and groundwater discharge into river channels. Isotopes in general can also be good indicators of large-scale changes in a river system because they are often sensitive to land use or major hydroclimatic factors. Therefore, this chapter documents current isotopic conditions in the Danube which can serve as baseline data for evaluations of future impacts. It is also worth noting that the majority of the Danube isotope data discussed in this chapter (as well as many other world-wide isotope records) are publically available through the IAEA Water Isotope System for Data Analysis, Visualization, and Electronic Retrieval system (WISER, [1]) by accessing the Global Network of Isotopes in Precipitation (GNIP) and Global Network of Isotopes in Rivers (GNIR) databases through the IAEA Water Resources Programme website (http://www.iaea.org/water/).

2 Examples and Discussion

2.1 Sources of Water in the Danube

The stable isotopes of oxygen and hydrogen ($\delta^{2}H$ and $\delta^{18}O$) can provide valuable details about where and how water is moving through a river system compared to simple flow observations and maps. This subsection discusses how these isotopes can be used to fingerprint water sources and to reveal important information about the dynamics of water movement though the Danube system. Oxygen and hydrogen isotopes in precipitation from central Europe show a high degree of seasonality, where values become more negative during the winter and shift to less negative values during the summer. Temperature and other effects drive these differences especially during the process of condensation of water vapor in the atmosphere. Although the amplitude shifts are not as great as in precipitation, the seasonality of isotope values is carried through to Danube River water as shown Fig. 1. The peaks and valleys of the Danube time series are governed by the differences in summer and winter isotope values. What one would expect based on the precipitation isotope values and local air temperatures would be less negative values in summer

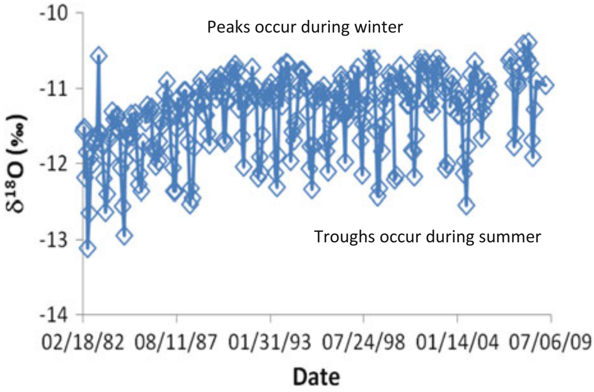

Fig. 1 Time series of Danube River $\delta^{18}O$ values at Vienna from 1982 to 2009 from the IAEA GNIR database

and more negative values in winter. However, the Danube River time series shows the opposite situation where more negative values occur during summer and less negative values during winter. What the river isotope series is reflecting are summer time inputs of snow- and ice-melt sources from alpine areas that reach the Danube during the May–August period. These variations can also be used to quantify hydrological residence times in the basin as discussed in Sect. 2.4. The $\delta^{18}O$ profile collected during the second Joint Danube Survey (JDS2) demonstrates the alpine inputs quite well (Fig. 2; [3]; Rank et al. 2009). In the upper Danube, values are in the −9 to −10 ‰ range reflecting lowland drainage from the headwater area. However, when the Inn enters the Danube, there is a substantial shift to lower isotope values. This shift is driven by the Inn's alpine drainage area and substantial discharge that contributes large amounts of ice- and snowmelt sourced water to the Danube channel. Further downstream there is a slow increase in isotope values reflecting continuing additions of tributary waters that have less negative isotope values from lower elevation sources which dilute the Inn alpine input. There is also a small increase in values near the Iron Gate area (labeled high water influence in Fig. 2) that is not related to tributary inputs, but rather to an extreme precipitation event in central Europe during JDS2 sampling. This event increased $\delta^{18}O$ values slightly between Iron Gate and the river mouth at the Black Sea.

In addition to providing information about the sources of water in the Danube system, the isotope measurements from JDS2 are a useful benchmark for monitoring future climatic or land use changes in the basin (e.g., see discussion by Rank and Papesch [4] and Rank et al. 2013 [9]). For example, potential changes in the timing and duration of snowmelt will likely be observable in the Danube isotope record. There is only one other synoptic water isotope record for the Danube [5], and so these two time series serve as important archives about conditions in the Danube Basin that complement time-based data collected in the IAEA Global Network of Isotopes in Rivers database [6] and national isotope monitoring programs.

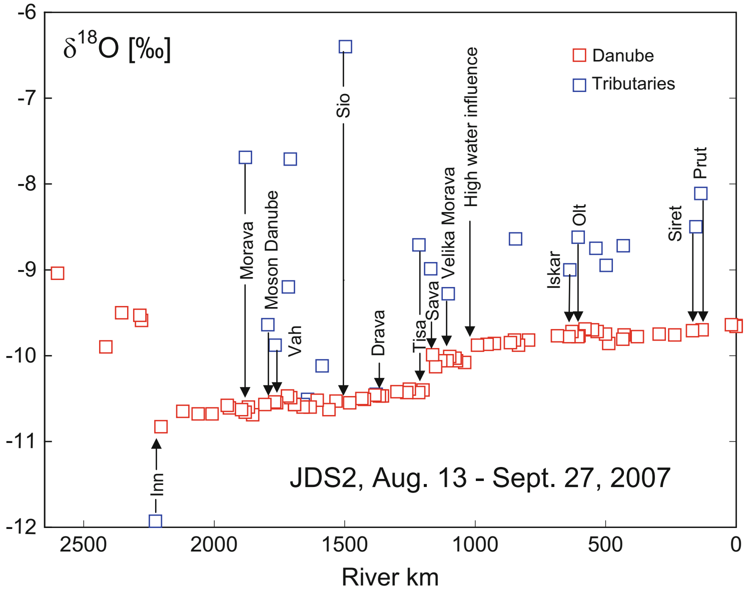

Fig. 2 Profile of $\delta^{18}O$ along the Danube from JDS2 (August 13 to September 27, 2007). *Red boxes* are data from the Danube River, and the *blue boxes* are from tributaries ([2]; Rank et al. 2009 [8])

2.2 *Evaporation*

Surface water evaporation can be an important water loss process and should be evaluated because of the potential to affect river water balances. The stable isotopes of water (δ^2H and $\delta^{18}O$) are sensitive to evaporation where evaporated waters show a characteristic deviation to the right of the meteoric water line on a $\delta^{18}O$-δ^2H diagram. The meteoric water line describes the typically linear pattern that precipitation stable isotope data plot along throughout the year [7]. A multiyear record of monthly data for the Danube River at Vienna (Fig. 3) shows that river values lie along the meteoric water line, rather than being shifted to the right of the line, indicating that surface water evaporation along the river course is relatively minor.

This conclusion is reinforced by the isotope results from JDS2 (Fig. 4) which included measurements along almost the entire length of the Danube in addition to some tributaries [3, 8, 9]. These samples were taken during the late summer/early fall period when evaporation effects should be most evident. Like the GNIR data in Fig. 3, the JDS2 Danube main channel data plot along the meteoric water line, thus indicating minimal evaporation. The Sio does show a strong evaporation influence because it contains discharge from Lake Balaton. The Ipoly, Morava, and Prut also show some evaporation effects because the data plot to the right of the meteoric line. The higher deuterium excess values ($d = \delta^2H - 8\delta^{18}O$) in the upper sections of the Iskar, Jantra, and Arges cause values to plot to the left of the meteoric water line

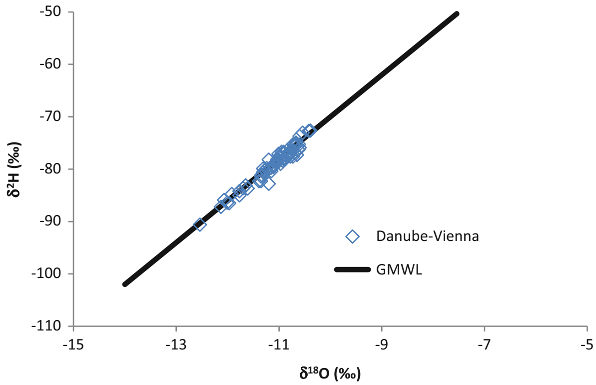

Fig. 3 Monthly water isotopes in the Danube River at Vienna from 2002 to 2008 (IAEA Global Network of Isotopes in Rivers database; [6]). The *black line* represents the global meteoric water line [7]

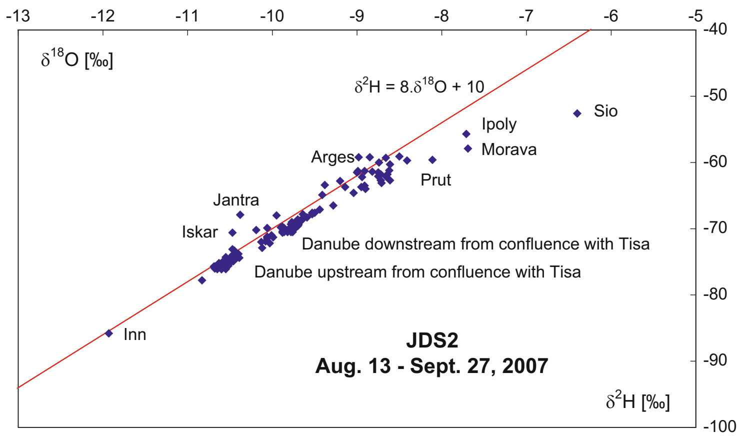

Fig. 4 $\delta^{18}O$–$\delta^{2}H$ diagram for river water samples from JDS2. The *solid line* indicates the global meteoric water line ([2]; Rank et al. 2009 [8])

and are probably due to local orographic (mountainous) conditions (see discussion in [4]). Overall, the data in Figs. 3 and 4 suggest that evaporation losses from the Danube River are minor in terms of the river water balance.

2.3 *Groundwater Discharge*

An important aspect of the hydrology of the Danube River is the relative importance of groundwater discharges to the Danube main channel versus surface inflow through tributaries. To investigate this issue, a set of radon-222 data were collected during JDS2 [3]. Radon-222 is a naturally occurring radioactive isotope with a half-life of 3.8 days. Although radon-222 in natural waters can be affected by geology and other factors, elevated radon-222 has repeatedly been shown to be an effective indicator of groundwater discharges in rivers, lakes, and along coastal zones [10–12]. It is an effective isotope approach because groundwater values tend to be much higher than in rivers; therefore locations within the river channel that have elevated radon-222 are likely to be at or near groundwater discharge zones. River reaches that do not have groundwater discharge zones have low radon-222 because surface waters loose radon quickly to the atmosphere and because radon-222 production in rivers is typically much lower than in groundwater. For JDS2, radon-222 data were collected at the official sampling sites to identify potential locations with significant groundwater inputs. The radon-222 profile along the Danube has some interesting features (Fig. 5). Overall, the values are low, and the lowest values are effectively at the limit of detection as is typical for surface water without groundwater discharges. However, there are 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). Rank et al. [13] indicated groundwater inputs to the Danube upstream from Passau were relatively high which supports the radon-222 data. Some of the tributaries (e.g., the Sava, Velika Morava, and Siret) also have relatively high radon-222 values which suggest they have

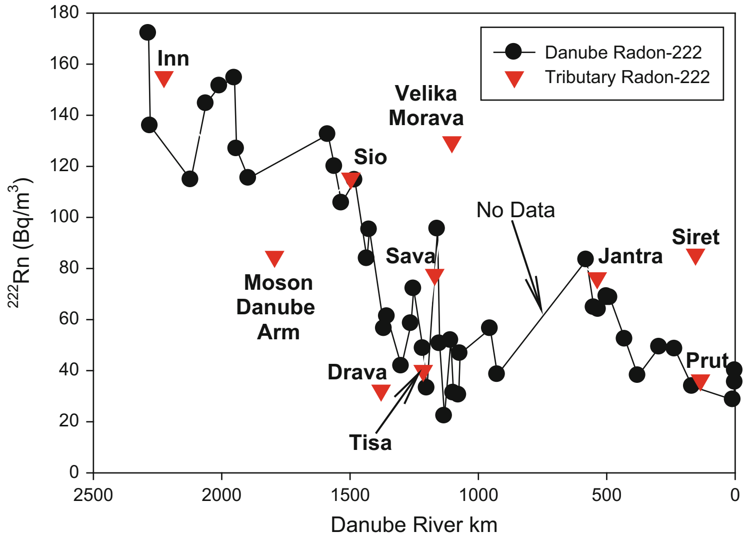

Fig. 5 Radon-222 in the Danube basin. *Black circles* indicate Danube River measurements, and *red triangles* represent tributary measurements. The *left side* of the *x*-axis is at the Danube headwater area, and the *right side* (0 km) is at the entrance to the Black Sea

groundwater inputs in the vicinity of the JDS2 sampling points. Because the JDS2 sampling sites focused on mid-channel samples and because of the large river volume, the survey was probably not optimal for identifying smaller, localized areas of groundwater discharge. Nevertheless, the overall interpretation from the generally low radon-222 values is that groundwater inputs to the Danube main channel are minor. This interpretation is consistent with discharge observations where major tributary inflows account for 80–90% of the total flow in the Danube (e.g., [14, 15]). It should be stressed that while it appears that groundwater discharges to the Danube main channel are minor in terms of total river discharge, they may still be important biogeochemically and as potential nutrient/pollutant sources. Groundwater discharges are also likely to be important sources of flow in some tributaries as suggested by the radon-222 data, and as will be discussed later in the residence time section of this chapter, groundwater does play a major role at the basin scale. This distinction between the groundwater contributions to the main channel and to the overall basin hydrology should not be overlooked.

2.4 Hydrological Residence Times

An understanding of hydrological residence times (or transit times) in the basin is one of the most critical aspects of sustainable management of Danube hydrological and ecological resources. Conceptually, residence time distributions in river basins reflect how long it takes for water or solutes to be cycled through the basin. The timescales over which the basin hydrologic system operates will strongly impact how quickly nutrients or pollutants will be flushed from the basin and thus has major implications on how quickly water quality improvements can be made, as well as on Water Framework Directive monitoring strategies. Unfortunately, residence time distributions are often difficult to quantify especially at basin scales. However, isotope approaches can be used to constrain mean residence times and sometimes the residence time distribution. In the case of the Danube, the extensive time series of isotopes in precipitation and Danube river water (tritium, oxygen-18, and deuterium) for Vienna (through the on-line IAEA WISER system) provide a way of quantifying residence times for the upper part of the basin (Fig. 6).

Rank et al. [13] compared interannual variations of Vienna precipitation stable isotope (oxygen-18 and deuterium) time series to those of Danube water at Vienna (see discussion in Sect. 2.1) as a way of constraining the fast-flow component (e.g., surface flow) of the upper basin. They found that the interannual variations of river water reflected those of precipitation inputs (as discussed in Sect. 2.1); however, the river water variations were lagged in time versus precipitation. By adjusting the river time series to match the peaks and valleys of the precipitation time series, Rank et al. estimated a mean transit time of about 1 year. They then examined the slow-flow (e.g., subsurface flow) component using tritium time series of Vienna precipitation and Danube river water (Fig. 6). The dynamics of the tritium time series are different than the stable isotopes, and so a lumped parameter modelling

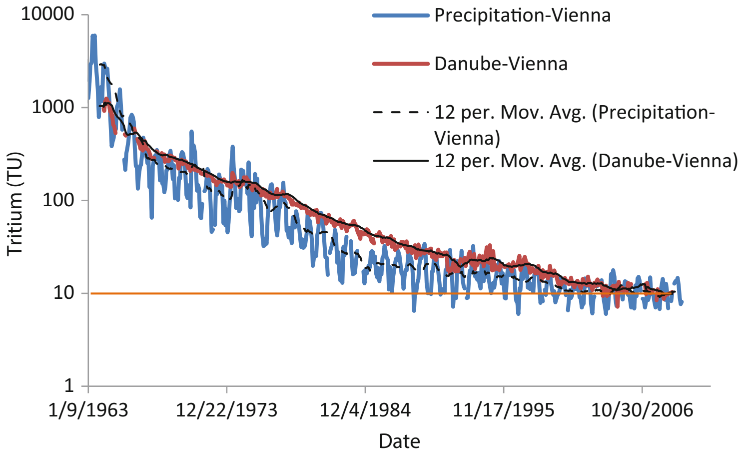

Fig. 6 Annual time series and moving averages for Vienna precipitation and Danube River water from 1963 to 2009. The decrease of tritium released during atmospheric nuclear weapons testing in the early 1960s is apparent until approximate background levels were reached in the late 1990s/early 2000s period. The *orange line* at 10 TU is the current "background" value

approach was used [16]. Tritium in precipitation was dominated for decades by inputs from atmospheric testing of nuclear weapons in the late 1950s to 1960s. The lumped parameter model uses the tritium in precipitation time series as input and then calculates a transit time distribution function to transform the precipitation input to match the river water tritium time series. This so-called "black box" approach has the advantage of not requiring spatially explicit hydrogeological parameters and is well suited for situations where high-quality input and output tracer time series are available. Using an exponential function to constrain the lumped parameter model produced an estimated mean residence time for the upper Danube basin slow-flow component of 3 years. Rank et al. [13] noted that the 1960s and 1970s part of the river water time series was not well fit, and thus the 3-year estimate has substantial uncertainty.

Yurtsever [17] used the same Vienna isotope data as above (Fig. 6) but used two alternative modelling approaches. The first approach assumed fast- and slow-flow components using a mixing cell approach (also known as a bucket model) with the tritium precipitation time series as input. The estimated mean transit time for the fast (surface) flow component was 0.8 years and the estimated slow (subsurface) component was 11.7 years. The average flow-weighted travel time for the upper basin combining both slow and fast components was about 4.7 years. These results indicated a much broader travel time distribution than suggested by the results of Rank et al. [13]. The second approach incorporated a neural network model which involves a model training procedure prior to final simulations. This approach did not have a slow-flow/fast-flow conceptualization. The weighted-mean transit time produced was the same as the mixing cell approach at about 4.7 years.

The results of Yurtsever [17] and Rank et al. [13] suggest that there are fast (mainly surface related) flow paths that have residence times of around 0.8–1 year. There are also slow paths (mainly subsurface flow related) that have mean transit times between 3 to almost 12 years. The combined mean residence times range from a couple of years up to nearly 5 years. However, one needs to be careful in interpreting these results. First, the estimates have substantial uncertainties. Second, they reflect the mean residence times and not the residence time distribution. It is well known from tracer work and modelling studies that residence time distributions are typically skewed, long-tail type distributions. Thus, for example, pollutant concentrations may not fully return to prerelease or background values until well after the mean residence time period has passed. Alternative modelling approaches that use new extended tritium records are currently being developed which should help reduce uncertainties about the basin residence times.

2.5 Tributary Mixing

The rate and spatial scale of mixing between tributary waters and the Danube River has important implications for understanding nutrient and pollutant dynamics in the river as well as for developing sound monitoring plans. Tritium sampling in the center, right side, and left side of the Danube channel (i.e., a transect perpendicular to flow) at a few locations along the river during JDS2 coupled with a release of tritium in the Vah tributary provide a valuable example of mixing dynamics in the Danube. Current, post-bomb background tritium in precipitation in Central Europe is about 10 TU. However, tritium is released into rivers of the Danube basin through nuclear power plants and industrial activities which can temporarily raise tritium levels. These releases are at low levels and are below health concern values. They are however excellent tracer inputs to track water movement through the river system. Tritium levels in the Vah and Danube during JDS2 are a good example. Locations of tritium samples in the Vah channel and above and below the Vah confluence with the Danube are shown in Fig. 7. Tritium values from these locations (and other Danube and tributary locations) are shown in Fig. 8. Power plant tritium was released into the Vah and detectible at over 70 TU during JDS2 (Fig. 8). Upstream of the Vah values were at about 10 TU. Samples collected 5 km downstream from the Vah had over 40 TU on the left (Vah) side of the Danube, but the center and right-side samples only had about 10 TU. These results indicate that the Vah discharge was not well mixed with the Danube for at least 5 km below the confluence. Sampling was not done at a fine enough spatial scale to fully quantify the mixing length of the Vah water, and it may have been much longer than 5 km. Other large river isotope studies have demonstrated mixing lengths of 100s of km for tributary inflows [18, 19]. Although we do not know if such long lengths occur in the Danube, the Vah results do demonstrate that spatial heterogeneity can occur in the Danube below tributary confluences for considerable distances. Thus, if tributaries have different pollutant or nutrient loads than the Danube, then

Fig. 7 Image of Vah/Danube confluence and locations of tritium sampling sites

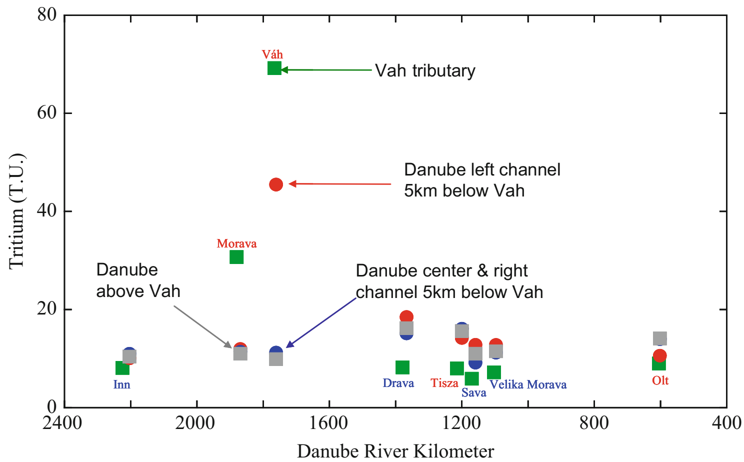

Fig. 8 Tritium from the Danube and Vah rivers showing incomplete mixing for at least 5 km downstream from the confluence. *Green squares* indicate tributary samples, *red circles* indicate samples from the left side of the Danube, *blue circles* indicate samples from the right side of the Danube, and *gray squares* indicate samples from mid-channel (center) locations in the Danube

tributaries can impact ecosystems on the confluence side of the river more strongly than on the opposite side of the river. Thus, the potential for long mixing zones should be considered when designing surface water sampling plans and when evaluating riparian zone impacts below tributary confluences.

2.6 Nitrate in the Danube

Nitrate is one of the most important water quality issues in the Danube basin. A substantial fraction of reaches in the Danube River and its tributaries are ecologically at or potentially at risk from nutrient pollution [20]. A great deal of effort is being made to monitor nitrate and other nutrients in the Danube system through various national programs and through coordinated international activities such as the ICPDR Transitional Monitoring Network and Joint Danube Surveys (e.g., ICPDR [2, 14]). In addition, a modelling approach using MONERIS (MOdelling Nutrient Emissions in RIver Systems; [21]) is being utilized to estimate nutrient inputs into the basin by point sources and various diffuse pathways. Despite these efforts, a great deal of uncertainty exists regarding nutrient sources and biodegradation in the Danube system [20]. In particular, there are uncertainties related to the various sources of nitrate and their impacts on the river. The isotopes of nitrate (δ^{18}O and δ^{15}N) can be diagnostic of nitrate sources and biodegradation [22]. However, we are unaware of any published nitrogen isotope data sets from the Danube. A reconnaissance set of nitrate isotope samples was collected at a series of locations during JDS2, but results were not available for publication in the JDS2 report [2]. They are therefore discussed for the first time here. Results from JDS2 show remarkably little variation in the δ^{15}N isotope composition of nitrate along the main river channel, whereas tributary compositions are much more variable (Fig. 9). Plots of nitrate δ^{15}N versus nitrate δ^{18}O have been shown to be useful

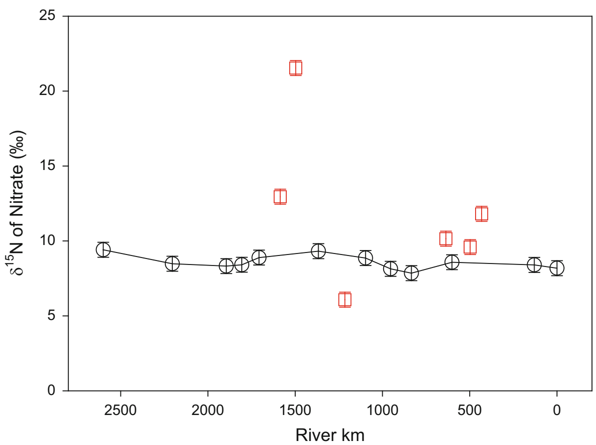

Fig. 9 JDS2 profile of δ^{15}N (nitrate) along the Danube. *Black circles* are values from the Danube main channel, and *red squares* are values from tributaries

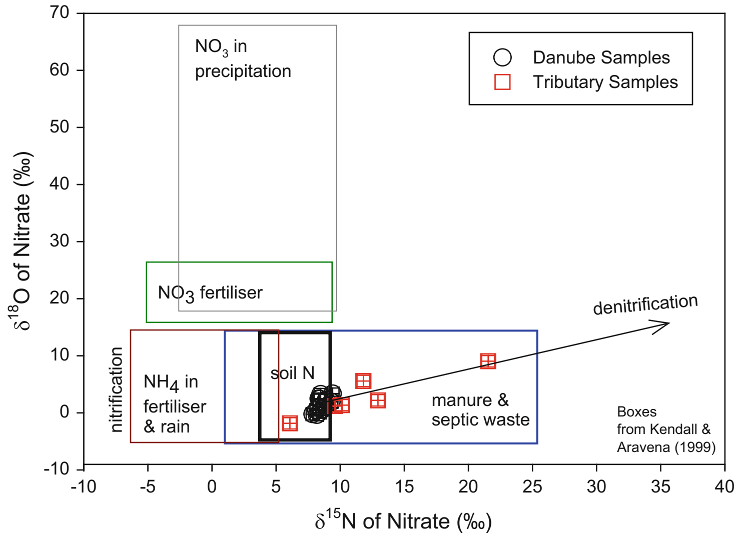

Fig. 10 Nitrate isotope diagram showing JDS2 results relative to nitrate source fields. *Black circles* are from the Danube main channel, and *red squares* are from tributaries. The *black arrow* represents the theoretical denitrification line

for understanding nitrate sources because different sources tend to plot in different fields on a nitrate isotopes diagram [22]. When the JDS2 nitrate isotope data are plotted in this way, some clear patterns about the various sources of nitrate in the basin emerge (Fig. 10). For the Danube main channel, isotope results suggest that inputs of atmospheric nitrate and nitrate-based fertilizers to the river are minimal. Instead, the river appears to be dominated by inputs of soil nitrate, nitrate from sewage/manure, or a mixture of the two. One of the limitations of this technique is that some source fields partially overlap which prevents, for example, discrimination between soil nitrate and that from sewage/manure for some samples. A few tributaries do indicate clear sewage/manure sources because they plot well to the right of the soil nitrate field. These tributary waters also plot along the theoretical denitrification line indicating biodegradation of nitrate may have occurred. However, it is not possible to say with this limited data set whether denitrification happened locally in the sampling area or in some other spot along the nitrate transport pathway. A recommendation for future work is to conduct a more thorough nitrate isotope assessment of tributary inputs since many tributaries were not sampled, and no samples were collected upstream from the JDS2 tributary sample locations. Additional insights about the nitrogen sources could be gained by analyzing nitrogen isotopes from source materials such as manure used on agricultural fields in the basin. Boron isotopes may also be useful for examining the relative importance of sewage versus other nitrate sources (e.g., [23]).

3 Summary

The oxygen and hydrogen stable isotope data show that water in the Danube main channel is fed by lowland areas until a large alpine-sourced discharge arrives through the Inn tributary, whereupon a significant decrease in Danube isotope values occurs. Discharges from tributaries further downstream cause a slow increase in Danube isotope values reflecting their relatively lower elevation sources compared to the Inn. They also show that evaporation losses from the Danube are minor, although this is not the case for some tributaries. Groundwater discharges to the Danube also appear to be minor, except in the headwater area based on radon-222 and tributary discharge data. However, groundwater discharges are of major importance at the basin scale and at least in some tributaries based on models of tritium time series which indicate a significant subsurface flow component with an estimated mean residence time of 3 to nearly 12 years. Nitrogen isotope results suggest that soil nitrate and manure/sewage are the most important sources of nitrate in the Danube and that some tributaries have a clear manure/sewage signature. These various isotope applications demonstrate how isotopes can be used to obtain very useful and insightful quantitative and conceptual information about the Danube and can be applied in other river basins. The upcoming JDS-3 activity as well as other ongoing efforts in the basin would be a good way of collecting additional valuable isotope data. For example, the Vah tritium mixing analysis indicates that there is much more to be learned about tributary mixing in the Danube and that additional isotope studies of confluence areas could greatly clarify how extensive mixing zones are. Such information could be used to improve surface water monitoring strategies as well as better understand relative ecosystem impacts on the confluence and non-confluence sides of the river. Likewise, nitrogen isotope work, especially in tributaries, could be used to define the extent of manure/septic pollution in different river reaches and would help to better constrain nitrate sources to the Danube. Finally, continuing to build on existing Danube water isotope data sets using synoptic sampling approaches like those of the Joint Danube Survey will benefit future assessments of climate and land use change impacts in the basin.

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