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The Sava River

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Volume 31

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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; geo-ecology; 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

Preface

The Sava River is the major drainage basin of the Southeastern Europe and the greatest tributary to the Danube River. It is 945 km long, and with 97,713 km^2 large catchment area, it is extended over Slovenia, Croatia, Bosnia and Herzegovina and Serbia. The Sava River and its main tributaries have significant ecological and socioeconomic impact on the Danube River Basin. In Slovenia, the Sava is alpine river, which turns at the Slovenian-Croatian border into a typical lowland river.

The climate within the Sava watershed varies from alpine, pannonian to continental. Floods are typical for the springtime. A great part of the basin is covered by forest and agricultural areas. In the upper Sava region, hydroelectric power plants are located, while in the flat land area, the Sava is navigable for 593 km from Sisak to Belgrade. In the middle and lower Sava Basin, heavy industry, oil refineries and untreated municipal waste discharges cause environmental pollution. The human activities have significant influence on flow, morphology, climate changes and ecological status of the river, which affected the biodiversity.

To maintain sustainable development of the region, International Sava River Basin Commission was established in 2006. It successfully supports transboundary cooperation of the riparian countries.

The book on *The Sava River* gathered the available knowledge on the functioning of the Sava River Basin. It is based mainly on the previous investigations within the European Union (EU) FP6-funded project SARIB (2004–2007), the project of bilateral cooperation between Croatia and Serbia entitled “Assessing the scale of biocontamination of large rivers in Croatia and Serbia” (2011–2012) and other national research projects.

The book contains 17 chapters covering topics related to transboundary water cooperation within the Sava River Basin, climate change impact on flood hazards and climate change projections, evaluation of chemical dynamics and anthropogenic pollution sources, chemical pollution of sediments (metals, persistent organic pollutants), assessment of the metal bioavailability and accumulation of metals in fish tissues, determinations of surfactants in water and ecotoxicological characterization of the river. Microbiological status of the considerable stretch of the Sava

River is also evaluated. The biology part of the book deals with all quality elements related to aquatic ecosystems (algae, macrophytes, zooplankton, macroinvertebrates and fish), including the life of riparian ecosystems (amphibian, reptiles, birds and mammals). The assessment of the general state of biodiversity along the Sava River, conservation practice, status assessment based on biological quality elements as well as review of protected areas within the basin area are presented. Invasive aquatic species are also covered by the book, as the issues of growing concern.

Authors hope that the book content will attract the interest of environmental chemists, geologists, biologists, students, river basin managers and stakeholders and that it will be of interest to the general public, as well. The book on *The Sava River* provides also the overview of the most important stressors within the basin, which will serve as a database for the further research within the ongoing EU FP7-funded project GLOBAQUA. We would like to thank all authors of this book for their valuable contributions and the time and efforts devoted to create the book chapters. Finally, we would like to thank Prof. Damia Barcelo for his kind invitation to prepare *The Sava River* book.

Ljubljana, Slovenia Radmila Milačič
Ljubljana, Slovenia Janez Ščančar
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Contents

Transboundary Water Cooperation for Sustainable Development of the Sava River Basin

Dejan Komatina and Samo Grošelj

Abstract Transboundary water cooperation is an essential prerequisite to implement the basin approach and the principles of integrated water resources management, as a basis for efficient and sustainable development and management of water resources in international basins. Principles of transboundary water cooperation within river basins were laid down in the UNECE *Water Convention* and further promoted by recent processes, led by European Union (e.g., development of the *EU Strategy for the Danube Region*). In the Sava River Basin, the cooperation framework has been provided by the development of the *Framework Agreement on the Sava River Basin* (*FASRB*) and the establishment of the International Sava River Basin Commission, as a joint body with responsibility to coordinate the implementation. The *FASRB* has already shown to be a good framework for cooperation of the Parties (Bosnia and Herzegovina, Croatia, Serbia, and Slovenia) on integrated water resources management, by adding a considerable value to the national efforts. The cooperation process based on the *FASRB* implementation, which is presented in this chapter, is perceived as a process providing multiple benefits for the Parties and a good basis for further progress toward the key objective—sustainable development of the region within the Sava River Basin.

Keywords Framework Agreement on the Sava River Basin • International Sava River Basin Commission • Sava River Basin • Transboundary cooperation • Water resources management

List of Abbreviations

AL	Albania
BA	Bosnia and Herzegovina
EC	European Commission
EU	European Union
EUSDR	EU Strategy for the Danube Region
FASRB	Framework Agreement on the Sava River Basin

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FRM	Flood Risk Management
GIS	Geographical Information System
HR	Croatia
ICPDR	International Commission for the Protection of the Danube River
INSPIRE	Infrastructure for Spatial Information in the European Community
ISRBC	International Sava River Basin Commission
ME	Montenegro
NHMS	National Hydro-meteorological Service
RBM	River Basin Management
RIS	River Information Services
RS	Serbia
SI	Slovenia
UN	United Nations
UNECE	UN Economic Commission for Europe
WFD	EU Water Framework Directive

1 Introduction

Water is a key driver of economic and social development, while it also has a basic function in maintaining the integrity of the natural environment [1]. However, water does not stop at national borders—many river and lake basins, as well as aquifers, are shared between countries. There are 263 large transboundary river basins around the world, 69 of which are located in Europe, covering 54 % of the European continental area [2]. Given these facts, transboundary water cooperation is an essential prerequisite to implement the basin approach and the principles of integrated water resources management, which have been accepted internationally as a good basis for efficient and sustainable development and management of water resources, coping with conflicting demands, reducing poverty, protecting natural resources, and preventing crises and conflicts worldwide [1]. Joint management of transboundary water resources can also encourage deepened cooperation among riparians that goes beyond the water sector.

For the first time, principles of transboundary water cooperation within river basins were laid down in the UNECE *Water Convention* [3]. So far, a great number of international basin organizations have been established to manage water resources in transboundary basins. Generally, they can be divided into two groups [4]: implementation-oriented basin organizations, responsible for development, implementation, and maintenance of joint projects, often having a development focus and going beyond pure water resources management, and coordination-oriented basin organizations, in charge of coordinating water resources management tasks that are developed and implemented on national level but coordinated and harmonized on transboundary level.

In Europe, given the nature and scope of the conventions dealing with transboundary basins such as the Danube, Rhine, Elbe, and others, the respective basin organizations are focused, either on sustainability issues (i.e., protection of the rivers) or on development activities (i.e., navigation or tourism). However, recent processes, led by European Union, namely, the *EU 2020 Strategy* [5] and the *EU Strategy for the Danube Region* [6], provided new frameworks that tend to integrate sustainability and development.

In comparison with other European river basins, however, the situation in the Sava River Basin was peculiar. The decay of the former Yugoslavia in the 1990s challenged the water resources management in the basin substantially, by turning the Sava River from the largest national river of the former country into an international river and causing fragmentation of its basic elements (e.g., data exchange, monitoring, and early warning systems) to the national level, unlike the integrated approach, emerging in Europe at the same time [7]. The region also experienced a sharp decrease of water-related economic activities, such as navigation, unlike the trends in other parts of Europe [8]. Therefore, a new international framework became necessary in order to ensure a sustainable use, protection, and management of water resources in the Sava River Basin and thus enable better life conditions and raising the standard of population in the region.

After a process of negotiations, the new cooperation framework has finally been provided by the development of the *Framework Agreement on the Sava River Basin* (*FASRB*) [9] and the establishment of the International Sava River Basin Commission (ISRBC), as an organization with the responsibility to coordinate the implementation of the *FASRB*. The overall objective of the *FASRB* is to ensure transboundary water cooperation, in order to provide conditions for sustainable development of the region within the basin. The cooperation process and its mechanisms, key features, and visions are presented in this chapter, while useful additional information can be found in other sources [10–12].

2 Background of Cooperation

The background of the transboundary water cooperation in the Sava River Basin is associated with the following two major challenges:

– The need, and a legal obligation, for environmental protection of the basin and, at the same time, a strong need for economic development of the countries in the region.
– The need for a new international framework for management of water resources on the basin level, following the geopolitical changes in the region in the 1990s,

which are elaborated in the following text.

Fig. 1 Location of the Sava River Basin [13]

2.1 *General Characteristics of the Basin*

The Sava River Basin is a major drainage basin of Southeastern Europe and one of the most significant subbasins of the Danube River Basin (Fig. 1) [14]. Its *total area* equals 97,713 km^2, which represents 12 % of the total Danube Basin area. The Sava basin is *shared among five countries*, with its negligible part also extends to the sixth country—Albania (Table 1)—and hosts the *population* of approximately nine million.

The basin is characterized by a diverse landscape. The *elevation* varies between approx. 71 m above sea level (m a.s.l.) at the mouth of the Sava River in Belgrade (Serbia) and 2,864 m a.s.l. (Triglav, Alps, Slovenia), while the mean elevation of the basin is approx. 545 m a.s.l. Regarding the *land cover/land use*, a major part of the basin is covered by forest and seminatural areas (54.7 %) and agricultural surfaces (42.4 %), while the share of artificial surfaces is 2.2 % [14].

The moderate *climate* of the northern hemisphere prevails in the basin. The average annual *air temperature* for the whole basin is 9.5 °C. Mean monthly

Table 1 Share of the countries belonging to the Sava River Basin [14]

	SI	HR	BA	RS	ME	AL
Total country area (km^2)	20,273	56,542	51,129	88,361	13,812	27,398
Share of national territory in the basin (%)	52.8	45.2	75.8	17.4	49.6	0.6
Area of the country in the basin (km^2)	11,734.8	25,373.5	38,349.1	15,147.0	6,929.8	179.0
Share of the basin (%)	12.0	26.0	39.2	15.5	7.1	0.2

Notation: SI, Slovenia; HR, Croatia; BA, Bosnia and Herzegovina; RS, Serbia; ME, Montenegro; AL, Albania

temperature in January falls to about −1.5 °C, while in July it can reach almost 20 °C.

The amount and the annual distribution of *precipitation* are very variable within the basin, with the basin average of about 1,100 mm/year. The average *unit-area-runoff* is equal to 18 l/s/km^2, which corresponds to *effective rainfall* and *evapotranspiration* for the whole basin of about 570 and 530 mm/year, respectively [14].

The Sava River is formed by two mountainous streams—Sava Dolinka and Sava Bohinjka. From their confluence at Radovljica (Slovenia) to its mouth to the Danube, the Sava River is 945 km long, thus being the third longest tributary of the Danube. Together with its longer headwater, the Sava Dolinka river (Fig. 2 left), it measures 990 km. Sava River represents the richest-in-water Danube tributary. Having the long-term *average discharge* at the mouth of about 1,700 m^3/s, it contributes almost 25 % to Danube's total discharge (Fig. 2 right). The longitudinal presentation of annual discharges along the Sava River is given in Fig. 3.

The basin hosts the largest complex of alluvial wetlands in the Danube Basin and large lowland forest complexes. It is unique for some of the floodplains being still intact, thus supporting biodiversity and flood alleviation (Fig. 4). For illustration, the drop of the 100-year high flow, shown in Fig. 3, is associated with hydraulic effects of Lonjsko Polje, the largest retention area along the river. There are 167 protected areas in total, including six Ramsar sites, eight national parks, as well as numerous important bird and plant areas, protected areas at the national level, and Natura 2000 sites [14].

The total annual *water use* in the basin is estimated at about 4.8 billion m^3. The overview of various types of the consumptive water uses is shown in Fig. 5, while the nonconsumptive uses include transportation (594 km of the Sava River is the waterway), hydropower use, recreation, and fishing.

2.2 *The Need for a Balanced Approach to Development*

The Sava River Basin is widely known for its high environmental and socioeconomic values [10]. These values are reflected in natural beauties all over the basin, in an outstanding biological and landscape diversity (Figs. 2 and 6), in large

Fig. 2 Source and mouth of the Sava River. *Left photo*: "The source of the Sava river below Planica," Author: Boško Tintor. *Right photo*: "Mouth," Author: Vlada Marinković. Credit: ISRBC

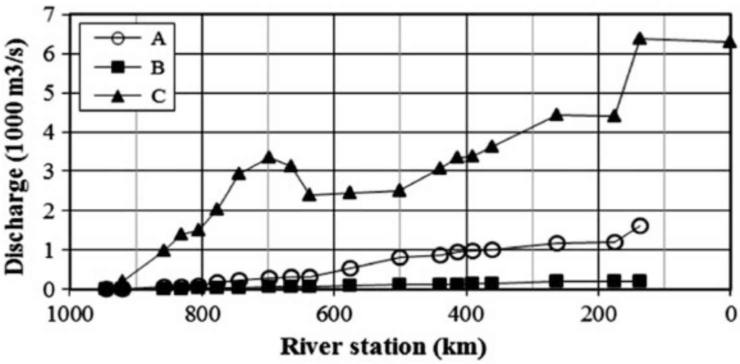

Fig. 3 Annual discharges along the Sava River [14]. *Notes*: A, mean values; B, 100-year return period low flows; C, 100-year return period high flows; the river station is measured in the upstream direction (the zero station corresponds to the river mouth)

Fig. 4 Lonjsko Polje—a nature park and retention area. *Left photo*: "Lonjsko Polje—Mužilovčica." *Right photo*: "Lonjsko Polje—flood." Author: Boris Krstinić. Credit: ISRBC

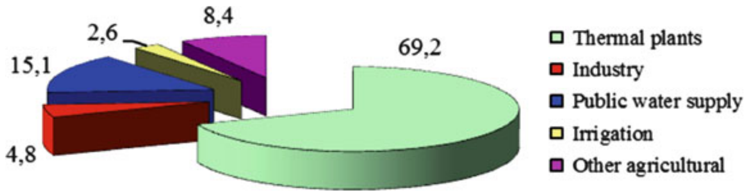

Fig. 5 Estimation of total water use in the Sava River Basin (in %) [14]

Fig. 6 Tributaries of the Sava River, Una and Drina. *Left photo*: "Una—Štrbački buk." *Right photo*: "Drina river at Loznica." Author: Miroslav Jeremić. Credit: ISRBC

retention areas along the river (Fig. 4), and in a high potential for the development of water-related economic activities, such as the waterway transport of cargo and passengers (Fig. 7), hydropower use (Fig. 8), and tourism and recreation (Fig. 9), as well as other activities related to the use of water.

A general challenge for the water cooperation in the Sava River Basin originates from two opposing needs, experienced by all countries in the basin. Although the basin is characterized by a relatively low degree of human intervention, there is a need, on one hand, to preserve the outstanding environmental values, existing in the basin. This preservation has also become a legal obligation, given the existing regulation on European and national level of the countries in the basin. On the other hand, there is a strong need for increased use of the potential for economic development, associated with water resources in the basin, which in turn may cause environmental impacts.

Therefore, a balanced approach is needed to use the potential and preserve the values simultaneously and thus provide a basis for sustainable development of the region. Managing water resources on the basin level is an important prerequisite to apply such approach in an efficient manner.

Fig. 7 Navigation on the Sava River. Author of the *left photo*: Branimir Butković. Author of the *right photo*: Jelena Marčetić. Credit: ISRBC

Fig. 8 Hydropower use. *Left photo*: "Under the weir," Author: Jelena Mihajlovska. *Right photo*: "Piva lake," Author: Miroslav Jeremić. Credit: ISRBC

Fig. 9 Recreational tourism. Credit: ISRBC

2.3 The Need for an International Framework for Cooperation

The establishment of the new countries has significantly influenced the water resources management in the Sava River Basin [10]. Beforehand, the institutional framework for implementation of water policy was based on the national regulations, plans, and programs, developed on the basin level. The geopolitical changes seriously affected the existence and functionality of the basic elements of water management (e.g., data exchange, monitoring, and early warning systems), thus confining the water management to national level of the newly created countries, unlike the tendency to promote the integrated river basin management approach emerging in Europe at the same time (EU *WFD*) [7].

The changes have also caused a sharp decrease of economic activities in the basin, including navigation, unlike the trends in other parts of Europe, where the inland waterway transport has proven to be a competitive transport mode, being environmentally friendly and capable of reducing congestion on densely used roads [8]. Since then, the Sava River has been hardly used for transport, for a number of reasons, including a lack of infrastructure maintenance and investments and its consequences [12].

For these reasons, a new international framework became necessary to ensure sustainable use, protection, and management of water resources in the Sava River Basin and thus enable better life conditions and raise living standard in the region [10, 11].

3 Legal and Institutional Framework for Cooperation

The establishment of the Stability Pact for South Eastern Europe provided a solid basis for active cooperation of all stakeholders in the region and paved a way toward creation of a new approach to water resources management in the Sava River Basin. The four countries of the basin—Bosnia and Herzegovina, Federal Republic of Yugoslavia (later on Serbia and Montenegro, and then Republic of Serbia), Republic of Croatia, and Republic of Slovenia—entered into a process of negotiations, with the primary aim to establish an appropriate framework for transboundary cooperation in the water sector and thus foster sustainable development of the region [10, 11].

The process begun by considering the rehabilitation and development of navigation on the Sava River as a potential area of cooperation. However, in accordance with different priorities of the countries, other issues, such as flood protection, maintenance of water quality and quantity, tourism development, energy production, etc., were brought in, as well.

The process successfully ended by developing the *Framework Agreement on the Sava River Basin* (*FASRB*), a unique international agreement integrating all aspects

Table 2 Establishment of the cooperation—important dates

Activity/event	Date
Launch of the Sava River Initiative	June 2001
Signing the Letter of Intent on cooperation within the Sava River Basin	November 29, 2001
Signing of the *FASRB*	December 3, 2002 (Kranjska Gora, Slovenia)
Establishment of the Interim Sava Commission	March 12, 2003 (Brussels, Belgium)
Entry into force of the *FASRB*	December 29, 2004
Establishment of the ISRBC	June 25–27, 2005 (Zagreb, Croatia)
Start of work of the ISRBC secretariat	January 9, 2006 (Zagreb, Croatia)

of water resources management, and by establishing the International Sava River Basin Commission (ISRBC), as an organization responsible for implementation of the *FASRB* (Table 2).

3.1 Framework Agreement on the Sava River Basin

The *Framework Agreement on the Sava River Basin* [9] is an international agreement integrating all aspects of water resources management, e.g., the different kinds of water use, the water and aquatic ecosystem protection, as well as the protection against harmful effects of water due to extreme hydrologic events and accidents involving water pollution. It is the first development-oriented multilateral agreement concluded in the region after the *Dayton Peace Agreement* and the *Agreement on Succession Issues* [10].

The strategic objective of the *FASRB* is to ensure transboundary cooperation in the water sector, in order to provide conditions for sustainable development of the region within the Sava River Basin [10, 11]. The particular objectives of the *FASRB* include the establishment of:

- International regime of navigation on the Sava River and its navigable tributaries;
- Sustainable water management in the basin, and
- Sustainable management of hazards in the basin (i.e., floods, droughts, ice, accidents involving the water pollution),

thus addressing aspects of both sustainability and development. The *FASRB* is sufficiently broad to provide also a good framework for cooperation on other development issues, such as the river tourism or other water-related economic activities (e.g., hydropower use), i.e., for the integrated water resources management.

The basic principles of the *FASRB* are [10]:

– Cooperation based on sovereign equality, territorial integrity, mutual benefit, and good faith in order to achieve the goals of the *FASRB* as well as based on regular exchange of information within the basin, cooperation with international organizations, and being in accordance with the *WFD* and other EU directives and UNECE conventions
– Reasonable and equitable use of the water resources, applying measures aimed at securing the integrity of the water regime in the basin and reduction of transboundary impacts caused by economic and other activities of the Parties, and respecting the "no harm rule."

The *FASRB* implementation is being undertaken by the national institutions, officially nominated by the Parties (e.g. ministries responsible for water management, environment and transport), and is coordinated by the ISRBC.

3.2 International Sava River Basin Commission

The International Sava River Basin Commission (ISRBC) is a joint body, established as an organization with the international legal capacity necessary for exercising its functions. Thanks to its integrated nature, the *FASRB* provides the ISRBC with the broadest scope of work among European international basin organizations (i.e., river/lake commissions), making it responsible for coordination of the following activities [10, 11]:

– Preparation and implementation of *joint plans* for the basin (e.g., river basin management plan, flood risk management plan)
– Preparation of *development programs*, e.g., for rehabilitation and development of navigation in the basin
– Establishment of *integrated systems* for the basin, such as geographical information system (GIS), river information services (RIS), data exchange, monitoring, forecasting and early warning systems, etc.
– Harmonization of national regulation with the EU regulation
– Development of protocols for regulating specific aspects of the *FASRB* implementation

In accordance with its mandate and responsibilities, the ISRBC is a central point in identification and implementation of projects of regional importance, aiming to strengthen the cooperation of the Parties and facilitate the fulfillment of the *FASRB* objectives. The ISRBC has the capacity for making decisions (that are obligatory for the Parties) in the field of navigation and providing recommendations on all other issues, i.e., in water protection and hazard management (Fig. 10). It also provides recommendations to the Meeting of the Parties, a ministerial-level body which makes decisions related to strategic issues of the *FASRB* implementation and performs a general monitoring of the implementation process [10].

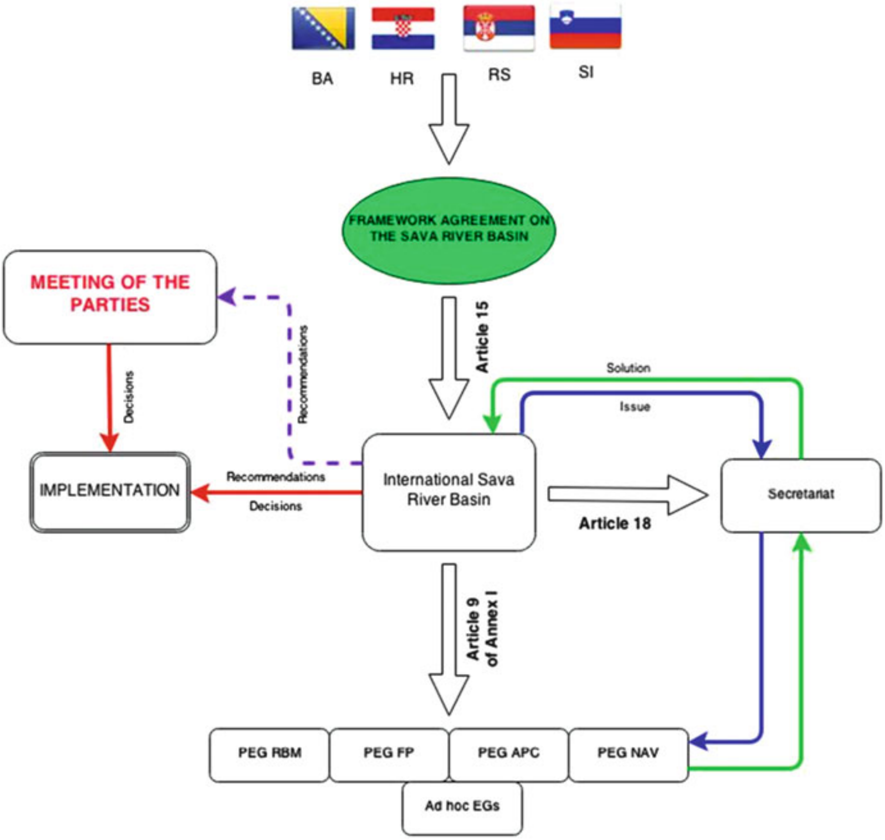

Fig. 10 Decision-making process in the implementation of the *FASRB* [15]

In order to foster cooperation and ensure synergy in achieving its goals, the ISRBC has established permanent and ad hoc expert groups, composed of delegated experts from each Party (e.g., from water and environment agencies, national hydro-meteorological services, port master offices). The key issues in the basin—river basin management, accident prevention and control, flood prevention, and navigation—are considered by the permanent expert groups, while the specific issues, GIS, RIS, legal, financial, or hydro-meteorological issues, are a responsibility of the ad hoc expert groups [10]. The secretariat is an administrative and executive body of the ISRBC. Also, the expert groups are chaired by the officials of the secretariat.

4 Approach to Cooperation

The achievement of the principal objectives of the *FASRB*, taking its broad scope into account, requires an integrated and sustainable approach to balancing the needs for development of economic activities (i.e., navigation, tourism) and the needs of other water management subsectors (i.e., other water uses, protection against detrimental effects of water, and protection of water and aquatic ecosystem). The main features of the approach, as applied by the ISRBC, are illustrated below.

4.1 The Scope of Cooperation

4.1.1 River Basin Management

The EU *WFD* [7] establishes a legal framework to protect and enhance the status of all waters and protected areas including water-dependent ecosystems, prevent their deterioration, and ensure long-term, sustainable use of water resources. The Parties to the *FASRB* are committed to respecting the *WFD*, although some of them, i.e., the non-EU member states, are not legally bound to do so. Accordingly, the preparation of the *Sava River Basin Management Plan* (*Sava RBM Plan*), in line with the *WFD* and under the coordinating role of the ISRBC, represents the most important task in reaching one of the ultimate goals of the *FASRB*—the establishment of sustainable water management in the Sava River Basin.

An important first step in this regard was the development of the *Sava River Basin Analysis Report* [14], a comprehensive document dealing with both water quality and quantity issues and hydrology and hydromorphology of the basin and providing the first overview and thematic GIS maps of the basin [13]. The process, which continued with the support of the European Commission, resulted in the first *Sava RBM Plan* [16], which is currently undergoing national procedures prior to the adoption by the Parties. The *Plan* provided a thorough basin-wide analysis of the present water status and an overview of measures to be implemented on the basin scale in order to achieve the agreed environmental objectives. The *Plan* also established several integrative principles for water management, including the integration of water protection into other water management subsectors. Thus, the issues of flood protection, navigation, hydropower use, agriculture, and climate change were elaborated in the *Sava RBM Plan*.

Results of the regional climate modeling suggest an overall reduction of around 15–30 % in mean annual runoff in the Sava River Basin by the middle of this century, which could be challenging for all investments made in the basin. Taking this into account, the development of *Water and Climate Adaptation Plan for the Sava River Basin* has been undertaken. Through the elaboration of alternatives for adaptive management actions in water management subsectors such as navigation, hydropower, agricultural water use, flood protection, and environmental protection, the *Adaptation Plan* aims to fill the knowledge gap on the climate change impact on water management in the basin and show how to increase the climate resilience of critical water management infrastructure investments and of integrated water resources management in the basin [10].

In addition to these activities, the *Protocol on Sediment Management to the FASRB* has been prepared, stipulating the preparation of a sediment management plan for the basin in accordance with the *Sava RBM Plan* [10]. The *Protocol* is undergoing a process of harmonization prior to the signing by the Parties.

4.1.2 Flood Management

In accordance with the *Flood Action Programme for the Danube River Basin* of the ICPDR, the *Flood Action Plan for the Sava River Basin* [17] has been prepared, providing the first program of measures for each Party to achieve the defined targets for flood management in its part of the Sava basin until 2015 [10, 11].

The legal basis for cooperation of the Parties in line with the EU *Flood Directive*, including the preparation of the *Flood Risk Management Plan for the Sava River Basin* (*Sava FRM Plan*), is provided by the *Protocol on Flood Protection to the FASRB* [18], which is currently under ratification. Preparatory activities toward the *Sava FRM Plan*, performed so far, include an analysis of the present status of flood management (including current practices and the existing flood protection facilities in the Parties), preparation of a GIS-based, indicative flood extent map for the whole Sava River (Fig. 11), development of a preliminary hydrological model of the Sava River Basin and the hydraulic model of the Sava River, as well as efforts to make a link between the flood risk management planning and the climate change assessment in the basin [10].

4.1.3 Accident Prevention and Control

In order to provide conditions for efficient accident prevention and control in the Sava River Basin, participation in testing of the existing Accident Emergency Warning System of the ICPDR is continuously being done. The efforts are focused to the improvement of work of the Principal International Alert Centers in the Parties to the *FASRB*, including the organization of training courses for the operational staff of the Alert Centers, in cooperation with the ICPDR. The *Protocol on Emergency Situations to the FASRB* has been drafted and entered the process of harmonization by the Parties. The *Protocol* aims to enhance prevention, preparedness, response, and mutual assistance of the Parties in case of emergency situations. In the future, a water contingency management plan for the basin is planned to be developed and implemented [10, 11].

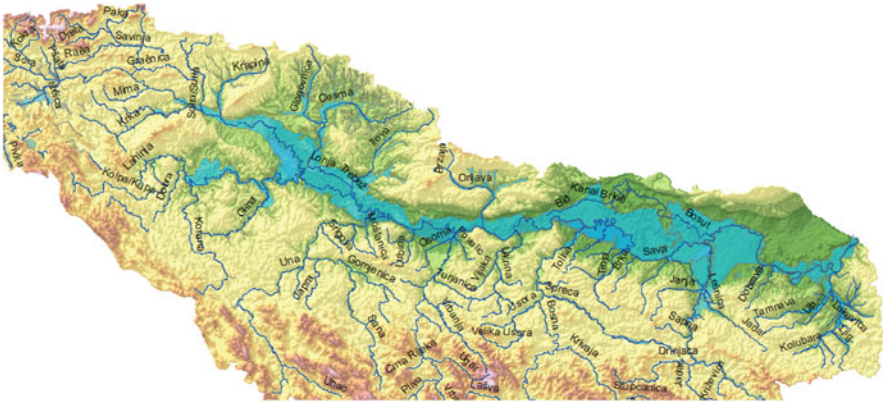

Fig. 11 Indicative map of important flood-prone areas along the Sava River [14]

4.1.4 Information Exchange and Management

With the aim to establish a broad platform for *information management*, the *Sava GIS Strategy* [19] has been developed, in accordance with the EU *INSPIRE Directive* and the Water Information System for Europe. Subsequently, the implementing documents for the establishment of Sava GIS have been prepared, and the initial phase is being implemented [10, 11].

In cooperation with the relevant national institutions of the Parties (ministries for environment and/or water management, water agencies, and the national hydrometeorological services—NHMSs), a platform for the *exchange and use of the hydrological and meteorological information* in the Sava River Basin has been established. Within the platform, values of the agreed hydrological and meteorological parameters, measured at relevant gauging stations in the basin and provided by the NHMSs, are collected and presented, either in the form of annual summary of the water regime in the basin, as a *Hydrological Yearbook*, which is published annually [20], or through a data exchange system, which automatically uploads the values from the national databases and displays them continuously on the ISRBC website [21, 22].

4.1.5 Rehabilitation and Development of Navigation

The collapse of the former Yugoslavia influenced economic activities in the region, as well as the waterway and port infrastructure, significantly. Consequently, cargo traffic dropped from around ten million tons in 1982 to less than one million tons in middle 1990s [10, 12]. Since then, the Sava River has been hardly used for transport, primarily due to lack of maintenance and investments, which resulted in a poor quality of the infrastructure, low level of navigation safety due to unexploded ordnances, and poor intermodal road and railway connections.

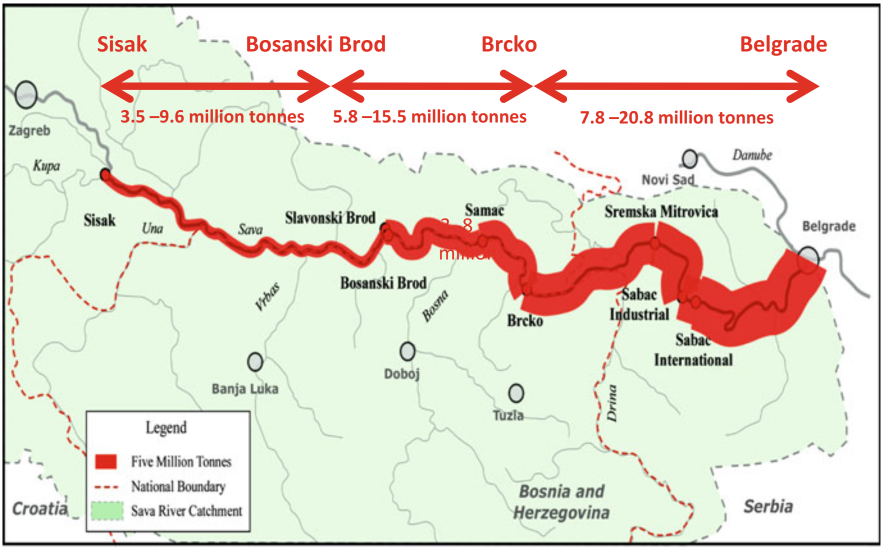

Fig. 12 Estimated margins of traffic volume on the Sava River for year 2027 [24]

Navigation conditions have been unfavorable due to a limited draft during long periods, a limited width of the fairway, and a limited height for passages under some bridges, as well as insufficient marking [10, 12].

Since the beginning of implementation of the *FASRB*, as well as the *Protocol on Navigation Regime to the FASRB* [23], the ISRBC and the Parties have invested considerable efforts to provide conditions for making the Sava River an important, environment-friendly, and navigation-safe lifeline for inland transport, focusing particularly on [10, 12]: (a) planning for rehabilitation and development of the Sava River waterway infrastructure and (b) improvement of technical standards and safety of navigation aiming to prevent the environmental risks associated with navigation. The waterway planning, based on the estimated transport demand (Fig. 12), is in the final phase. The waterway marking system has been fully restored after 20 years, the unexploded ordnances removed from the river banks, and the initial phase of establishment of the Sava RIS done, in accordance with the EU *RIS Directive*. A set of rules and other documents related to technical issues and safety of navigation, harmonized with the corresponding EU and UNECE regulations, have been developed [25, 26]. The *Protocol on Prevention of Water Pollution Caused by Navigation to the FASRB* [27] has been developed and signed and is currently undergoing ratification.

In order to ensure environmental sustainability of the navigation rehabilitation and development, the ISRBC is actively involved in the relevant processes on Danube and European levels, i.e., the implementation of *Joint Statement on Guiding Principles for the Development of Inland Navigation and Environmental Protection in the Danube River Basin* [28] and *Manual on Good Practices in Sustainable Waterway Planning* [29], where the issue of navigation development is continuously discussed by a variety of stakeholders from navigation and environmental sectors [10, 12].

4.1.6 Development of River Tourism

In line with the key objective of the *FASRB*, i.e., sustainable development of the region (through transboundary cooperation), the ISRBC has been mandated by the Parties to coordinate activities on the development of sustainable river tourism in the Sava River Basin.

As an initial activity on the development of *nautical tourism*, the first ever *Nautical and Tourist Guide for the Sava River* [30] has been prepared and published in cooperation with the Forum of Chambers of Commerce from the Parties [10–12]. This publication is expected to serve as a basis for further steps, such as development of a master plan and of the infrastructure for nautical tourism in the Sava River Basin.

A multi-stakeholder process has been initiated to foster development of *ecotourism* in the basin. The process resulted in the *Transboundary Ecotourism Guidelines for the Sava River Basin* [31], as a first step toward a strategy of ecotourism development in the basin and the implementation of concrete, "quick-win" projects in the field.

The starting activities on the development of *recreational tourism* have focused on the establishment of bicycle lanes along the Sava river, given a high interest for such development, expressed by cities and local communities along the river. These activities have been undertaken in close coordination with biking associations as direct end users, in addition to a broad range of other stakeholders. An international cycling tour from the Sava source to its mouth, which was organized in 2013 within the celebration of the Sava River Day, contributed to the promotion of this project.

To demonstrate a high potential for the development of *culturally and/or socially conscious tourism* in the basin, a number of activities promoting local tradition and culture, traditional food and drinks, as well as handicraft articles have been performed, mostly within the celebrations of the Sava River Day.

A common feature of all the activities on tourism development is a combination of the "top-down" and "bottom-up" approaches by involving, from the very beginning of the process, a variety of stakeholders from governmental, civil, business, and academic sectors, as well as local communities.

4.1.7 Promotion of Cooperation

Considerable attention of the ISRBC is paid to the issues of cooperation, public participation, and stakeholder involvement.

Cooperation with a number of international organizations and institutions has been established and maintained by the ISRBC, including the UN organizations, European Commission, river commissions, governments, financial institutions, and other organizations. In order to enable close cooperation and coordination of activities with those performed on the Danube level, memorandums of understanding on cooperation have been signed with the ICPDR and Danube Commission [10, 11], providing a basis for periodic consultations on all issues of cooperation, exchange of data and information on regular basis, and undertaking of projects of mutual interest. There is also a permanent cooperation with the national institutions responsible for the *FASRB* implementation as well as with other institutions in the Parties, such as agencies, offices, services, institutes, and universities.

In order to ensure *public participation and stakeholder involvement* in the *FASRB* implementation, cooperation with nongovernmental organizations and other institutions and local actors from the Sava River Basin has been established, and a network of observers to the ISRBC has been created. The following tools are used by the ISRBC for information provision and consultation of stakeholders and/or wide public [10, 11]: the official website; the Sava NewsFlash bulletin; publications and promotion material; celebration of the Sava River Day (June 1); press releases and media briefings; consultation workshops; public presentations and other meetings with stakeholders; organized by the ISRBC; or events; projects; publications; and the websites of other organizations/institutions that the ISRBC attended or contributed to.

4.2 Key Features of the Approach

4.2.1 Cohesiveness

Generally, the *FASRB* has proven to be a good platform for *intensified contacts* and an *improved cooperation* among the Parties, providing opportunities for *exchange of experiences* and an *additional training* of a broad range of experts from the Parties.

Majority of the activities within the cooperation process are performed through *implementation of joint, basin-wide projects*. The projects are agreed upon by all Parties [15, 32] and are in full conformity with the EU directives (*WFD, Flood Directive, INSPIRE, RIS*) and strategies [5, 6], which all largely contributes to a successful fund-raising. As a result, 87 % of the funds for the projects, implemented or commenced so far, were provided from external financial sources, while the remaining 13 % are the means of the Parties. As for the planned priority projects, given their relevance for the implementation of the *EU Strategy for the Danube Region*, it is likely that the percentage of the externally funded projects will further increase in the future.

The approach is oriented toward *harmonization of the national regulation with the EU regulation* and *harmonization of methodologies and procedures* (e.g., monitoring system of water quality, hydrological and meteorological data exchange system), and it also provides for an *enhanced cross-sectoral cooperation* on national level, especially among the competent authorities within a Party.

4.2.2 Integrated Nature

The approach is entirely based on the principles of integrated water resources management. It does consider the *whole river basin*, being focused on the preparation of plans (i.e., for river basin management, flood risk management, sediment management, climate change adaptation) and the establishment of integrated systems (i.e., data exchange, monitoring, forecasting, early warning systems) for the whole basin.

From the perspective of the *scope of work*, both the environmental protection, the protection against the water-related hazards, and the issues of development (e.g., navigation, tourism) are addressed simultaneously, thus ensuring the water resources management in an integrated manner.

Permanent efforts are made to integrate *all societal sectors* (governmental, nongovernmental, business, academic) into the mechanisms of implementation of the *FASRB*. The process of preparation of the *Sava RBM Plan* represents a good example of involving different stakeholders through their participation at workshops and consultation throughout the preparation process.

There is a tendency to combine, whenever possible, the "*top-down*" and "*bottom-up*" approaches, using the principle that governmental sector provides initial ideas and directions on a subject, while solutions are sought through multi-stakeholder processes, led on transboundary level. For example, most of the ISRBC activities in the field of river tourism development are based on this approach, as described in Sect. 4.1.6.

4.2.3 Alignment with the UNECE and EU Regulations and Strategies

The approach is fully aligned with the UNECE conventions and EU directives (*WFD*, *Birds*, *Habitat*, *Floods*, *INSPIRE*, *RIS*) and strategies [5, 6]. It is also considered as relevant to the processes on a wider (Danube and EU) scale, such as those associated with the *EU Strategy for the Danube Region* (*EUSDR*) and the *EU 2020 Strategy*, for several reasons [10, 11]:

- The overall objective of the *EUSDR* and *FASRB* is identical—sustainable development of the region they refer to.
- There is an obvious conformity of the ISRBC approach and its priority projects with the *EUSDR* priorities, and a high potential for synergy, as the implementation of the ISRBC projects within the *EUSDR* framework can contribute to implementation of both *EUSDR* and *FASRB*.
- The subregional level, such as the Sava River Basin level, is likely to be the most effective level from the viewpoint of the *EUSDR* implementation.
- A majority of the activities within the cooperation process, led by the ISRBC, fully match the three main priorities of the *EU 2020 Strategy*, i.e., sustainable, smart, and inclusive growth.

4.2.4 Complementarity with the Processes on the Danube Basin Level

As mentioned before, the process of the *FASRB* implementation is completely in line with the ongoing processes at the Danube Basin level. However, the issues are considered at a more detailed scale, and the results of a finer resolution are provided, thus being complementary to those obtained on the Danube level. For illustration, the *Danube RBM Plan* [33] dealt with the rivers with catchment areas larger than 4,000 km^2, while the *Sava RBM Plan* considered the rivers with catchment size greater than 1,000 km^2. Nevertheless, the *Sava RBM Plan* has been prepared in accordance with the *Danube RBM Plan* in terms of the significant water management issues, the environmental objectives, and the program of measures.

4.2.5 Pragmatism and Practicality

The process of the *FASRB* implementation under the coordinating role of the ISRBC is oriented to provision of concrete "products" to the Parties, such as strategic plans (e.g., for river basin management, flood risk management), integrated systems (data exchange, monitoring, forecasting, early warning), infrastructure for the development of economic activities (navigation, river tourism), or harmonized regulation (e.g., rules on navigation and minimum requirements for vessels and boat masters). In addition to coordination of activities of the Parties, which is a primary role of the ISRBC, concrete projects are also implemented, thus providing tangible benefits for the countries.

4.2.6 Educative Character

The approach provides different forms of "*nonformal*" and *"informal" education*. The nonformal education is mainly based on *capacity building*. The permanent capacity building is focused on the implementation of EU directives (*WFD*, *Floods*, *INSPIRE*, *RIS*) and UNECE regulations, thus mostly targeting experts from national institutions. The ad hoc capacity building includes trainings, courses, and workshops (e.g., dealing with new methodologies, procedures, and models) and targets various groups of stakeholders. The informal education is based on *raising the awareness* of the wide public, or some of its groups, on the outstanding values and potential of the basin; the need for using the potential while preserving the existing values; and the importance of regional water cooperation and its benefits. It is performed through a variety of mechanisms, described in Sect. 4.1.7.

4.3 Challenges and Obstacles

A number of challenges and obstacles have been identified in the process of the *FASRB* implementation. Most of them are, generally, associated with [10, 11]:

– Differences between the countries, in terms of EU membership status, eligibility for approaching funds, level of economic development, organizational structure in decision-making process, and environmental awareness of the public
– Securing funds for implementation of priority projects, preparation of strategic studies, and establishment of integrated systems for the basin
– Lack of human and financial resources of the Parties, necessary for realization of the activities agreed on the basin level
– Lack of appropriate institutional arrangements, including the coordination of, and the information exchange among, the national institutions responsible for the *FASRB* implementation
– Lack of harmonization of legislation with the EU *acquis*
– Lack of capacity for a proper implementation of legislation or for an adequate scientific research to support achievement of the *FASRB*-related goals
– Possibilities for improvement of bilateral cooperation, where the ISRBC is perceived as a possible mediator
– Limited access to basic data (i.e., topographic, hydrologic, etc.) needed for preparation of studies of common interest under the umbrella of the ISRBC, especially when the data are owned by the national institutions not officially nominated as responsible for implementation of the *FASRB*
– Different perceptions of requirements in the field of water protection and hazard management by competent authorities of the Parties, where the requirements toward the Parties are based on recommendations and conclusions of the ISRBC (unlike the ISRBC decisions in the field of navigation, which have a binding character for the Parties)
– Resolving conflicts of interests of different users of water on both transboundary and national levels, especially as these conflicts are likely to increase in future due to climate change

Some challenges are associated with specific fields of the *FASRB* implementation [10]. For example, on national level, the inland navigation is generally underestimated in comparison with other modes of transport, although being the most efficient and environmentally friendly mode of transport.

4.4 Vision of the Future Cooperation

A wide range of activities have been undertaken or launched since the beginning of the *FASRB* implementation. In order to respond to a steady progress in the *FASRB* implementation during the last years, as well as to recent processes and initiatives on the Danube level [28, 33, 34] and European level [5, 6], relevant for the *FASRB* implementation, an updated *Strategy on Implementation of the FASRB* [15] and the accompanying *Action Plan for the Period 2011–2015* [32] have been developed to govern the future implementation.

According to the *Strategy*, the future efforts should be oriented to:

- Preparation/upgrade of strategic plans, such as the RBM plan and FRM plan for the Sava River Basin and implementation of measures to achieve the objectives agreed upon by the Parties
- Further development of integrated systems which provide platforms for the exchange of information, forecasting, and early warning on the occurrence of extreme events (floods, droughts) and accidents involving the pollution of water in the basin, as well as the harmonization of national methodologies related to these issues
- Efficient completion of the planning process for the Sava River waterway and, subsequently, launching the works on the waterway rehabilitation, in order to establish, as soon as possible, the navigation on the Sava River in accordance with the safety, technical, and environmental standards of the EU
- Fostering development of different forms of sustainable river tourism, through preparation of strategic documents and implementation of projects on the ground
- Considering other development activities in the basin (e.g., hydropower use, water supply, agriculture) and taking care of their environmental sustainability and possible impacts of climate change on these activities
- Further strengthening of stakeholder involvement into all relevant processes within the *FASRB* implementation
- Further investigation of possibilities to introduce the legally binding character of the ISRBC decisions to certain fields of water management

To this end, further progress should primarily be made in relation to [10]:

- Providing adequate financial instruments for realization of the respective activities and projects, especially those to be performed under the umbrella of the ISRBC
- Exchange of information within the basin (e.g., hydrological and meteorological data)
- Harmonization of national methodologies (e.g., related to collection of hydrometeorological data, hydrological and hydraulic analyses, flood risk and damage assessment, etc.)
- Development of data policies securing an access to basic data (topographic, hydrologic, etc.) needed for preparation of specific studies of common interest under the coordination of the ISRBC, particularly with regard to the data owned by national institutions not officially nominated as responsible for implementation of the *FASRB*
- Development of the legal background and institutional arrangements in the Parties
- Capacity building in the fields of work, related to the *FASRB*

- Involvement of stakeholders in the *FASRB* implementation and, especially, broadening the multi-stakeholder platform to further improve the involvement of the nongovernmental, academic, and business sectors
- Raising the awareness of stakeholders on benefits and importance of the existing cooperation on the *FASRB* implementation, with a special emphasis on the institutions responsible for the implementation and other national institutions

5 Conclusion

Generally, the *FASRB* has proven to be a good framework for cooperation of the Parties on integrated water resources management, by adding a considerable value to the national efforts in the water sector, including:

- Intensified contacts and improved cooperation among the Parties
- Implementation of joint, basin-wide projects
- Harmonization of regulation, methodologies, and procedures
- Integration of sustainability and development as well as integration across the river basin and the sectors of society
- Orientation toward provision of concrete products to the Parties, as well as education
- Conformity with the processes and frameworks for cooperation on a wider level (Danube, EU, UNECE), as well as complementarity with the cooperation processes on the Danube level

It seems that the "Sava model of cooperation" is perceived in other regions (i.e., rest of the Southeastern Europe, Mediterranean region, Western Europe, Central Asia) as an attractive example of a platform for transboundary cooperation, sufficiently broad to integrate all aspects of water management and provide opportunities for the Parties to meet their specific interests through the cooperation.

However, a number of challenges and existing or potential obstacles for further cooperation have been identified. Additionally, due to a broad scope of the *FASRB*, the cooperation process has shown to require many focal points and a good cross-sectoral coordination and communication within a Party, thus being rather demanding in terms of the need for resources and continuous joint efforts of the Parties.

Despite all the challenges, water cooperation in the Sava River Basin is perceived as a process providing multiple benefits for the Parties and a good basis for further progress toward the key objective—sustainable development of the region within the basin.

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Climate Change Impact on Flood Hazard in the Sava River Basin

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Abstract In the past few years, the topic of climate change impact on the water regime of the Sava River basin has been presented in several studies. Average seasonal precipitation and temperature data were calculated and presented, but results are not useful for climate change impacts on floods. The maximum daily precipitation data for each season and temperature data from the meteorological report are taken for the hydrological analysis. Maximum daily precipitations were provided with twenty-year and hundred-year return periods. The hydrological analysis was derived using a hydrological model calibrated for the flood event in 1974 before large flood protection scheme was developed along the Sava River. Flood peak discharges were calculated for autumn season by twenty- and hundred-year return period daily precipitation for the periods 2011–2040, 2041–2070 and 2071–2100. Changes in peak discharge probability functions were developed for the water station along the river for each period. The peak discharges will increase by the end of the twenty-first century for the 100-year return period from 9 % at the mouth up to 55 % at the head part of the river basin.

Keywords Climate Change • Probability of Floods • Sava River

1 Introduction

In the past few years, the topic of climate change impact on the water regime of the Sava River basin has been presented in several studies. The studies focus mainly on the trends of temperature and mean discharge values. Climate trends in the Sava River basin were analysed in the World Bank study [1]. The study focused on mean values based on observations and empirical analyses. In the study, peak flood flows and droughts were not analysed. Notably, mean yearly temperatures show stronger trends over shorter periods (trends of the last 10 years) and are weaker in the long term. In the study conducted by Jupp [2], the climate change impact was analysed by the results calculated using a series of model simulations. Average seasonal

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precipitation data were calculated and presented. In the forecast, the mean seasonal precipitation mainly decreases, except in winter time. The results are not useful for flood prediction.

Each country in the basin produces its own country report on climate change, which is submitted for the United Nations Framework Convention on Climate Change with scenarios A1B and C. In Slovenia's Fourth and Fifth National Communication under the United Nations Framework Convention on Climate Change [3, 4], it is mentioned that weather extremes will be more frequent. Floods are not specifically referred in the reports. In the Second, Third, Fourth and Fifth National Communications of the Republic of Croatia under the United Nations Framework Convention on Climate Change [5, 6], there is a short note on the Danube river flood in 2003. Furthermore, the reports predicted more frequent flood events. Also, the evident concern regarding the increase of erosion in the head water parts of watersheds is expressed in the report. However, specific measures to be adopted are not listed. The last report stresses the importance of decreasing precipitation and corresponding decrease of run-off. In the Initial National Communication of Bosnia and Herzegovina under the United Nations Framework Convention on Climate Change, Banja Luka, October 2009 [7], it is mentioned that the intensity and frequency of storms, floods and droughts will increase from 50 years to 5 to 10 years. The Ministry for Spatial Planning and Environment published the report the Initial National Communication on Climate Change of Montenegro to the United Nations Framework Convention on Climate Change, in 2010 [8]. Generally they take the statement that "lack of water and severe droughts are expected as main issue for water management and more frequent floods are also expected". A few chapters in the Initial National Communication of the Republic of Serbia under the United Nations Framework Convention on Climate Change [9] deal with hydrology and climate change. The trends and changes of mean values of precipitation, evapotranspiration and discharges are well documented. It is clearly exposed "that the above projections show that climate change might cause more intense flood and drought episodes, greater both in scope and duration".

The International Commission for the Protection of the Danube River (ICPDR) [10] study country reports for Middle Danube River Basin and stress impacts on the increase in frequency and magnitude of flood events in head parts of watersheds. In the same study only Serbia is addressing floods and for other countries in the Sava River basin no data are available.

The topic of climate change impacts is broad. Various scenarios are being examined, based mainly on increase of air temperature. The reports that we reviewed were mainly related to mean yearly or seasonal values and not to extremes.

The formation of flood run-off is a complex non-linear process that cannot be easily transformed from precipitation data. For the transformation of extreme precipitation data, we developed a hydrological model and then incorporated the precipitation data calculated for different projections for the A1B scenario.

2 Hydrological Model of the Sava River Watershed

The Sava River watershed, from its source to the discharge into the Danube, extends over an area of around 95,000 km^2. The south-east border of watershed is in the Dinaric Karst region and could not be precisely determined. To ensure the rigidity and robustness of the model, the subbasins were generated to be as large as possible while covering not more than one major tributary stream. As a result, the watershed was divided into 13 subbasins with areas ranging from 2,000 to 14,000 km^2 (Table 1, Fig. 1). The subbasins are linked together, and the outflow from the upstream ones is routed through the downstream ones.

All the subbasins were divided into elevation (three were chosen) and vegetation zones. The upper and south-east part of the Sava River watershed is mountainous; as a result, the subbasins in that area have three elevation zones (Fig. 2). The subbasins in the plain area (north-west part of the watershed), where altitudes generally do not exceed 200 m, have two elevation zones (Fig. 2). Each elevation zone was then further divided into two areas according to land coverage (Fig. 2), i.e. into the so-called vegetation zones: forest and field (non-forest). The division into elevation and vegetation zones is especially important for the snow calculating routine.

It is based on the simple degree–day relation. In this routine, a threshold temperature (TT), which is usually close to 0 °C, is used to define the temperature above which snowmelt occurs. The threshold temperature usually decides whether the precipitation falls as rain or as snow. Within the threshold temperature interval (TTI), the precipitation is assumed to be a mix of rain and snow (decreasing linearly from 100 % snow at the lower end to 0 % at the upper end). The snowpack is assumed to retain meltwater as long as the amount does not exceed a certain fraction of the snow. When the temperature decreases below TT, the water

Table 1 List of subbasins

#	Subbasin number	Subbasin name	Stream	Subbasin area (km^2)
1	I	Sava I	Sava	10,073
2	II	Sava II	Sava	3,481
3	III	Kolpa/Kupa	Kolpa/Kupa	9,501
4	IV	Sava III	Sava	6,701
5	V	Una	Una	9,907
6	VI	Sava IV	Sava	1,880
7	VII	Vrbas	Vrbas	5,295
8	VIII	Sava V	Sava	4,403
9	IX	Bosna	Bosna	10,261
10	X	Sava VI	Sava	5,021
11	XI	Drina I	Drina	13,781
12	XII	Drina II	Drina	5,979
13	XIII	Sava VII	Sava	8,424
			Watershed total	94,708

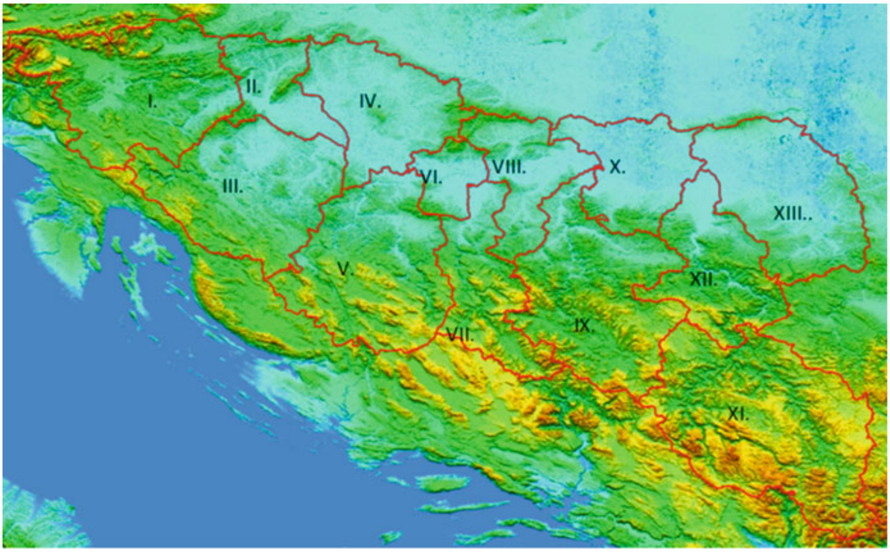

Fig. 1 Modelled Sava River watershed—from its source to its confluence with the Danube—with orographic subbasin and watershed borders

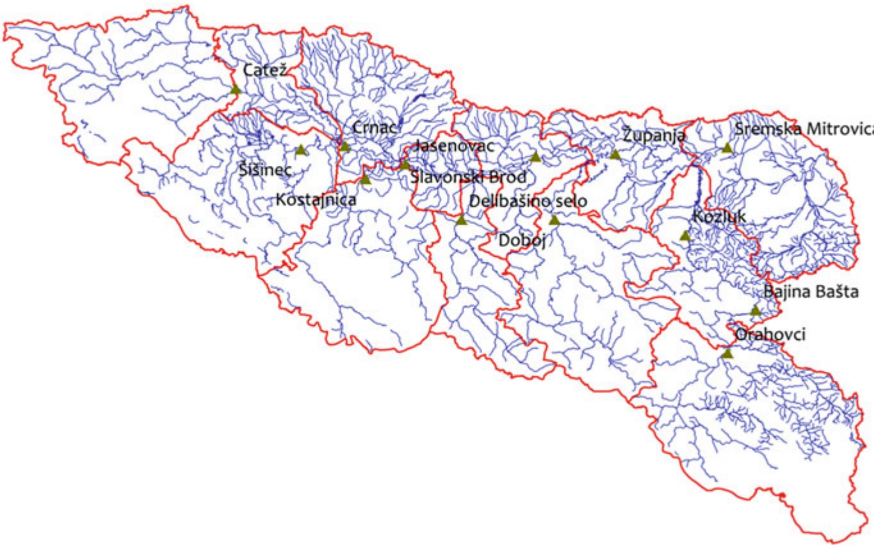

Fig. 2 Sava River watershed with discharge stations (used for model calibration)

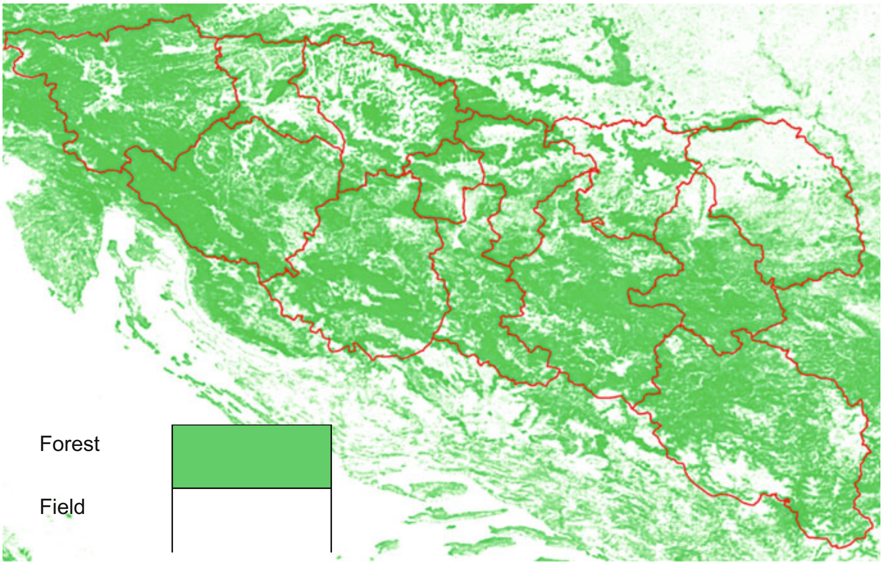

Fig. 3 Modelled Sava River watershed—from its source to its confluence with the Danube—with all the subbasins and the forest coverage [11]

refreezes. Different melting and refreezing factors are used for forest and non-forest zones (Fig. 3) [11].

The following input data are required to calibrate/run the model:

– Precipitation (32 measurement stations were chosen) (Fig. 4)
– Temperatures (8 measurement stations were chosen)
– Discharge data (12 measurement stations were chosen)
– Potential evapotranspiration (8 measurement stations were chosen)

The temperature and precipitation data were prepared as a set of data with a 1-day time step. The time step of evapotranspiration data is usually greater than that of the model. So a transformation to the model time step is required. This is done automatically by the model. In this case, average monthly values (mm/day) are transformed to the 1-day time step by linear interpolation.

To describe areas of influence of points (which represent different stations), Thiessen polygons were used. Precipitation data were obtained from Meteorological Yearbooks 1974 and 1978 [12, 13], discharge data from Hydrological Yearbooks 1974 and 1978 [14, 15], and temperature and potential evapotranspiration data from the database collected for the World Bank report [1].

Model calibration and validation were developed with data for flood events from years 1974 and 1978, for the period of time before a large flood protection system has been developed on the watershed and modified flood events. The number of parameters normally used in the model is in the order of 20–33. While in most cases

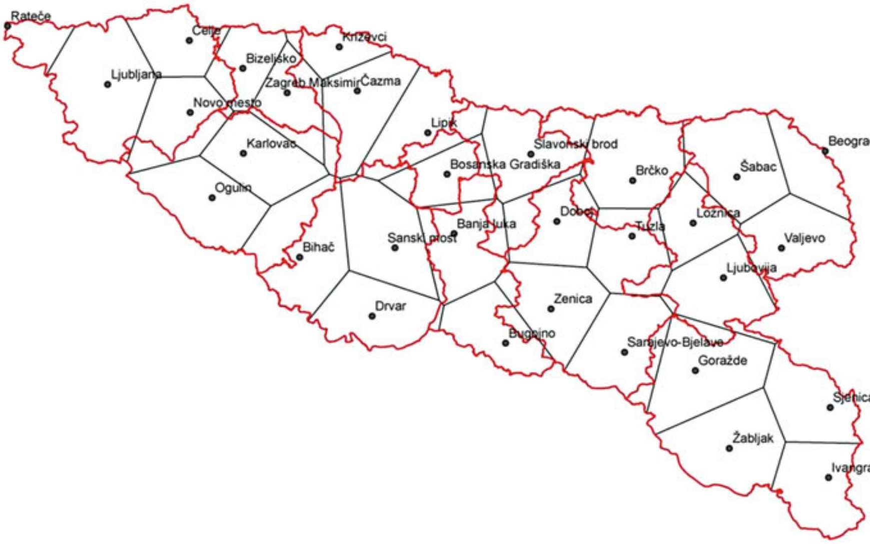

Fig. 4 Sava River watershed with precipitation stations and Thiessen polygons

five of them are set to standard values, it is very important to calibrate approximately 15 of the parameters.

Three main criteria of fit are used while calibrating: visual inspection of the computed and observed hydrographs, Nash/Sutcliffe criterion R^2 and inspection of the accumulated error. The R^2 efficiency criterion was introduced by Nash and Sutcliffe [16] and is commonly used in hydrological modelling. R^2 has a value of 1.0 if the simulation and the observations agree completely and 0 if the model does not perform any better than the mean value of the run-off record. In practice, values between 0.8 and 0.95 can be achieved if the quality of observed data is good. Negative values can be the result of poor model performance or poor data. In addition to the R^2 criterion, there is another very important performance indicator: the accumulated error.

The calibration is an interactive process. First, one must carefully observe the hydrographs where the differences appeared. Then it is necessary to determine if there is a problem of volume or a problem of shape. After this, one has to look at the conditions during the period of poor results (temperature, presence of snow, precipitation, maximum discharge before, droughts) and change the relevant parameters. Finally, the R^2 value is checked. Sometimes the result is better with the R^2 criterion a bit less strong because the peaks are better modelled.

For the calibration purposes, we collected the data (input data: precipitation, temperature, evapotranspiration, discharge) for the period from June 1 to December 31, 1974 (Table 2). An important characteristic of the 1974 flood event was major rainfall that moved with time from the east to the west part of the Sava River basin. In the east, head part of the watershed, maximum rainfall occurred on September 25 and in the west part on September 27, 1974 [12, 14].

Table 2 Model calibration peak discharges in m^3/s (1974)

Subbasins	WS	Area	Measured	Calibrated	%
Sava I	Čatež	10,173	2,294	2,308	0.6
Kolpa	Šišinec	7,321	1,250	1,419	13.5
Sava II	Crnac	23,102	2,147	2,295	6.9
Una	Kostajnica	9,171	1,370	1,445	5.4
Sava III	Jasenovac	29,565	2,580	2,515	−2.5
Vrbas	Delibašino selo	5,469	691	762	10.3
Sava IV	Slavonski Brod	54,134	3,460	3,422	−1.1
Bosna	Doboj	9,618	1,095	753	−31.3
Sava V	Županja	62,22	3,930	4,057	3.2
Drina I	Bajina Bašta	14,797	3,359	2,715	−19.2
Drina II	Kozluk	17,735	3,041	2,640	−13.2
Sava V	Sremska Mitrovica	87,996	6,275	6,540	4.2
Confluence in Danube				6,653	

Table 3 Model performance

Watershed no.	Watershed name	Calibration		Verification		Station name
		R^2	Acc. diff. (mm)	R^2	Acc diff. (mm)	
I	Sava I	0.8183	−23.7937	−0.4213	20.8903	Čatež
III	Kolpa/Kupa	0.9029	−19.8823	0.7461	−25.4299	Šišinec
IV	Sava III	0.7689	−27.8047	0.4193	4.7807	Crnac
V	Una	0.7921	18.8697	−3.2602	63.4986	Kostajnica
VI	Sava IV	0.6361	−180.7203	0.6881	−24.1327	Jasenovac
VII	Vrbas	0.3133	−10.3829	−1.5449	46.8637	Delibašino Selo
VIII	Sava V	0.8646	−46.2497	−0.4608	24.1783	Slavonski Brod
IX	Bosna	0.2735	−91.3311	−2.9617	102.6221	Doboj
X	Sava VI	0.8553	−14.7998	−2.0815	48.1689	Županja
XI	Drina I	0.7999	−45.7861	−3.3535	4.6146	Bajina Bašta
XII	Drina II	0.7830	−19.3865	−5.2540	22.571	Kozluk
	Sava VI + Drina	0.8561	10.1821	−3.1442	48.0747	Sremska Mitrovica
XIII	Sava VII					Confluence

The selected verification period was from September 1, 1978, to November 30, 1978 [13, 15]. The peak discharges are quit high and data form weather stations was available for modelling.

The results of calibration and verification of the model are not impressive, especially for sub-watersheds (Table 3). The sub-watersheds were modelled as homogenised areas except for the Drina River basin. The main task of the calibration was flood peaks, not water balance. In Figs. 5 and 6, the comparison of the

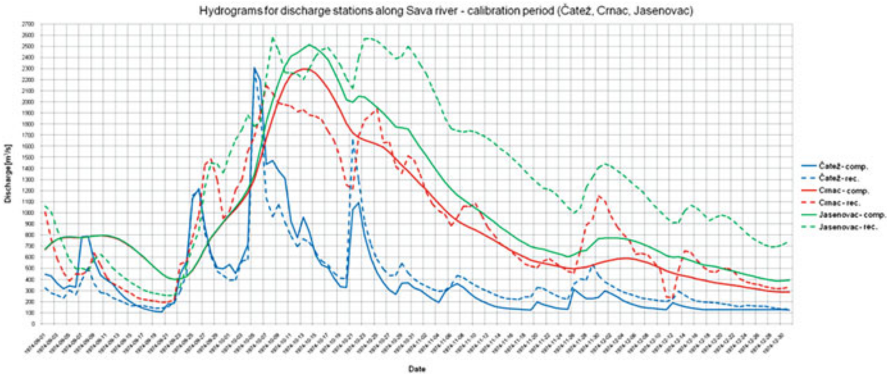

Fig. 5 Measured and modelled discharges at the selected stations in the upper part of the Sava River Basin (calibration period)

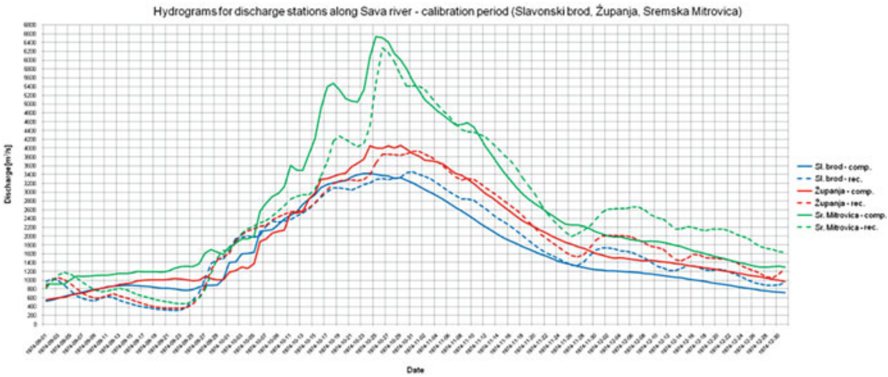

Fig. 6 Measured and modelled discharges at the selected stations in the lower part of the Sava River Basin (calibration period)

measured and modelled discharges for selected water stations is shown as a result of the hydrological model calibration procedure for the calibration period June 1–December 31, 1974.

3 Data Transformation for Hydrological Forecasts of Climate Change Impacts

The precipitation and temperature data from the meteorological report [17] are taken from figures based on the position of rain gauge stations and used for the hydrological model. Observed data from the grid database of the European observation system (E-OBS) are extracted E-OBS [18] and shown in Table 4. These data have been designed to provide the best estimate of grid box averages to enable a

Table 4 Daily maximum seasonal precipitation derived for weather station from E-OBS data for the period 1971–2010 with 20-year return period in mm

Longitude	Latitude	Station	Max. prec. [14]	Spring E-OBS	Summer E-OBS	Autumn E-OBS	Winter E-OBS
13° 43′ E	46° 30′ N	Rateče	42.6	98.2	99.0	131.9	99.6
14° 31′ E	46° 04′ N	Ljubljana	95.8	69.0	90.9	88.5	75.4
15° 15′ E	46° 15′ N	Celje	66.7	62.3	82.4	85.4	58.2
15° 42′ E	46° 01′ N	Bizeljsko	68	47.0	62.9	64.3	49.2
15° 11′ E	45° 48′ N	Novo Mesto	55	57.6	75.0	79.7	62.8
16° 33′ E	46° 02′ N	Križevci	26.5	34.2	47.0	47.1	38.6
15° 14′ E	45° 16′ N	Ogulin	63.2	58.0	85.6	86.6	70.9
15° 33′ E	45° 30′ N	Karlovac	42.5	46.3	61.0	62.0	52.1
16° 02′ E	45° 49′ N	Zagreb-Maksimir	34.5	34.6	47.2	43.6	36.4
16° 38′ E	45° 45′ N	Čazma	29.3	28.2	43.6	40.1	36.6
17° 10′ E	45° 25′ N	Lipik	49.3	27.2	39.9	32.3	35.1
18° 00′ E	45° 10′ N	Slavonski Brod	31.6	25.9	30.6	31.1	27.2
17° 16′ E	45° 09′ N	Bosanska Gradiška	38.4	27.7	33.5	31.7	31.4
15° 53′ E	44° 49′ N	Bihać	82.9	45.8	58.3	69.7	58.1
16° 24′ E	44° 23′ N	Drvar	58.6	39.9	47.9	54.9	42.3
16° 42′ E	44° 46′ N	Sanski Most	61.5	32.4	37.7	47.9	35.5
17° 13′ E	44° 47′ N	Banja Luka	56.2	25.2	29.9	34.0	29.0
17° 28′ E	44° 04′ N	Bugojno	40.4	25.9	32.6	38.0	30.1
17° 54′ E	44° 13′ N	Zenica	21.4	23.8	29.2	34.7	31.9
18° 06′ E	44° 44′ N	Doboj	24.2	25.5	30.2	30.7	28.9
18° 42′ E	44° 33′ N	Tuzla	21.5	25.9	33.5	31.7	29.7
18° 50′ E	44° 53′ N	Brčko	23.5	28.7	36.4	33.3	29.8
18° 26′ E	43° 52′ N	Sarajevo-Bjelave	36	26.2	34.6	37.6	38.2
18° 59′ E	43° 40′ N	Goražde	29.2	27.3	34.3	42.2	41.2
19° 14′ E	44° 33′ N	Loznica	26.5	33.5	50.5	34.6	32.9
19° 23′ E	44° 11′ N	Ljubovija	50.9	31.8	42.5	35.5	36.5
19° 41′ E	44° 46′ N	Šabac	46.8	34.4	52.2	36.0	31.5
19° 55′ E	44° 17′ N	Valjevo	49	39.5	49.7	39.3	38.5
20° 28′ E	44° 48′ N	Beograd	39.4	39.6	51.7	36.0	32.9
20° 01′ E	43° 16′ N	Sjenica	45.1	32.6	51.9	42.9	34.3
19° 08′ E	43° 09′ N	Žabljak	83.9	27.1	37.5	37.1	34.3
19° 52′ E	42° 50′ N	Ivangrad	39.2	31.5	48.6	44.0	33.5
		Average	46.2	37.9	49.6	49.5	42.0
		Max.	95.8	98.2	99.0	131.9	99.6
		Min.	21.4	23.8	29.2	30.7	27.2

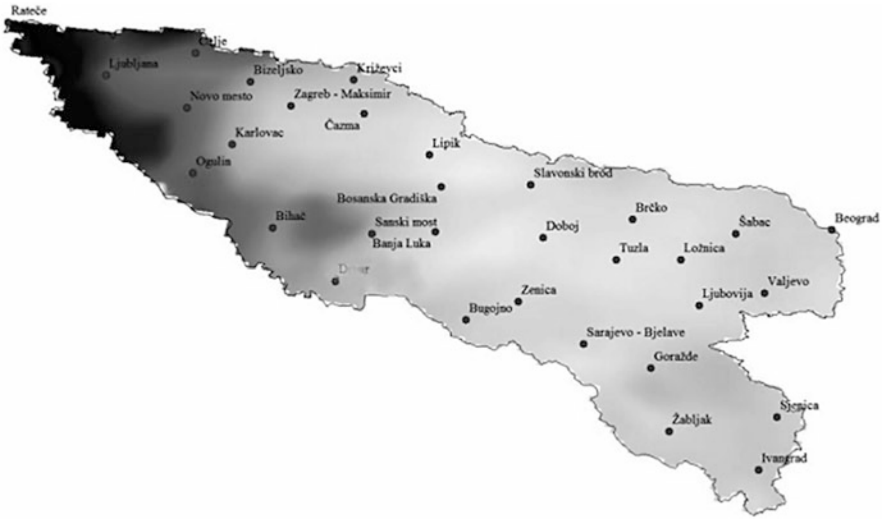

Fig. 7 E-OBS data. Precipitation distribution for the 100-year return period [17]

direct comparison with RCMs. The E-OBS data set was defined on the same 0.25° grid resolution, and data collected between 1961 and 2010 were used in this study. An example of the data set is on the map in Fig. 7.

The precipitation data in the meteorological report are in raster format, and we collected the data from the cell in which the precipitation station was positioned. Maximum daily precipitation values from E-OBS data are highest in summer and slightly lower (0.1 mm) in autumn.

The maximum daily values of the precipitation measured in 1974 are mainly slightly lower than the values of E-OBS. There is a high discrepancy between the E-OBS data and the measurements in the area of the Dinaric Mountains, especially in Montenegro (Fig. 7). The value at the Žabljak station is two times higher than that in E-OBS data with the 20-year return period and even the 100-year return period (Table 5). A concern is that for the E-OBS data set, precipitation from Montenegro was not used. The flood event in 1974 is one of the highest floods measured before large flood protection construction works started on the Posavina, and precipitation on all stations of basin has low probability.

Summer daily precipitation is slightly higher than in autumn. However, run-off in the autumn season is much higher, due to higher evaporation, and for further calculations and analysis, we chose the autumn values (Table 5).

Forecast data for the periods of 2011–2040, 2041–2070 and 2071–2100 are represented in Table 5 and show interesting dynamics. Data for some stations increase with time, while with other stations, first an increase and then a decrease

Table 5 Autumn rainfall values with 20- and 100-year return periods based on the E-OBS data with forecasts

	EOBS_20	EOBS_100	20_11-40	20_41-70	20_71-2100	100_11-40	100_41-70	100_71-100
Rateče	131.9	171.1	149.6	147.5	155.7	206.5	191.3	201.9
Ljubljana	88.5	110.0	99.1	110.0	113.3	131.1	148.0	153.2
Celje	85.4	105.3	92.7	105.9	111.1	122.4	140.1	149.8
Bizeljsko	64.3	77.1	71.1	83.2	86.8	94.5	119.5	126.9
Novo mesto	79.7	101.5	86.4	100.7	108.4	117.8	148.6	164.3
Križevci	47.1	55.9	50.3	56.5	59.7	61.9	73.1	80.4
Ogulin	86.6	103.8	89.8	102.6	110.8	108.8	138.6	148.7
Karlovac	62.0	71.9	67.0	74.1	82.0	81.9	94.5	111.7
Zagreb-Maksimir	43.6	50.3	46.0	52.0	56.3	56.2	67.4	80.4
Čazma	40.1	45.5	42.5	47.2	50.1	48.5	56.7	62.4
Lipik	32.3	34.3	36.4	37.9	37.3	40.5	42.4	38.9
Slavonski brod	31.1	38.6	36.2	36.3	36.8	48.1	47.8	45.0
Bosanska Gradiška	31.7	39.2	36.4	37.0	37.1	47.3	48.1	46.2
Bihać	69.7	83.4	76.3	81.0	88.4	95.8	101.8	114.2
Drvar	54.9	69.3	60.0	65.6	64.7	78.0	91.5	86.6
Sanski most	47.9	68.6	53.8	55.6	56.5	81.5	84.3	82.1
Banja Luka	34.0	44.0	38.2	38.9	39.1	51.9	53.4	50.7
Bugojno	38.0	50.4	43.1	44.8	43.9	61.6	66.6	62.2
Zenica	34.7	42.4	41.0	43.6	40.3	54.1	60.9	51.2
Doboj	30.7	34.9	36.9	38.2	35.8	46.4	51.3	41.6
Tuzla	31.7	35.2	39.0	40.7	39.3	50.1	51.6	48.6
Brčko	33.3	39.4	39.6	40.4	40.6	50.7	51.4	49.0
Sarajevo-Bjelave	37.6	42.6	45.1	49.6	44.5	58.8	66.5	52.8
Goražde	42.2	52.6	46.7	52.8	50.3	61.3	74.2	66.5
Ložnica	34.6	37.5	41.5	44.7	41.6	51.0	54.6	46.0

(continued)

Table 5 (continued)

	EOBS_20	EOBS_100	20_11-40	20_41-70	20_71-2100	100_11-40	100_41-70	100_71-100
Ljubovija	35.5	39.5	42.1	48.0	42.5	52.2	64.6	50.6
Šabac	36.0	43.4	43.9	47.2	43.3	59.5	62.1	53.0
Valjevo	39.3	47.2	43.5	51.1	47.2	55.1	70.3	59.4
Beograd	36.0	46.1	41.9	46.4	44.8	58.3	66.7	61.0
Sjenica	42.9	51.3	44.9	55.9	52.6	54.6	77.6	66.1
Žabljak	37.1	45.7	40.4	49.3	44.1	54.1	75.0	61.6
Ivangrad	44.0	53.1	49.8	63.5	58.5	62.2	98.7	76.6
Average	49.5	60.3	55.4	60.9	61.4	72.0	82.5	80.9

Table 6 Probability of maximum daily precipitation (mm) based on the report (Meerbach et al. 2010) in 1974 [12] and data from Table 4

Station name	Return period			Max. prec. in 1974	V1	V2	V3	V4
	1,000	100	20		EOBS_20	EOBS_100	20_41-70	100_41-70
Ljubljana	190.7	106.3	72.2	95.8	88.5	110.0	110.0	148.0
Rateče	214.9	121.2	83.2	42.6	131.9	171.1	147.5	191.3
Zagreb	117.2	65.9	45.2	34.5	43.6	50.3	52.0	67.4
Slavonski brod	104.1	59.1	40.9	31.6	31.1	38.6	36.3	47.8
Bihać	155.3	89.5	62.8	82.9	69.7	83.4	81.0	101.8
Bugojno	119.9	66.2	44.5	40.4	38.0	50.4	44.8	66.6
Sarajevo	120.0	67.0	45.5	36.0	37.6	42.6	49.6	66.5
Banja luka	86.0	57.4	45.8	56.2	34.0	44.0	38.9	53.4
Beograd	126.8	66.3	41.9	39.4	36.0	46.1	46.4	66.7
Sjenica	89.9	53.3	38.5	45.1	42.9	51.3	55.9	77.6

can be observed. Average values for rainfall with a 20-year return period show a very small increase between the periods 2041–2070 and 2071–2100 and an even smaller decrease for the 100-year return period.

The probabilities in Table 6 are based on the Gumbel probability distribution and were calculated using the data on precipitation from the report by Meerbach et al. (2010). The period of observation varied from 1908 or 1951 to 2009. The differences of values of precipitation with the 20-year return period calculated using the Gumbel distribution function and E-OBS varied. At some stations, the values calculated using the Gumbel distribution function were higher than those calculated using the E-OBS data, and vice versa. For the 100-year return period, only the values from Slovenia are lower if calculated using the Gumbel distribution function than those calculated using the E-OBS data. All other stations have higher values. Finally, the 100-year return period values for the forecast between 2041 and 2070 are lower than the values with the 1,000-year return period for all rainfall stations.

Temperature data are given in Table 7. Temperature data vary significantly inside the Sava River watershed. However, the forecast variation is rather small. For further calculations, we chose an increase of 0.8 °C in autumn in the period 2011–2040, 1.8 °C for autumn in the period 2041–2070 and 2.9 °C in the period 2071–2100, for watershed as whole.

Table 7 Temperature data and climate change forecast in °C

Station	EOBS temperature data for 1971–2010				Increase of temperature		
	Spring	Summer	Autumn	Winter	2011–2040	2041–2070	2071–2100
Rateče	4.8	14.0	6.4	–3.2	0.9	1.9	3.0
Ljubljana	8.9	17.9	9.5	–0.3	0.9	1.9	2.9
Celje	8.4	17.2	9.1	–0.8	0.8	1.8	2.9
Bizeljsko	10.2	18.8	10.4	0.5	0.9	1.8	2.9
Novo mesto	9.2	17.9	9.8	0.0	0.9	1.8	2.9
Križevci	11.0	19.7	11.1	1.0	0.8	1.8	2.8
Ogulin	8.4	17.4	9.6	0.2	0.8	1.7	2.7
Karlovac	10.8	19.7	11.4	1.7	0.8	1.7	2.7
Zagreb-Maksimir	11.2	19.9	11.4	1.5	0.8	1.8	2.8
Čazma	11.5	20.3	11.7	1.7	0.8	1.7	2.8
Lipik	10.9	19.8	11.3	1.2	0.9	1.7	2.8
Slavonski brod	11.3	20.2	11.5	1.2	0.9	1.8	2.8
Bosanska Gradiška	11.1	20.0	11.6	1.5	0.8	1.7	2.7
Bihać	8.5	17.5	9.5	0.0	0.8	1.6	2.7
Drvar	7.1	16.3	8.7	–0.6	0.9	1.8	3.0
Sanski most	10.1	19.2	11.0	1.4	0.7	1.6	2.5
Banja Luka	10.7	19.8	11.5	1.7	0.7	1.6	2.5
Bugojno	7.2	16.3	8.9	–0.5	0.8	1.8	3.0
Zenica	8.8	17.6	9.8	0.1	0.8	1.8	2.9
Doboj	11.0	19.8	11.4	1.3	0.8	1.6	2.6
Tuzla	10.1	18.8	10.4	0.4	0.8	1.7	2.8
Brčko	11.4	20.1	11.3	1.2	0.8	1.7	2.8
Sarajevo-Bjelave	8.1	16.9	9.2	–0.5	0.9	1.9	3.2
Goražde	8.2	17.0	9.4	–0.6	0.9	1.9	3.2
Ložnica	10.6	19.4	10.8	0.7	0.8	1.7	2.8
Ljubovija	9.1	17.9	9.8	–0.3	0.9	1.8	3.0
Šabac	11.5	20.3	11.4	1.1	0.9	1.8	2.9
Valjevo	10.2	19.1	10.6	0.4	0.8	1.8	2.9
Beograd	11.8	20.8	12.1	1.5	0.9	1.9	3.1
Sjenica	5.5	14.2	6.7	–3.5	0.9	2.0	3.3
Žabljak	4.8	13.8	6.7	–3.0	0.9	2.1	3.4
Ivangrad	5.7	14.7	7.3	–2.7	0.9	2.0	3.2
Average	9.3	18.2	10.0	0.1	0.8	1.8	2.9
Stand. dev.	2.1	2.0	1.6	1.5	0.1	0.1	0.2

4 Results of Climate Change Modelling

The hydrological model was used for modelling of the impact of climate change forecasts on the Sava River discharges at selected stations. For modelling of the impact of climate change, the same input data as those for the calibrated model for the flood in 1974 were used. We only changed the rainfall data for the day with maximum precipitation and increase temperature (Table 4). Instead of using the measured maximum daily precipitation, we used the predicted maximum daily precipitation from Table 4. First, we calculated peak discharges for E-OBS (1971–2010) data with 20- and 100-year return periods. The calibrated and measured discharges with the E-OBS data modelling are represented in Table 8.

Peak calibrated discharges and central parts of the watershed, down to Sava III, are lower than those calculated by E-OBS data for the 20-year return period. Values of discharge in the lower part of the watershed are between the values calculated for E-OBS data for 20- and 100-year return periods. The Drina River flood peak discharges are much higher than those calculated by the E-OBS 100-year return period data.

We calculated the impact of climate change in the same way as in the model calibration, by taking into account the change of the maximum daily values of precipitation with the data from Table 4 and the increase in temperature using the data from Table 7. The results of modelling for E-OBS data for the 20-year return period and for forecasts in the periods 2011–2040, 2041–2070 and 2071–2100 are represented in Table 9 and Fig. 8, and for E-OBS data with the 100-year return period, the results are shown in Table 10 and Fig. 9.

Forecasted flood peaks with the 20-year return period, in the period 2071–2100, will increase in average 14 % and up to 36 % in the upper part of the basin and on some tributaries (Table 9). The calculated base flow drops a little on Fig. 8 due to higher temperatures. The flood peaks along the main stream will increase in the next 60 years from 8 % on the inflow in Danube to 33 % on the head water part of the catchment. Forecasted discharges, due climate change, increase in time. Only discharges on the Drina River WS and downstream WS Sremska Mitrovica on the Sava River have lower predicted discharge for the period 2071–2100 than for the period 2041–2070. Discrepancies in peak discharges on the Drina River basin could be the result of fewer predictions used for the 2071–2100 periods of precipitation forecasts. Some results of climate change modelling [17], which were used for the periods 2011–2040 and 2041–2070, were not available for the period 2071–2100 forecasts.

Forecasted flood peaks with 100-year return periods are in Table 10. Data are presented with peak discharge values and in percentage of increase relative to calculation using the E-OBS data. Percentages of increase of flood discharges with the 100-year return period of floods (Table 10) show higher increase than values with 20-year return period, as presented in Table 9. The average increase, for the period up to 2100, is 14 % for the 20-year return period of flood and 31 % for

Table 8 Result of modelling recent climate flood peaks (in m^3/s)

Subbasins	WS	Calibrated	E-OBS_ret20	E-OBS_ret100
Sava I	Čatež	2,308	2,308	2,780
Kolpa	Šišinec	1,419	1,473	1,522
Sava II	Crnac	2,295	2,350	2,510
Una	Kostajnica	1,445	1,382	1,407
Sava III	Jasenovac	2,515	2,561	2,718
Vrbas	Delibašino Selo	762	620	707
Sava IV	Slavonski Brod	3,422	3,411	3,573
Bosna	Doboj	753	742	767
Sava V	Županja	4,057	4,068	4,227
Drina I	Bajina Bašta	2,715	2,336	2,474
Drina II	Kozluk	2,640	2,276	2,407
Sava VI	Sremska Mitrovica	6,540	6,328	6,603
Confluence with Danube		6,653	6,432	6,715

Table 9 Result of modelling climate change flood peaks with E-OBS data for 20-year return period (in m^3/s)

Subbasins	WS	E-OBS (m^3/s)	11–40 (m^3/s)	41–70 (m^3/s)	71–2100 (m^3/s)	11–40/ E-OBSE	41–70/ E-OBSE	71–2100/ E-OBSE
Sava I	Čatež	2,308	2,552	2,859	3,073	1.11	1.24	1.33
Kolpa/ kupa	Šišinec	1,473	1,523	1,568	1,591	1.03	1.06	1.08
Sava II	Crnac	2,350	2,428	2,520	2,571	1.03	1.07	1.09
Una	Kostajnica	1,382	1,637	1,726	1,718	1.19	1.25	1.24
Sava III	Jasenovac	2,561	2,630	2,717	2,742	1.03	1.06	1.07
Vrbas	Delibašino selo	620	676	687	691	1.09	1.11	1.11
Sava IV	Slavonski Brod	3,411	3,623	3,742	3,788	1.06	1.10	1.11
Bosna	Doboj	742	912	931	1,010	1.23	1.25	1.36
Sava V	Županja	4,068	4,346	4,554	4,826	1.07	1.12	1.19
Drina I	Bajina Bašta	2,336	2,471	2,617	2,456	1.06	1.12	1.05
Drina II	Kozluk	2,276	2,427	2,586	2,425	1.07	1.14	1.07
Sava VI	Sremska Mitrovica	6,328	6,659	6,862	6,854	1.05	1.08	1.08
Confluence		6,432	6,757	6,960	6,944	1.05	1.08	1.08
					Average	1.08	1.13	1.14
					Max.	1.23	1.25	1.36
					Min.	1.03	1.06	1.05

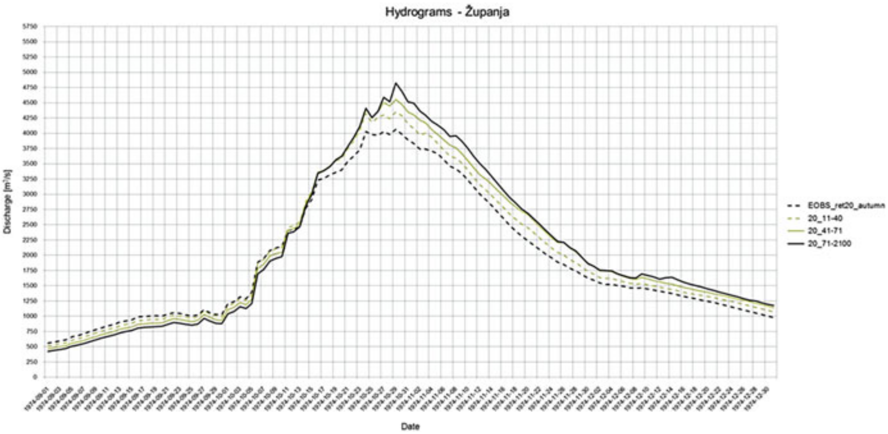

Fig. 8 Discharges calculated with E-OBS data for 20-year return periods for WS Županja, Sava V

Table 10 Results of modelling climate change flood peaks with E-OBS data of the 100-year return period (in m^3/s and %)

Subbasins	WS	E-OBS (m^3/s)	2011–40 (m^3/s)	2041–70 (m^3/s)	2071–2100 (m^3/s)	2011–40/ E-OBSE	2041–70/ E-OBSE	2071–2100/ E-OBSE
Sava I	Čatež	2,780	3,297	3,770	4,134	1.43	1.63	1.79
Kolpa/ kupa	Šišinec	1,522	1,595	1,664	1,722	1.08	1.13	1.17
Sava II	Crnac	2,510	2,670	2,817	2,929	1.14	1.20	1.25
Una	Kostajnica	1,407	2,060	2,245	2,188	1.49	1.63	1.58
Sava III	Jasenovac	2,718	2,863	2,993	3,086	1.12	1.17	1.21
Vrbas	Delibašino selo	707	813	845	825	1.31	1.36	1.33
Sava IV	Slavonski Brod	3,573	3,895	4,062	4,142	1.14	1.19	1.21
Bosna	Doboj	767	985	1,025	1,103	1.33	1.38	1.49
Sava V	Županja	4,227	4,699	4,957	5,270	1.16	1.22	1.30
Drina I	Bajina Bašta	2,474	2,683	3,087	2,719	1.15	1.32	1.16
Drina II	Kozluk	2,407	2,639	3,059	2,686	1.16	1.34	1.18
Sava VI	Sremska Mitrovica	6,603	7,143	7,580	7,409	1.13	1.20	1.17
confluence		6,715	7,253	7,695	7,509	1.13	1.20	1.17
					Average	1.21	1.31	1.31
					Max.	1.49	1.63	1.79

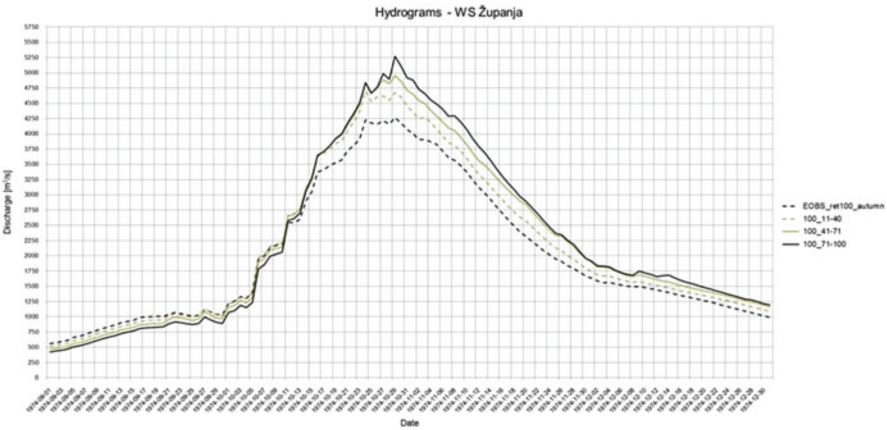

Fig. 9 Discharges calculated with E-OBS data for the 100-year return period for WS Županja, Sava V

Table 11 Probability of peak discharges for WS Čatež (m^3/s)

	E-OBS_20	E-OBS_100		
	26 %	3.05 %	1 %	0.1 %
1926–1965	2,308	2,780	3,027	3,400
2011–2040	2,551	3,296	3,694	4,056
2041–2070	2,859	3,770	4,248	4,627
2071–2100	3,072	4,133	4,687	5,060

100-year return period. The highest increase is observed at WS Rateče on the main stream with 79 %, followed by the Bosna River tributary (49 %) and the Una River tributary (58 %). Changes on the Drina River catchment and WS Sremska Mitrovica have similar anomalies as the discharges with the 20-year return period.

Calculated values in Table 11 are valid for the river mouth and not up to the most downstream water station, but percentage of increase could be used for watershed as a whole. The upper part of the watershed at WS Čatež has the greatest increase, up to 79 %. The Kolpa River tributary has much lower increase up to 17 %. The Una River tributary has a 63 % increase of discharge up to 2070 and then a smaller increase, because of smaller precipitation (Table 10). Similar is the dynamics of flood discharge with 100-year return period forecast for the Vrbas River tributary, which increases by 36 % and then decreases to 33 %. Flood discharge of the Bosna River tributary will increase by 49 % up to the end of the century. The Drina River has similar dynamics like the Una River and Vrbas River, but the drop, in the last period of forecast, is more significant. The flood discharge will increase up to 34 % and then drop to 18 %, which is similar to the increase in the first period of forecast. The forecasted discharges increase along the Sava River, indicating a drop from WS Čatež (79 %) to 25 % on WS Crnac and to 21 % on WS Jasenovac, which is the same value as that on WS Slavonski Brod. The percentage of discharges increases downstream down to WS Županja to 30 %. Downstream of the Drina River mouth, the percentage increases for the period 2041–2070 up to 20 % on the WS Sremska Mitrovica and then drops to 18 % for the period 2071–2100.

5 Climate Change Impact on Probability of Flood Peaks

The probability analysis was derived from the probability analysis represented in the report by Prohaska [19]. Probability analysis in the report was derived from the data collected in the period 1926–1965. There is no impact of flood protection measures in Central Posavina developed later on. Data about 10, 1 and 0.1 percentage of probability were used as basic relations for WS. Discharge values calculated for E-OBS data with 20-year return period and 100-year return periods were transformed based on the new probability according to the basic relations. In this way, we estimated the new probability for E-OBS_20 and EOS_100 according to the probability function from the report prepared by Prohaska [19].

The probability function for water station Čatež is in Fig. 10 and Table 11. The E-OBS_20 discharge has a probability of 26 % (instead of 5 %), and E-OBS_100 discharge has a probability of 3.05 % (instead of 1 %). The climate change values were then arranged in relation to the new estimated probability and in accordance with the basic relations from the report. New probability relations are estimated to be parallel to the basic ones published in the Prohaska report (2009). The hundred-

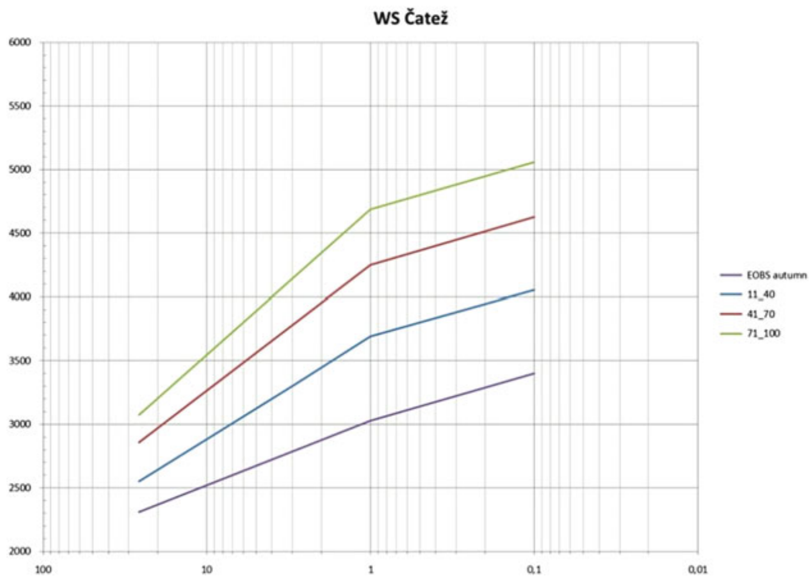

Fig. 10 Probability function (%) of peak discharges on WS Čatež for different periods of climate change forecast

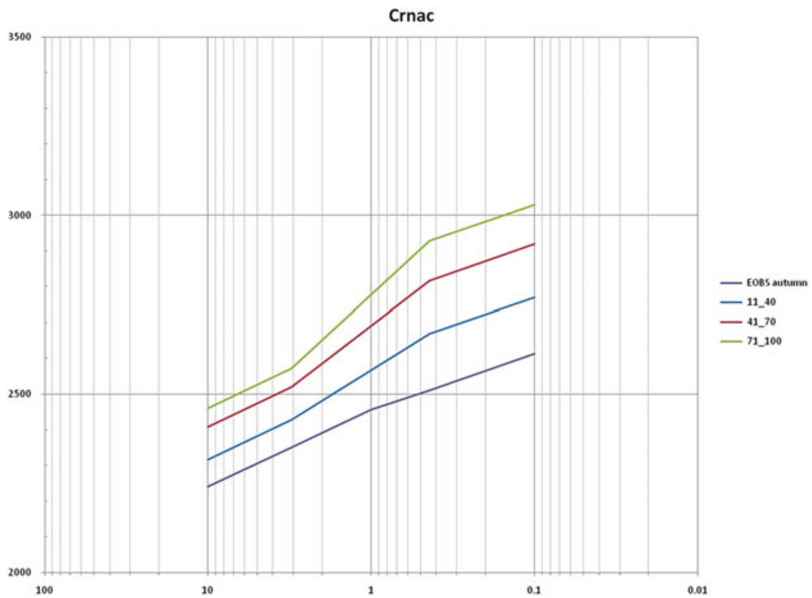

Fig. 11 Probability function (%) of peak discharges on WS Crnac for different periods of climate change forecast

Table 12 Probability of peak discharges for WS Crnac (m^3/s)

		E-OBS_20		E-OBS_100	
	10 %	3.10 %	1 %	0.44 %	0.10 %
1926–1965	2,240	2,350	2,456	2,510	2,613
2011–2040	2,317	2,670	2,570	2,428	2,770
2041–2070	2,409	2,817	2,690	2,520	2,920
2071–2100	2,460	2,929	2,780	2,571	3,030

year return period discharges (1 % in Table 11) will increase from 22 % in the first period 2011–2040 to 55 % in the last period 2071–2100, or the hundred-year return period of flood will increase, up to the year 2100, by 1.660 m^3/s, and the water level will increase by 225 cm.

The probability function for water station Crnac is in Fig. 11 and Table 12. The E-OBS_20 discharge has a probability of 3.1 % (instead of 5 %), and E-OBS_100 discharge has a probability of 0.44 % (instead of 1 %). The climate change values were then arranged in relation to the new estimated probability and in accordance with the basic relations from the report. New probability relations are estimated to be parallel to the basic ones published in the Prohaska report (2009). The hundred-year return period discharges (1 % in Table 12) will increase from 5 % in the first period 2011–2040 to 13 % in the last period 2071–2100. The huge inundation area of “Central Posavina” decreases not only flood discharges from the upstream part but also decreases significantly percentage of discharge increase due to the climate

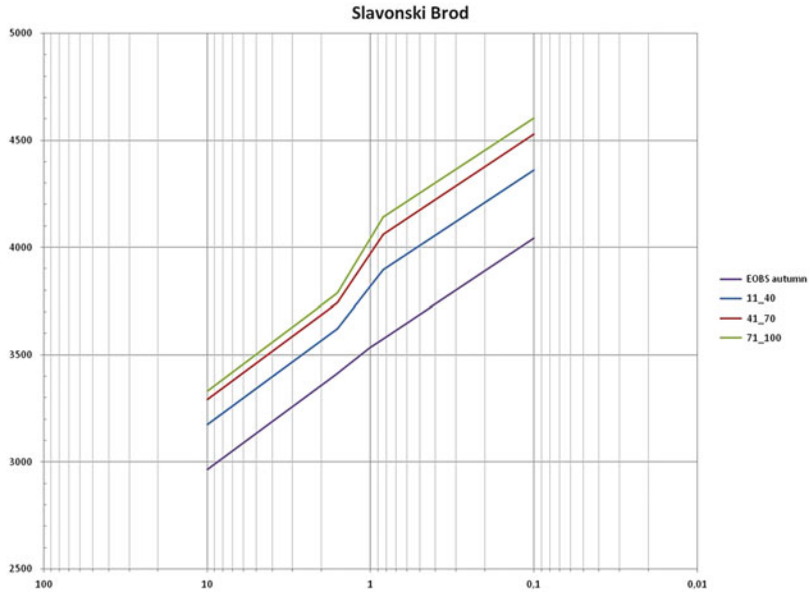

Fig. 12 Probability function (%) of peak discharges on WS Slavonski Brod for different periods of climate change forecast

Table 13 Probability of peak discharges on WS Slavonski Brod (m^3/s)

		E-OBS_20		E-OBS_100	
	10 %	1.62 %	1 %	0.84 %	0.10 %
1926–1965	2,966	3,411	3,535	3,573	4,041
2011–2040	3,175	3,623	3,825	3,895	4,360
2041–2070	3,291	3,743	3,975	4,062	4,530
2071–2100	3,332	3,788	4,050	4,142	4,605

change. The hundred-year return period of flood will increase, up to the year 2100, by 324 m^3/s, and the water level will increase by 82 cm.

The probability function for water station Slavonski Brod is in Fig. 12 and Table 13. The E-OBS_20 discharge has a probability of 1.62 % (instead of 5 %), and E-OBS_100 discharge has a probability of 0.84 % (instead of 1 %). The climate change values were then arranged in relation to the new estimated probability and in accordance with the basic relations from the report. New probability relations are estimated to be parallel to the basic ones published in the Prohaska report (2009).

The hundred-year return period discharges (1 % in Table 13) will increase from 8 % in the first period of 2011–2040 to 15 % in the last period of 2071–2100. The increase is similar to the one on the upstream WS Crnac. The hundred-year return

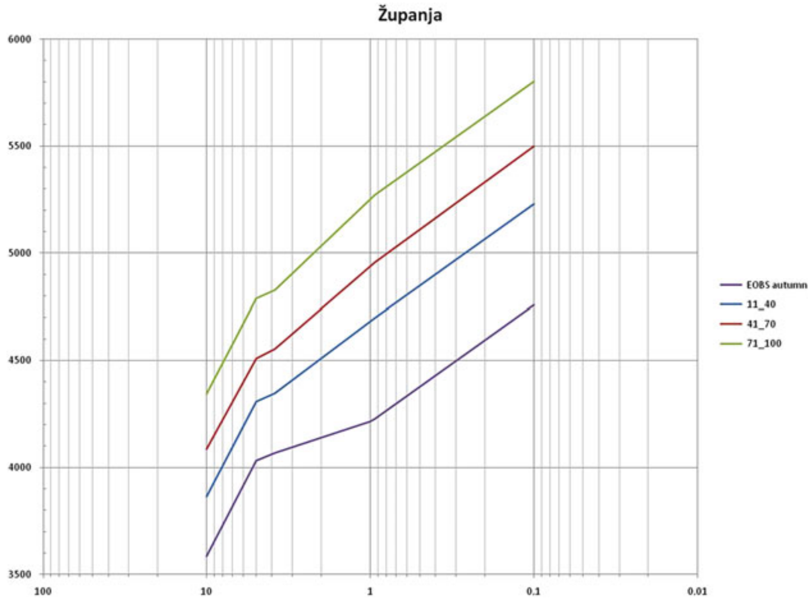

Fig. 13 Probability function (%) of peak discharges on WS Županja for different periods of climate change forecast

Table 14 Probability of peak discharges on WS Županja (m^3/s)

			E-OBS_20		E-OBS_100	
	10 %	5 %	3.85 %	1 %	0.94 %	0.10 %
1926–1965	3,585	4,031	4,068	4,215	4,227	4,759
2011–2040	3,863	4,309	4,346	4,687	4,699	5,231
2041–2070	4,086	4,510	4,554	4,945	4,957	5,500
2071–2100	4,343	4,789	4,826	5,268	5,270	5,802

period of flood will increase, up to the year 2100, by 515 m^3/s, and the water level will increase by 113 cm.

The probability function for water station Županja is in Fig. 13 and Table 14. The E-OBS_20 discharge has a probability of 3.85 % (instead of 5 %), and E-OBS_100 discharge has a probability of 0.94 % (instead of 1 %). The climate change values were then arranged in relation to the new estimated probability and in accordance with the basic relations from the report [19].

The hundred-year return period discharges (1 % in Table 14) in the WS Županja will increase from 11 % in the first period (2011–2040) to 25 % in the last period (2071–2100). The increase is higher than on the upstream WS Slavonski Brod. The hundred-year return period of flood will increase, up to year 2100, by 1,053 m^3/s, and the water level will increase by 181 cm.

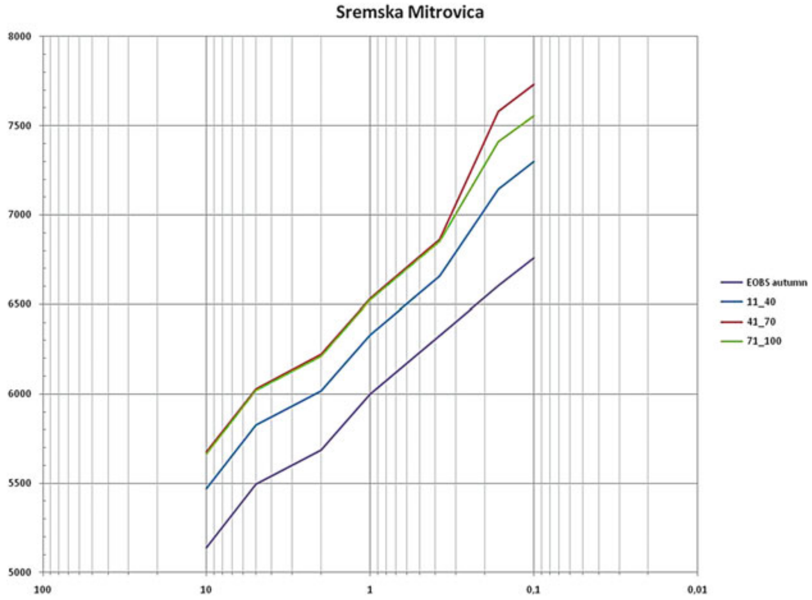

Fig. 14 Probability function (%) of peak discharges on WS Sremska Mitrovica for different periods of climate change forecast

Table 15 Probability of peak discharges on WS Sremska Mitrovica (m^3/s)

					E-OBS_20	E-OBS_100	
	10 %	5 %	2 %	1 %	0.38 %	0.16 %	0.10 %
1926–1965	5,140	5,495	5,687	6,000	6,328	6,603	6,760
2011–2040	5,471	5,826	6,018	6,331	6,659	7,143	7,300
2041–2070	5,674	6,029	6,221	6,534	6,862	7,580	7,731
2071–2100	5,666	6,021	6,213	6,526	6,854	7,410	7,556

The probability function for water station Županja is in Fig. 14 and Table 15. The E-OBS_20 discharge has a probability of 0.38 % (instead of 5 %), and E-OBS_100 discharge has a probability of 0.16 % (instead of 1 %). The climate change values were then arranged in relation to the new estimated probability and in accordance with the basic relations.

The breaks on the probability curves are caused by the logarithmic scale of probability on the abscissa. The hundred-year return period discharges (1 % in Table 15) will increase from 6 % in the first period (2011–2040) to 9 % in the last period (2071–2100). The increase is rather lower than on the upstream WS Županja. The hundred-year return period of flood will increase, up to the year 2100, by 526 m^3/s, and the water level will increase by 26 cm.

The discharges estimated as under the climate change impact are high but still much lower than the probability maximum flood of 7,081 m^3/s, calculated on the upper Sava for the Krško Nuclear Power Plant [20] and the discharge registered in 1896 on the lower part of the Sava River (in the extreme flood on the Drina River).

The process of reforestation decreases mean discharges on experimental river basin in Slovenia by 35 % [21]. The process of forestation will decrease flood discharges and mitigate the impact of climate change on floods in the Sava River basin. The process of reforestation should be researched in more detail for the Sava River basin as a whole.

On all water stations, the gradual increase of water levels of the 100-year return period floods over time is expected. The only exception is WS Sremska Mitrovica, where, at the first two periods up to year 2070, the water level rises and then it starts slightly to decrease. The largest increase in the level at the end of the century, i.e. more than 2 m, is expected in the upper part of the basin at WS Čatež. Downstream the Sava River, the water level rise is strongly reduced to 0.82 m at WS Crnac. Downstream of WS Crnac, the water level gradually increases up to 1.81 m at WS Županja. Then, downstream of WS Županja, the water level strongly drops to 0.27 m at WS Sremska Mitrovica. The modelling was derived from a model calibrated for the 1974 flood event when large construction on the system "Cenrealna Posavina" was not developed. The impact of the flood protection system "Central Posavina" and the impact of hydropower plant Mratinje on the Drina River could not be implemented in the model. The hydrological model presented seminatural conditions, without structures developed after 1974.

6 Conclusions

The reports on climate change impacts in the Sava River basin deal mainly with the average values of hydrological variables. All reports presented an expectation that in the future flood events will increase. There was no quantification of it [1–3, 5, 8, 9].

The E-OBS data set is useful for hydrological climate change forecasts of flood peak discharges in the Sava River basin. The assembly of data is not accurate enough on some parts of the basin, and additional improvements of the E-OBS data are required.

Climate change will increase peak discharges, mainly in the head part of the Sava River basin watershed. The peak discharges will increase by the end of the twenty-first century for the 100-year return period from 9 % at water station Sremska Mitrovica up to 55 % at water station Čatež.

There were some discrepancies in the Drina River basin that produced lower discharges in the forecast for the period 2071–2100 than those for the period 2041–2070. This also resulted in the lower discharge downstream of the confluence with the Sava River. Similar discrepancies, but not so strong, are presented on the following tributaries: Una River, Vrbas River and Bosna River.

The probability functions were derived for water stations, along the main stream of the Sava River, with an estimation of high flows up to the flows with the return period of 1,000 years. The climate change forecast was derived for the year periods 2011–2040, 2041–2070 and 2071–2100.

The impact of climate change on the water level forecasts with 100-year return period floods is quite high in the head part of the watershed, i.e. more than 2 m. Downstream, it first strongly decreases and then gradually increases up to 1.81 m and then drops tremendously to 0.27 m at water station Sremska Mitrovica.

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Climate Projections for the Sava River Basin

Andrej Ceglar and Jože Rakovec

Abstract Presented are climate change projections for the Sava river basin that follow from the ensemble of 16 combinations of the global climate models (GCM) and regional climate models (RCM). RCMs are normally configured to offer the optimal results for the region as a whole. Thus, they may have in some specific smaller domains also some systematic bias. Such eventual bias can be corrected by comparing the simulated values in smaller domain with measured values in that domain. That was done for the Sava river basin for precipitation amount and temperature for the twenty-first century and the results are presented for summer and winter conditions for two future standard climatological periods: 2011–2040 and 2071–2100 and compared with the reference period 1971–2000. In general, temperature is expected to increase over the basin area in all seasons, but the most pronounced increase can be observed for summer and winter. Precipitation is expected to decrease significantly in summer, whereas less pronounced decrease is expected in spring and autumn. Winter precipitation is expected to increase, especially in the northwestern part of the basin.

Keywords Climate model • Climate change • Bias correction • Sava river basin • Ensembles

1 Introduction

Reliable projections of weather variables from climate models are required for the assessment of future climate change impacts (e.g., flooding, drought, temperature-related mortality, crop yield). Assessments of such impacts are made by driving impact models with relevant weather variables from climate model simulations

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(e.g., precipitation cumulatives for flood assessment) (e.g., [1, 2]). In general it is necessary to adjust (calibrate) the simulated variables to correct for climate model biases rather than to drive impact models with raw climate model output (e.g., [3, 4]). Climate models are imperfect representations of reality; therefore, systematic discrepancies occur between climate model simulations and observations. Model discrepancies arise from many sources, such as structural uncertainty caused by representing the atmosphere by a finite number of variables, uncertainties in physical and sub-grid-scale parameterization schemes, and uncertainty in procedure to choose the model parameters [5].

1.1 The Role of Global Climate Models in Impact Assessment

The most common method to estimate the climate in the future is the use of global climate models (GCMs). GCMs represent the most important tool in the studies of climate variability and climate change (e.g., [6]). These models are state-of-the-art numerical coupled models that represent several subsystems of the Earth's climate (atmosphere, oceans, sea ice, land surface processes). GCMs should reproduce reasonably well climate features on large scales (global and continental), but their accuracy decreases when proceeding from continental to regional and local scales because of the lack of resolution. This is especially true for surface fields, such as precipitation and surface air temperature and their extremes, which are critically affected by topography and land use. At planetary scales, GCMs are able to simulate reliably the most important mean features of the global climate [7]. Also in these cases, with scales of a few grid distances, GCMs show deficiencies in simulating basic local climatic variables like surface air temperature and precipitation.

Outputs from GCMs cannot be used directly to force hydrological or other impact models without some form of prior bias correction, especially if realistic output is sought [4, 8, 9]. Outputs from GCMs are therefore downscaled, where a dynamical approach or statistical approach can be used [8, 9]. Dynamic and statistical downscaling techniques are often presented as mutually exclusive, but they can often be used together [10]. Statistical downscaling relies on the stationarity assumption regarding the relationship between local or regional climate variability and simulated climate variability on a large scale. This, however, is not a trivial assumption [11].

1.2 The Dynamical Downscaling: Regional Climate Models

Dynamical downscaling is a common procedure in meteorological numerical modeling; it was introduced in the 1970s (e.g., [12–14]) and is now used for several purposes; Žagar et al. [15] show an example of downscaling of low-level winds over complex terrain. In the case of climate studies, regional climate models (RCMs) represent the limited-area atmospheric models (LAMs) (e.g., [16, 17]) of spatial extent in the order of 10^7 km^2 with a spatial resolution better than ~20 km that use the large-scale fields simulated by the GCMs as boundary conditions. Regional characteristics, such as topography, are taken into account. An increased resolution in the region of interest may improve important aspects of the regional climate simulation. For instance, orographically induced precipitation and cyclonic activity at midlatitudes are better reproduced [5]. It is expected that an increased resolution may lead to better regional simulations [18]. Nevertheless, some systematic errors still remain. These are probably associated with the parameterizations of sub-grid processes, which are taken over from the parent GCMs, and with the large-scale errors of the coarse-resolution GCMs themselves [5].

There are several reasons for the failure of the models at the regional scale. The spatial resolution provides an inadequate description of the structure of the Earth's surface. The land-sea distribution is heavily smeared out and the mountains appear as broadened hills. For spectral models the truncated representation of the topography is also a source of additional difficulties, which may be severe at the local scale [5]. Also, the hydrodynamics of the atmosphere are nonlinear and the energy, which is fed into the system at the cyclonic scale, is cascaded through nonlinear interactions to the smallest scales. Because of the numerical truncation, this cascade is interrupted and the flow to the smallest scales is parameterized. These parameterizations affect the smallest resolved scales most strongly. The problem of the representation of the sub-grid-scale processes, such as cloud formation, rainfall, infiltration, evaporation, runoff, etc., is related to the model resolution as well. These processes have to be parameterized. Climate models can therefore be subject to parametric uncertainty induced by poorly confined model parameters of parameterized physical processes. Uncertain climate model parameters are typically calibrated in order to increase the agreement of the climate model with available observations over larger spatial scale. Manual adjusting of model parameters usually lacks objectivity and transparency in the use of observations. These shortcomings often haze model intercomparisons and hinder the implementation of new model parameterizations [19].

Different RCMs (as well as GCMs) offer different results, mainly due to different parameterizations of sub-grid processes and partly also due to other differences between the models, namely, RCM formulations and physiographic characteristics (topography, land/sea and land/lake contrasts, vegetation, surface albedo, soil type, and other fields related to such quantities) [20]. It is in principle not clear in advance which of the results is more reliable and which is less reliable. The most probable realization of the climate evolution often relies on the ensembles of models, where average and spread are computed based on the comparison of simulations of several models. Multi-model ensemble combination has become a standard technique to improve ensemble forecasts on all time scales, including climate time scales (decades or centuries). The multi-model ensemble can locally outperform a best-model approach, but only if the single-model ensembles are overconfident [21]. The reason is that multi-model combination reduces overconfidence (ensemble spread is widened) while average ensemble mean error is reduced. No single model is best at representing all climate processes and variables. Moreover, the quality of model results usually depends on location and time. It is therefore important to apply a weighting methodology, which is relevant to robustness and uncertainty in model performance. Impact assessment using RCM output should ideally use at least two or more RCMs forced by two or more GCMs to ensure that they do not undersample uncertainty [6].

1.3 The Uncertainty Cascade

Different sources of uncertainty should be addressed in the climate change impact studies. There are three major sources of uncertainty which enter into impact assessment at different stages of impact modeling: emission scenario, climate model structure, and parameterization schemes [22]. Simulations of RCMs are influenced by spatial and temporal resolution, numerical scheme, physical parameterizations, and boundary conditions [23]. Impact assessment models add a new source of uncertainty, which originates from the simulation of physical processes in the impact models.

The uncertainty cascade in impact studies can be addressed with an ensemble approach. The ensemble approach addresses the impact of climate change, whereby the uncertainties from CO_2 emission scenario, climate change scenarios, and physical processes in impact assessment models can be taken into account [24]. It is, however, very unlikely that any experiment ensemble can represent the full range of uncertainties related to the future greenhouse gas emissions and the choice of GCM and RCM. Furthermore, RCM simulations can be a subject to considerable biases when comparing the simulated control climate to observations. The use of these simulations on regional and local spatial scales to force the impact models can therefore result in unrealistic outputs [4, 8]. Methods which would allow a systematic calibration of climate model parameters are often not applicable to state-of-the-art climate models, especially due to computational constraints facing high dimensionality and nonlinearity of the problem [19]. Even though it is customary for climate modelers to present future global or regional temperature or precipitation changes in terms of relative changes, we still need a realistic representation of climate variables to force the impact models [1, 2]. RCM simulations over a subcontinental area, like the Sava river basin, should therefore be bias-corrected by statistical post-processing of simulated weather variables, which can increase their reliability as an input for impact models.

A realistic representation of precipitation fields in the future climate projections from climate models is crucial for impact and vulnerability assessment [1, 2, 25]. The resolution of RCMs is often not enough for most hydrological models;

thus they need to be further downscaled and bias-corrected [3], since most of RCMs are subject to a systematic error in precipitation. Systematic biases may result in too many days with very low precipitation intensity and too few dry days. Therefore, impact modelers often use bias-correction techniques that correct all ranges of the intensity histogram [4, 26]. This involves derivation of transfer functions from observed and simulated cumulative probability distributions. When applying a hindcast-derived correction to simulations of projected climate, we have to assume that the transfer function has the same form [27]. The transfer function between raw and corrected climate model simulations should therefore be robust, which is the case when it depends on fewer parameters to be derived from the data.

1.4 Bias Correction of RCM Simulations

A statistical bias-correction method was used in this study to correct simulated precipitation for systematic errors [27]. The method is based on adjusting cumulative probability distribution function of simulated precipitation to cumulative distribution function of observed precipitation. The fundamental assumption is that both observed and simulated daily precipitation probability distributions are well approximated by theoretical probability distribution.

Correction for precipitation was done simultaneously for precipitation frequency and intensity [9]. The correction includes truncating the RCM rainfall intensity distribution at a point that approximately reproduces the long-term observed relative frequency of rainfall and mapping the truncated RCM rainfall onto a gamma distribution fitted to the observed intensity distribution in the observation data set. We applied the two-step procedure for each of the 12 calendar months.

The frequency of daily RCM rainfall was corrected by fitting a threshold value to truncate the empirical distribution of the simulated daily RCM precipitation under the condition that the mean frequency of rainfall above the threshold matches the observed rainfall frequency. All simulated precipitation values below the threshold value were set to zero. The resulting time series of precipitation data were then used for correcting the precipitation intensity by mapping the cumulative distribution function (CDF) of simulated precipitation intensity onto the observed precipitation intensity distribution (Fig. 1).

A similar bias-correction procedure was applied to simulated daily temperatures as well. In this case, normal probability distribution was used to fit the temperature data. The bias-corrected temperature was calculated as the inverse of cumulative distribution function of observed temperature data at the value of cumulative distribution of simulated daily air temperature.

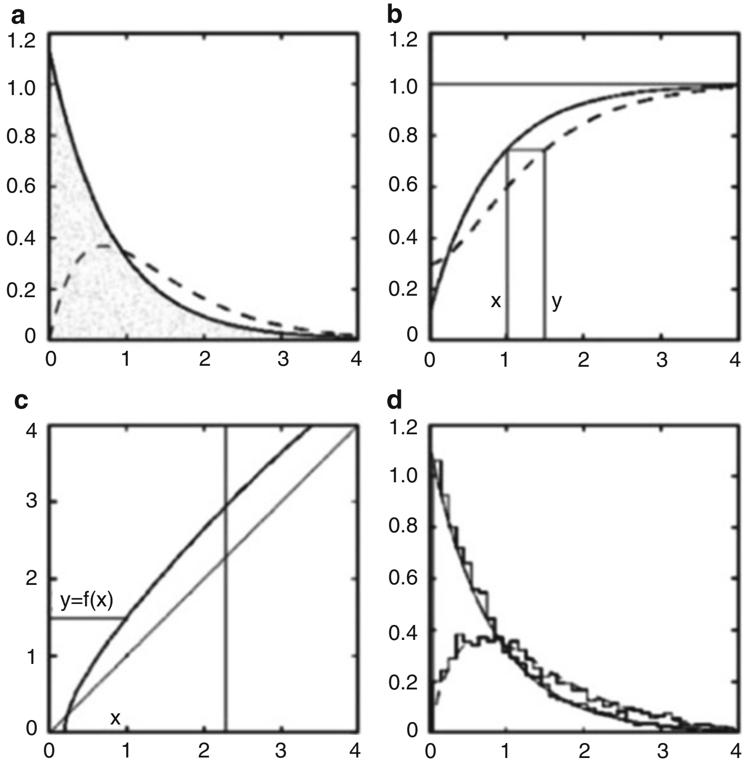

Fig. 1 Statistical correction applied to synthetic data set. (**a**) Synthetic probability density function (PDF) of simulated daily precipitation (*solid line*), synthetic PDF of observed daily precipitation (*dashed line*). (**b**) Cumulative distribution functions (CDF) obtained by integrating the corresponding PDFs in (**a**). (**c**) Transfer function obtained graphically from (**b**) by solving $CDF_{obs}(y) = CDF_{sim}(x)$ (*thick solid line*). (**d**) Histogram of synthetic data set given by the *x*-coordinate of points evenly scattered under solid PDF in (**a**) superimposed onto dashed PDF from (**a**) (*thin dashed line*) (after [27])

2 The Data

Meteorological data from simulations of 16 different ENSEMBLES GCM–RCM model runs [6] were used for preparation of projections (Table 1). The main value and core of the ENSEMBLES project was to run multiple climate models, which resulted in an ensemble of climate simulations over Europe. The ensembles method is known to improve the accuracy and reliability of forecasts. Using an ensemble of climate model simulations allows a systematic estimation of the uncertainty in climate projections. Different institutions therefore ran their RCMs with boundary conditions from five different GCMs (Tables 1 and 2). All simulations for the twenty-first century were done using only IPCC SRES A1B emission scenario [28], since it has been recognized that choice of the emission scenario is less relevant

Table 1 The pairs of GCM–RCM in ENSEMBLES simulations on a spatial resolution of 0.25° used in this study. For UKMO-HC GCM, there are standard, low-, and high-sensitivity runs (for details, see [6])

RCM–GCM	C4I RCA3	DMI HIRHAM5	ETHZ CLM	GKSS CLM	KNMI RACMO2	DNMI HIRHAM	UKMO HadRM	MPI REMO	SMHI RCA
UKMO low							1951–2100		1951–2100
UKMO std			1951–2100			1951–2050	1951–2100		
UKMO high	1951–2100	1951–2100					1951–2100		
MPIM ECHAM5		1951–2100			1951–2100			1951–2100	1951–2100
BCM		1951–2100				1951–2050			1951–2100
ARPEGE									
IPSL				1951–2050					

Table 2 ENSEMBLES GCM–RCM pairs and acronyms used in figures

RCM–GCM	C4I RCA3	DMI HIRHAM5	ETHZ CLM	GKSS CLM	KNMI RACMO2	DNMI HIRHAM	UKMO HadRM	MPI REMO	SMHI RCA
UKMO low							UKMO-HC_HadRM3Q3_HadRM3Q3		SMHI RCA Had CM3Q3
UKMO std			ETHZ_CLM			DKMI HIRHAM HadCM 3Q0	UKMO-HC_Had RM3Q0_HadRM 3Q0		
UKMO high	C4I RCA3_HadCM3Q16						UKMO-HC_HadRM3Q16_HadRM3Q16		
ECHAM5		DMI-HIRHAM5_ECHAM5			KNMI RACMO2			MPI-M-REMO	SMHI RCA ECHAM5
BCM		DMI-HIRHAM5_BCM				DKMI_HIRHAM_BCM			SMHI RCA BCM
ARPE GE		DMI-HIRHAM5-ARPEGE							
IPSL				GKSS-CCLM4					

until the middle of the twenty-first century [6]. The horizontal resolution of RCM simulations is 0.25° and they cover most of Europe. Simulations generally cover a time period between 1961 and 2100, with the exception of three model runs (Table 1), where the period 1961–2050 is covered. Simulations of two different meteorological variables were used in this study: daily precipitation and daily mean air temperature.

In addition, daily precipitation from the E-OBS data set [29] was used as a reference (observational) data set for comparison and bias-correction procedure. This data has been designed to provide the best estimate of grid box averages to enable a direct comparison with RCMs. E-OBS data set was defined on the same 0.25° grid resolution and covers the period between 1950 and 2012. Only the data between 1961 and 2010 were used in this study.

3 The Ensemble Climate Projections for Sava River Basin

Climate projections for the Sava river basin were calculated based on derived transfer functions for the period 1961–2000. Transfer functions were applied to climate projections for the twenty-first century from the ENSEMBLES RCM simulations. Two periods were used for assessing future climate change: 2011–2040 and 2071–2100. For each of the periods, absolute values for seasonal precipitation and extreme precipitation were determined as well as differences from the reference period (1971–2000) values. Results are provided in forms of images, where spatial distributions for the Sava river basin for each of the variables are shown.

3.1 Validation of Precipitation and Temperature Simulations

In the first step, the validation of corrected climate model simulations was performed. For each season, the mean daily precipitation was calculated as well as the mean seasonal precipitation from the raw climate model simulations and compared to the bias-corrected values. For this purpose, transfer functions were calculated for the period 1961–1990 and applied to RCM simulations for the period 1991–2010 (validation period). In general, raw model simulations underestimated the mean daily precipitation over the whole domain. The highest deviations can be observed in the northwestern part of the Sava river basin, where also the highest mean daily precipitation occurs. Ensemble spread indicates that the highest difference between models occurs in the northwestern part of the domain (including Julian Alps, Dinaric Alps, and Kamniško–Savinjske Alps in Slovenia) and along the Dinaric Alps toward the southeastern part of the basin. Highly complex orography prevails along that region, which influences precipitation occurrence and intensity in all seasons. Moreover, orography can locally significantly influence

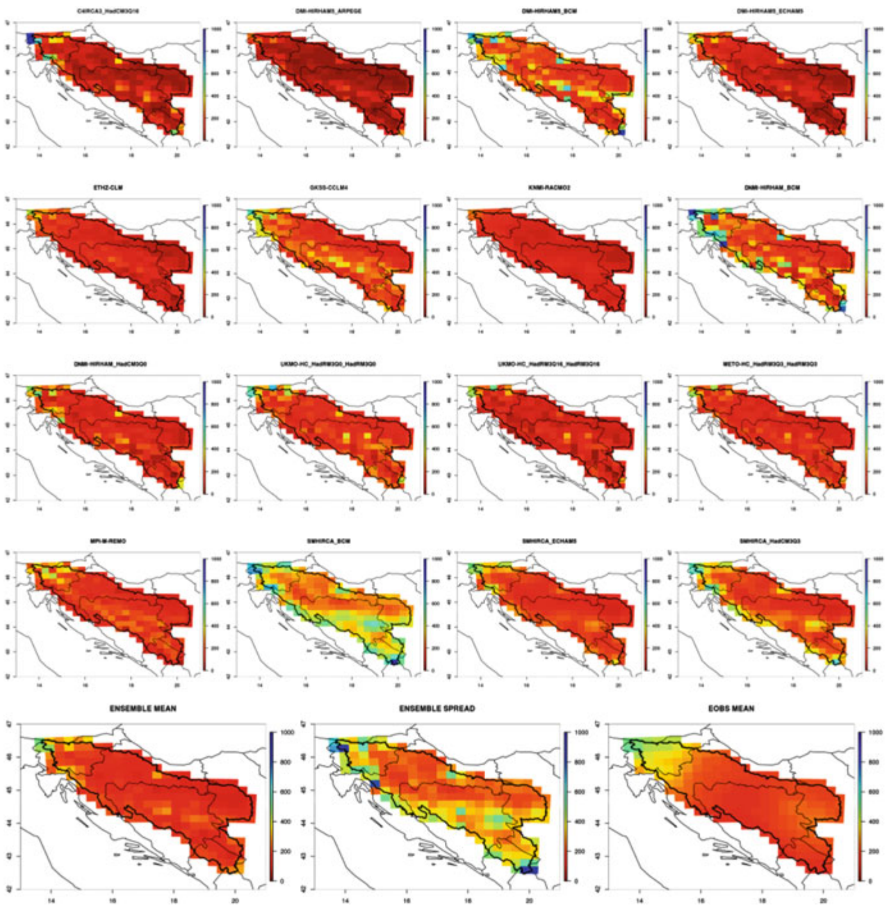

Fig. 2 Mean seasonal precipitation for summer for the validation period 1991–2010. Upper 16 panels show raw simulations with different climate model runs (see Table 3 for details); on the lowest three are ensemble mean, ensemble spread, and observed mean (E-OBS MEAN). On abscise and ordinate are geographical longitude and latitude in degrees; the unit for precipitation on all images is mm

climatic features of the region, which cannot be resolved in climate model simulations due to limited resolution. Ensemble mean (mean of 16 ensemble members) tend to underestimate daily precipitation as well, since all ensemble members systematically underestimate mean daily precipitation. Highest deviations between simulated and observed precipitation generally occurs in autumn.

Similar spatial patterns can be observed for seasonal precipitation. Models in general correctly reproduced east–west decreasing precipitation gradient over the basin, but significant differences occur between them. Model comparison reveals that models are generally underestimating the precipitation in autumn and summer (Fig. 2) in the northeastern part of the basin.

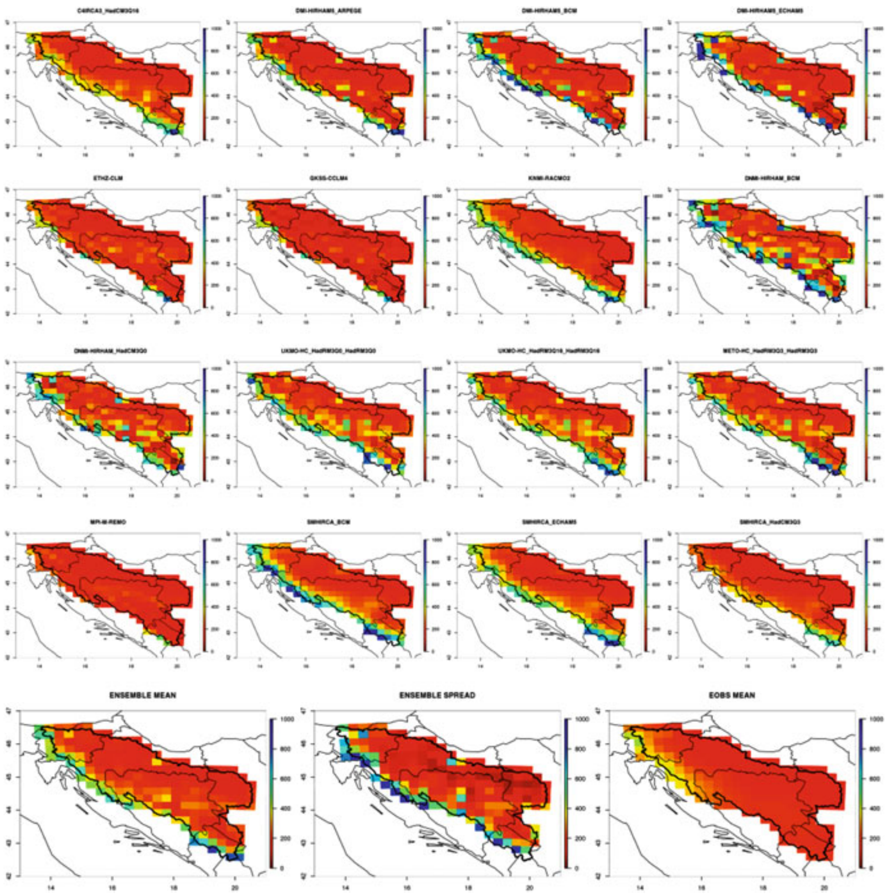

Fig. 3 Same as Fig. 2, but for winter

Seasonal precipitation is spatially highly variable, which is not the case with observed data. The highest ensemble spread can be seen again over the complex orography (western border of the basin), which was also the case with simulated daily precipitation. Ensemble mean most closely resembles observed values in all seasons, especially regarding the spatial precipitation variability. Models in general overestimate precipitation in winter (Fig. 3) and spring. We can conclude, according to the simulation of mean daily precipitation intensity on wet days in these seasons (it was underestimated), that the number of wet days in raw climate model simulations was overestimated.

Bias-correction procedure significantly improved the quality of modeled precipitation simulations over the basin, except over the central part of Bosnia and Herzegovina. This could be related to the stationarity of the bias-correction procedure; this is the main assumption, stating that the transfer function does not change in future climate. This assumption could be violated in the central part of Bosnia

Table 3 Information and references on ENSEMBLES RCMs

Institution	Acronym	Climate model	Reference
The Community Climate Change Consortium for Ireland	C4I	RCA3	[30]
Danish Meteorological Institute	DMI	HIRHAM5	[31]
Swiss Federal Institute of Technology	ETHZ	CLM	[32]
Spanish Meteorological Agency	AEMET	RCA3	[30]
The Royal Netherlands Meteorological Institute	KNMI	RACMO2	[33]
The Norwegian Meteorological Institute	NMI	HIRHAM	[34]
UK Met Office, Hadley Centre for Climate Prediction and Research	UKMO	HadRM3Q0 (3,16)	[35]
Max Planck Institute for Meteorology	MPIM	REMO	[36]
Swedish Meteorological and Hydrological Institute	SMHI	RCA3	[30]
National Centre for Meteorological Research	CNRM	RM4.5	[37]
International Centre for Theoretical Physics	ITCP	RegCM3	[38]
Czech Hydrometeorological Institute	CHMI	ALADIN	[39]

and Herzegovina, where the lowest improvement or slight worsening of the simulation quality was obtained after bias correction. Another possible reason for the low degree of improvement could be in the simulations of climate models (large-scale as well as convective precipitation). Since bias correction generally improved the quality of precipitation simulations over the Sava river basin, it was applied as well to raw climate model simulations for the twenty-first century.

The quality of bias-corrected temperature simulations was generally improved in spring, summer, and autumn, whereas in winter there were no significant differences across the basin. In spring, summer, and autumn, the highest degree of improvement can be seen for areas with a complex orography (northwestern part and western border of the basin). The highest improvement in quality can be observed in summer.

It has to be emphasized that an additional source of uncertainty can be introduced by interpolation of measured precipitation data into the reference grid (E-OBS data set), which are used for derivation of transfer functions for bias correction. The quality of interpolation depends on the station density as well as the interpolation technique, which is important especially over the highly complex orography. The station density over the Sava river basin is spatially highly variable (higher in the northern part and significantly lower in the southern part) [29]. Low station density over the southern part significantly affects the quality of interpolated precipitation, especially over the highly complex terrain of the Dinaric Alps.

4 Seasonal Climate Projections and Extremes

4.1 Seasonal Precipitation

Projections of seasonal precipitation were made for two periods: 2011–2040 (P1) and 2071–2100 (P2). Figures 4, 5, 6, and 7 show seasonal changes in precipitation during the two periods for summer and winter. Shown are ensemble mean changes (absolute and relative changes according to the reference period). Grid points, where at least 80 % of the models agree in the sign of change relative to the reference period, are marked with black dots. All projections were made using bias-corrected precipitation simulations, where transfer functions were calibrated for the period 1961–2000.

Pronounced precipitation changes are expected in the summer (Figs. 4 and 5). Precipitation is expected to decrease for 10 % in the southeastern part of the basin during the period P1. High ensemble spread can be observed for the same period in parts of central Slovenia, where the sign of change is uncertain. In the period P2 precipitation is expected to decrease between 20 % in the northwestern part and 40 % in the southeastern part of the basin. All models agree in the sign of change, even though a high ensemble spread can be observed in the northwestern part of the basin.

In winter, precipitation is expected to increase during the twenty-first century (Figs. 6 and 7), especially in the northwestern part of the basin (around 10 % in period P1 and 30 % in period P2). A significant increase of precipitation variability up to 40 % is expected over the basin toward the end of the century; moreover, models agree also in the sign of change. Less significant changes are expected in

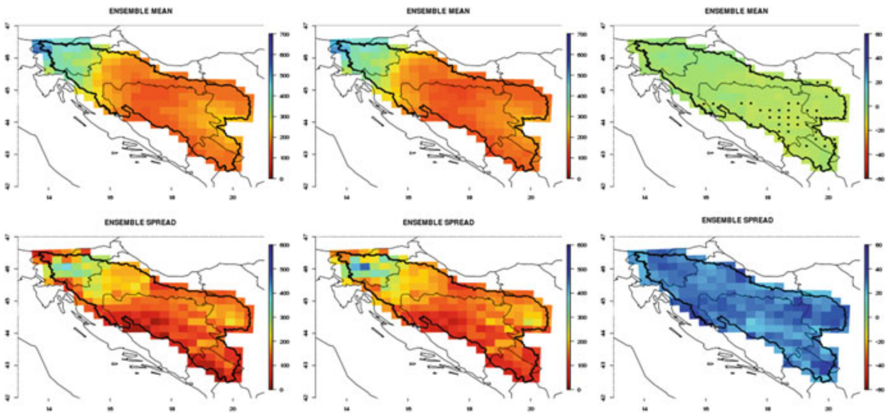

Fig. 4 Projections of summer precipitation. Shown are ensemble mean and spread for the reference period (*left column*, unit is mm), ensemble mean and spread for period P1 2011–2040 (*middle column*, unit is mm), and ensemble mean and spread of changes relative to the reference period (*right column*, unit is %). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

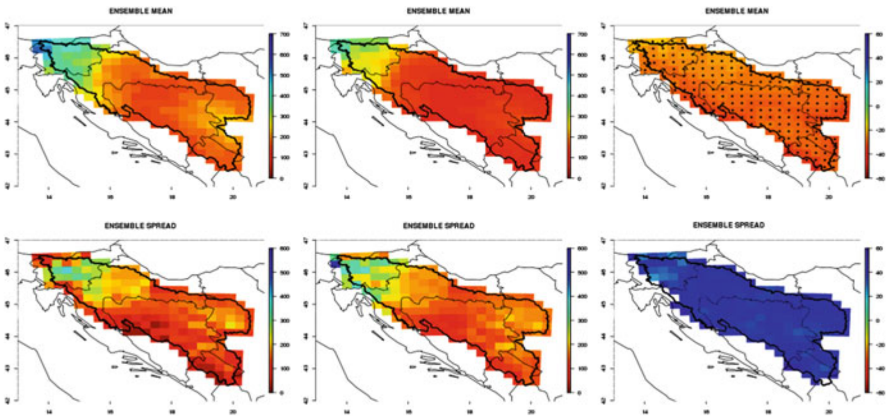

Fig. 5 Same as Fig. 4, but *middle* and *right panels* for period P2 2071–2100

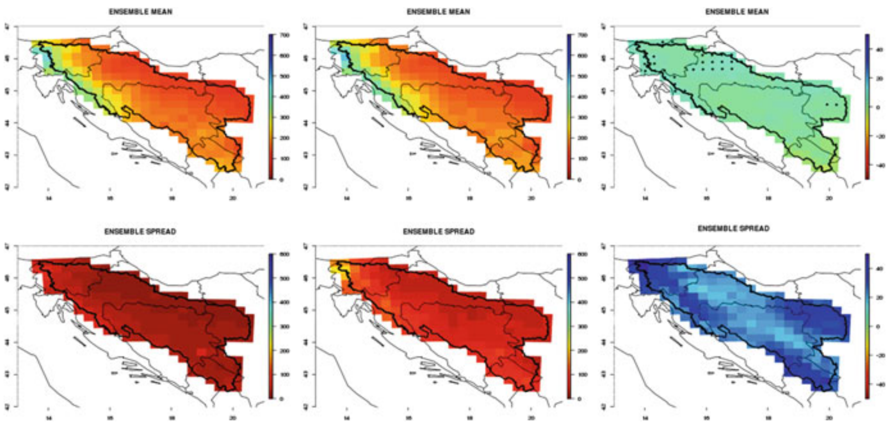

Fig. 6 Projections of winter precipitation. Shown are ensemble mean and spread for the reference period (*left column*, unit is mm), ensemble mean and spread for period P1 2011–2040 (*middle column*, unit is mm), and ensemble mean and spread of changes relative to the reference period (*right column*, unit is %). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

spring and autumn precipitation, especially during the period P1. High regional differences, however, exist between model simulations. The highest ensemble spread of projected values in all seasons can be observed in the northeastern part of the basin.

A different methodology was used by Jupp [40] to estimate the precipitation projections over the Sava river basin. The results of 24 GCMs were weighted according to their ability to simulate both the mean state and the variability of precipitation over the Sava river basin at the end of the twentieth century. The aim was to down-weight those models which simulate a climate whose mean value is far

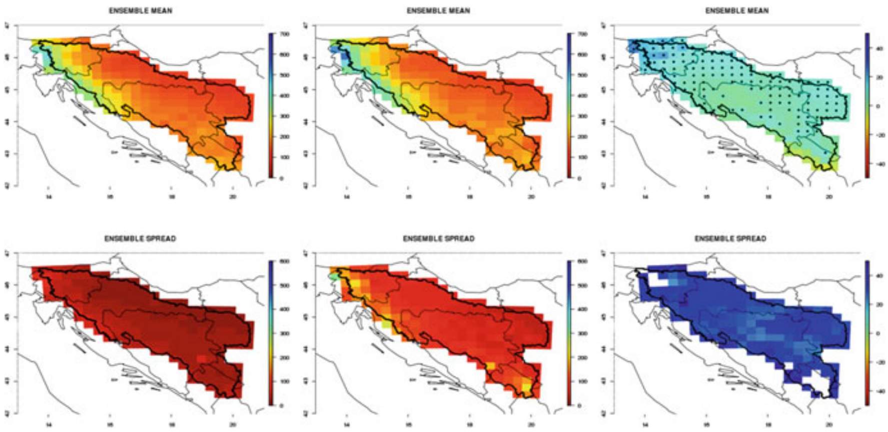

Fig. 7 Same as Fig. 6, but *middle* and *right panels* for period P2 2071–2100

from the observed mean or a climate whose statistical distribution is poor to the observed distribution. The projections were made for 29 locations in the Sava river basin. Results from Jupp's study indicate similar precipitation change patterns over the Sava river basin; the most significant changes are expected to occur during the summer, with decrease around 25 % during the course of the twenty-first century. Less certain results were reported for winter precipitation; some GCMs suggested an increase, whereas others a decrease of precipitation over the Sava river basin.

4.2 Seasonal Temperature

Figures 8, 9, 10, and 11 represent summer and winter near-surface air temperature projections for two periods in the twenty-first century. All models in the ensemble agree in the sign of temperature change for all seasons over the whole basin area. In spring, the mean temperature is expected to increase between 2 and 4 °C by the end of the century. The highest increase can be expected over the southern part of the basin.

Temperature increase in summer is the most pronounced; it is expected to increase already in the first period P1 2041–2070 for approximately 2 °C (Fig. 8). An even more pronounced increase is expected toward the end of the century (period P2 2071–2100): between 3 °C in the central part of the basin and 5 °C in southern part of the basin (Fig. 9). In autumn, temperature is expected to increase in period P2 between 2.5 °C in the central and 3.5 °C in the southern part of the basin. Strong warming can be observed also in winter, when temperature is expected to increase in period P1 for approximately 2 °C and in period P2 between 3 °C in the central and 4 °C in the southern part of the basin (Figs. 10 and 11). In all seasons, the highest model spread for projections can be observed over complex orography (northwestern part and western part of the basin).

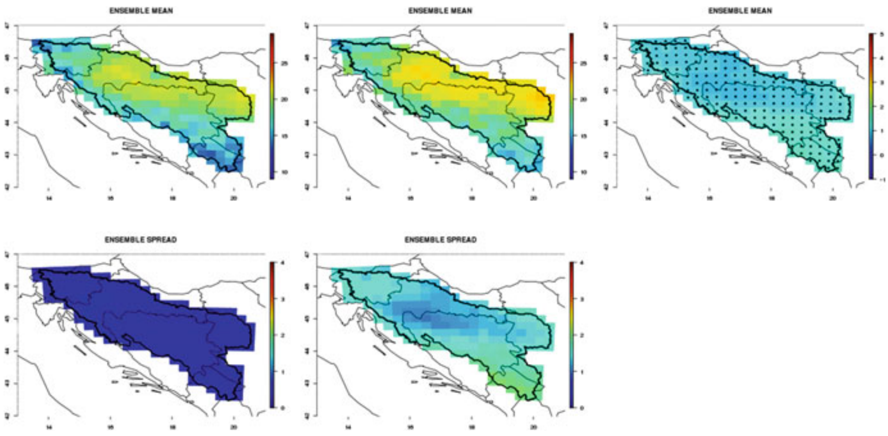

Fig. 8 Projections of summer temperature. Shown are ensemble mean and spread for the reference period (*left column*, unit is °C), ensemble mean and spread for period P1 2011–2040 (*middle column*, unit is °C), and ensemble mean of changes relative to the reference period (*right column*, unit is °C). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

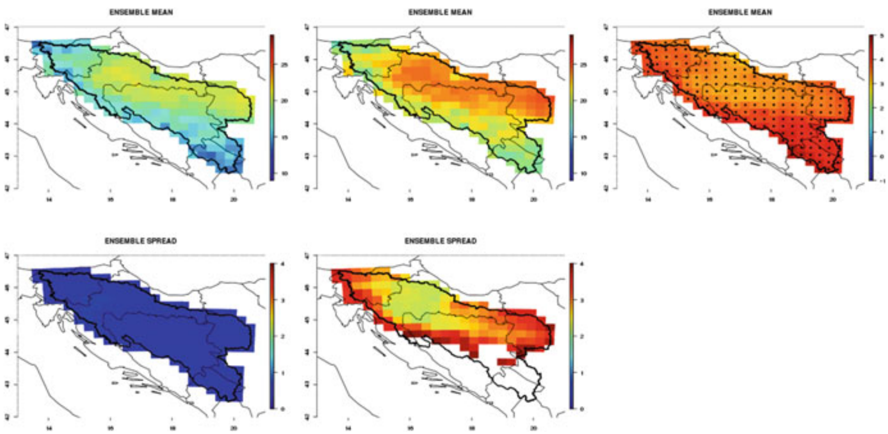

Fig. 9 Same as Fig. 8, but *middle* and *right panels* for period P2 2071–2100

4.3 Extreme Daily Precipitation Indices

Extreme precipitation events were characterized by 95th percentile of daily precipitation as well as maximum 24- and 48-h precipitation cumulatives for each season. Absolute values were calculated for two periods (P1 and P2) as well as changes relative to the reference period.

Figures 12, 13, 14, and 15 show 95th percentile of daily precipitation for summer and autumn. Each figure shows ensemble mean values for the reference period

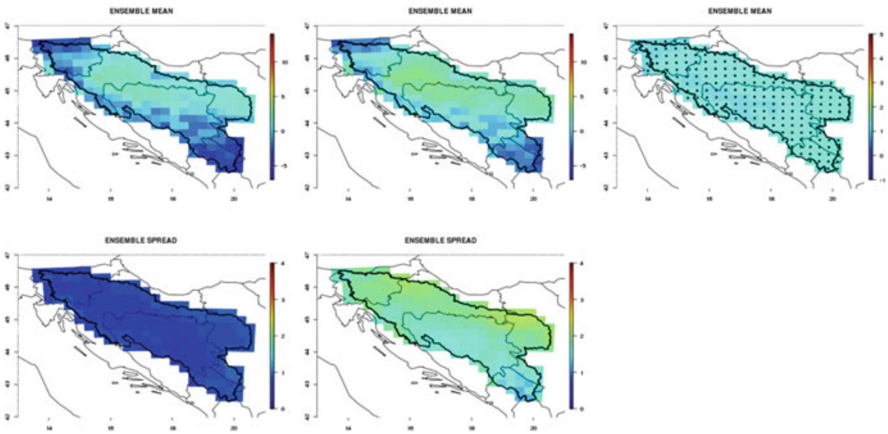

Fig. 10 Projections of winter temperature. Shown are ensemble mean and spread for the reference period (*left column*, unit is °C), ensemble mean and spread for period P1 2011–2040 (*middle column*, unit is °C), and ensemble mean of changes relative to the reference period (*right column*, unit is °C). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

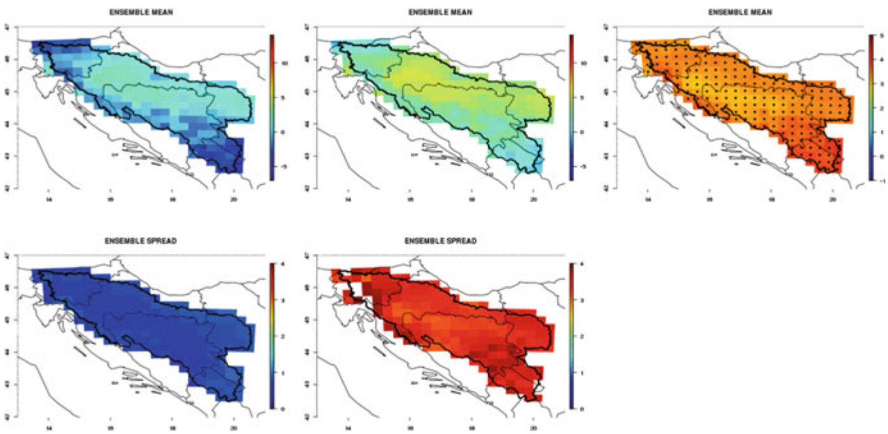

Fig. 11 Same as Fig. 10, but *middle* and *right panels* for period P2 2071–2100

(1971–2000) and scenario periods (P1 and P2) as well as relative changes according to the reference period.

In spring, 95th percentile values during the reference period ranged from 80 mm in the northwestern part of the basin to 20 mm in central Bosnia. 95th percentile of daily precipitation in spring is expected to increase throughout the twenty-first century. The ensemble mean indicates an increase of around 15 % in period P2 relative to the reference period. The change signal for 95th percentile of summer daily precipitation is less certain; it is expected to decrease in the western part of the

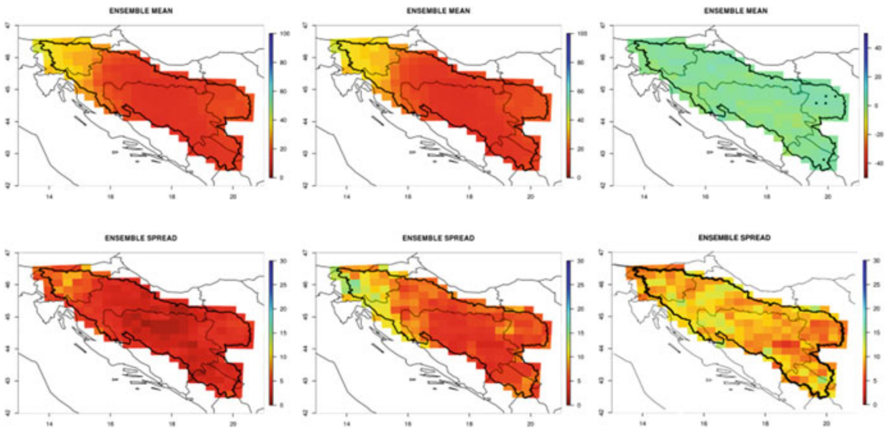

Fig. 12 Projections for 95th percentile daily precipitation on wet day in summer. Shown are ensemble mean and spread for the reference period (*left column*, unit is mm/day), ensemble mean and spread for period P1 2011–2040 (*middle column*, unit is mm/day), and ensemble mean and spread of changes relative to the reference period (*right column*, unit is %). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

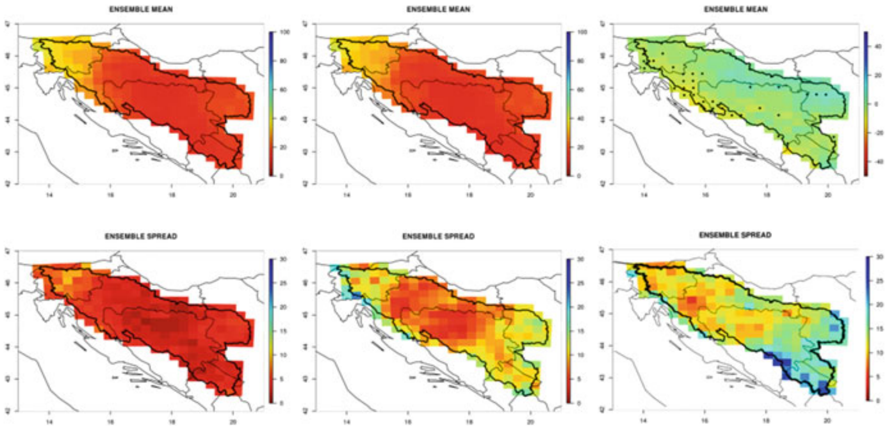

Fig. 13 Same as Fig. 12, but *middle* and *right columns* for period P1 2071–2100

basin toward the end of the century (approximately −10 %). The signal is less certain also in other parts of the basin, where models tend to disagree in the sign of change. There is an indication for an increase in the eastern part (Fig. 13) of the basin (for approximately 10 % in the period P2). In autumn, 95th percentile of daily precipitation is expected to increase toward the end of the century (Fig. 15). The change signal is very stable, since majority of models agree also in the sign of change for the whole basin. The most pronounced increase can be observed in the eastern and northern part of the basin (up to 30 % relative to the reference values). Similar change patterns are expected for winter, when 95th percentile of daily precipitation increases (up to 30 % at the end of the century).

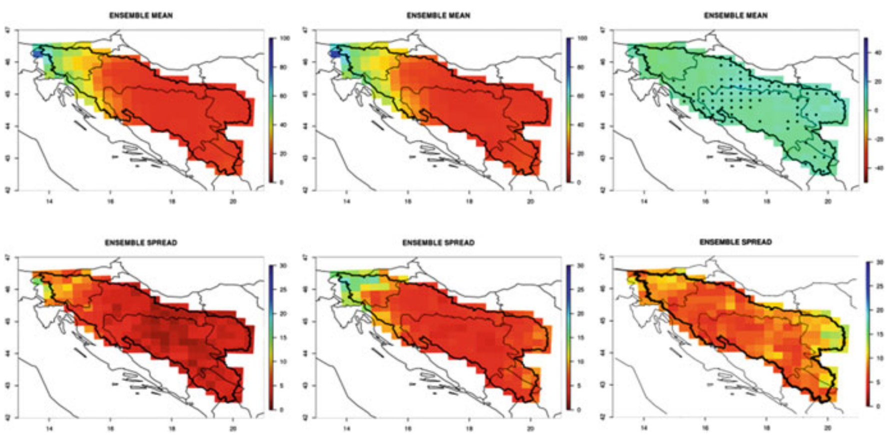

Fig. 14 Projections for 95th percentile daily precipitation on wet day in autumn. Shown are ensemble mean and spread for the reference period (*left column*, unit is mm/day), ensemble mean and spread for 2011–2040 (*middle column*, unit is mm/day), and ensemble mean and spread of changes relative to the reference period (*right column*, unit is %). Locations, where at least 80 % of models agree on the sign of change, are marked with a *black dot*

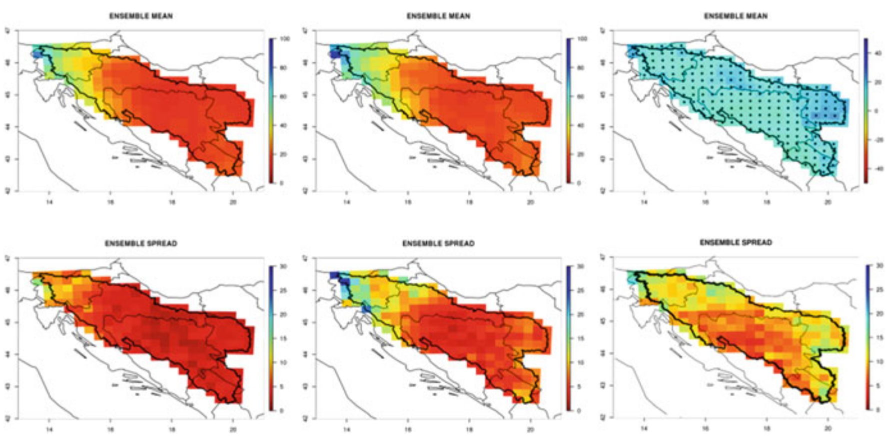

Fig. 15 Same as Fig. 14, but *middle* and *right columns* for period P1 2071–2100

Maximum 24- and 48-h precipitation is expected to increase throughout the twenty-first century in spring, autumn, and winter. Changes of summer maximums are spatially highly variable. In the western part of Croatia and Bosnia and Herzegovina, a decrease of up to −20 % is expected, whereas a slight increase (approximately +5 %) is expected in the northwestern part of the basin. Spatial patterns of changes are similar for 24 and 48 maximum precipitation. Highest ensemble spread can again be observed in the northwestern part of the basin.

5 Conclusions

ENSEMBLES climate model runs were used to produce climate projections for the Sava river basin. Statistical bias correction was used to correct raw model simulations for systematic biases. Validation procedure showed that statistical bias correction improved the quality of daily precipitation and temperature simulations over majority of the basin area and was dependent on the season. Transfer functions, derived for the period 1961–2000, were used to produce climate change projections for the basin area. In general, temperature is expected to increase over the basin area in all seasons (the most pronounced increase can be observed for summer and winter). Precipitation is expected to decrease significantly in summer, whereas a less pronounced decrease is expected in spring and autumn. Winter precipitation is expected to increase, especially in the northwestern part of the basin.

In general, the highest model simulation spread was observed over the most complex orography (Julian Alps, Kamniško–Savinjske Alps, and Dinaric Alps). This introduces some level of uncertainty in the simulation results over that area. In the future, climate model simulations of large-scale circulation patterns that influence the weather and climate in the basin should be verified. This will enable us to determine the primary causes of systematic model biases when simulating large-scale precipitation and other meteorological variables. A sensitivity study on convective parameterization schemes that are used in climate models to simulate sub-grid-scale convective precipitation would enable us to better understand and evaluate the uncertainty related to extreme precipitation events over the basin area. In addition, the impact of changing model resolution should be analyzed in the future climate modeling experiments.

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Integrated Approach to the Evaluation of Chemical Dynamics and Anthropogenic Pollution Sources in the Sava River Basin

Nives Ogrinc, Tjaša Kanduč, and David Kocman

Abstract A variety of approaches are presented to evaluating the geochemical dynamics and anthropogenic pollution sources of the entire Sava River Basin, a major tributary of the Danube River. The water chemistry is found to be controlled by the geological composition of the drainage area in the upper reaches of the river, influenced by agricultural activity and biological processes in the middle reaches, and related to industrial impact in the lower reaches. The Sava exported 1.9×10^{11} mol C year^{-1} as dissolved inorganic carbon (DIC) and emitted 2.5×10^{10} mol C year^{-1} to the atmosphere. Carbon isotope composition indicates that up to 42 % of DIC originated from carbonate weathering and 23 % from degradation of organic matter. Agricultural and industrial sources are shown by statistical analysis to contribute significantly to the increase in Na^+, K^+, Cl^-, SO_4^{2-} and NO_3^- concentrations in stream waters. Nitrate inputs are controlled by land use, and the elevated isotope composition of nitrate at some sites is attributed to sewage and/or animal waste. Contamination of suspended particulate matter by selected elements (Cu, Ni, Zn, Cd and Pb) in the main channel of the Sava River is low, while higher concentrations were observed in the main tributaries (Una, Vrbas, Bosna and Drina) due to industrial, mining and smelting activities.

Keywords Weathering • Pollution • Stable isotopes • Trace elements • Sava River Basin

List of Abbreviations

ARSO	Slovenian Environment Agency
BA	Bosnia and Herzegovina
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
EIONET	European Environment Information and Observation Network
GIS	Geographic Information System
HR	Croatia

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ICP-MS	Inductively coupled plasma mass spectrometer
IEA	Integrated environmental assessments
ME	Monte Negro
MRT	Mean residence time
N	Nitrogen
P	Phosphorous
POC	Particulate organic carbon
RS	Serbia
SARIB	Sava River Basin: Sustainable Use, Management and Protection of Resources
SEM/EDS	Scanning electron microscopy/energy dispersive spectroscopy
SI	Slovenia
SPM	Suspended particulate matter
SRB	Sava River Basin

1 Introduction

Sustainable water management is a critical element of the "green economy", a concept adopted within the Europe 2020 strategy, because healthy and resilient ecosystems provide the services needed to sustain human well-being and our economy [1]. Freshwater ecosystems are particularly important; however they are increasingly pressured by a multitude of environmental stressors. General drivers of anthropogenic stress impacting freshwater quantity and quality are various catchment disturbances, pollution, water resource development and different biotic factors [2]. These stressors are further affected by climate changes influencing general hydrometeorological conditions. Given the increasing multiplicity of environmental stressors associated with global change, there is an urgent need to better understand their effects on the freshwater ecosystem and thus to better predict the response of the latter to future environmental changes.

One of the most significant challenges for the science of global change is to determine how hydrological and biogeochemical cycles function at the land surface on regional to continental scales, where river basins are natural integrators of surface processes, and how human activities may influence their functioning. River water geochemistry is, to a large extent, a product of the interplay between lithology, climate and land use. It provides important information on chemical weathering of bedrock/soil and natural and anthropogenic processes that may control the dissolved chemical loads [3–7]. In a carbonate-dominated terrain, it is crucial to precise the contributions of different sources of water solutes and to estimate weathering rates of the continental crust and the associated CO_2 consumption [8, 9]. Rivers also reflect the biogeochemical processes occurring in their catchments, thus help material transport from land to oceans to be quantified [10]. Andersson et al. [11] estimated that the coastal ocean currently receives $\sim 1 \times 10^{15}$ g of inorganic and organic C year^{-1} from terrestrial sources, a significant part of the global C budget. The natural balance of chemical species can be influenced strongly by anthropogenic additions from domestic, agricultural and industrial activities. These anthropogenic stressors, from both point and diffuse sources, compromise the quality of water resources, particularly by microbial [12–14], sediment (e.g. [15, 16]) and nutrient (e.g. [17, 18]) pollution and, in particular, by contamination with pesticides and heavy metals (e.g. [19]). Diffuse pollutants pose a particular problem because they are hard to detect and their fluxes are highly variable in time [20–22].

Studies of river waters were initially focused on concentrations of particulate and dissolved constituents, enabling calculation of fluxes and mass balances for entire watersheds ([7] and references therein). Over the last few decades, data on inorganic and organic constituents have been complemented by isotope tracer measurements, including stable carbon and nitrogen isotopes. Stable carbon isotopes have been used mainly to determine the sources of dissolved inorganic or particulate organic carbon [7, 23–33], while stable nitrogen isotopes have been used to identify the sources of nitrate in surface waters [34–39].

The Sava River Basin (SRB) is an excellent area on which to investigate both natural and anthropogenic inputs influencing the chemical dynamics of a riverine ecosystem. Previous studies have indicated that the upper reaches are largely regulated by rates of high carbonate mineral weathering, the middle reaches by agricultural activity and biological processes related to eutrophication, and the lower reaches are influenced mainly by stressors related to high pollution from industrial processing, along with untreated municipal wastewater discharges [40]. In this chapter the following issues are addressed: (1) carbonate weathering and its impact on stream water carbonate geochemistry and geochemical fluxes, and determination of the sources of carbon using the stable carbon isotope approach, (2) nitrate pollution and its sources and (3) the extent of pollution with selected trace elements in suspended particulate matter. The aim was to understand the river water solute chemistry and anthropogenic impacts on the SRB by applying an integrated approach using geochemical analysis and specific geochemical methods (stable isotope techniques) in combination with the advantages of the Geographic Information System (GIS) as a tool for mapping and spatial data analysis.

2 Sava Catchment Characteristics

The characteristics of the SRB are described in more detail in earlier chapters; a brief description only of specific parameters related to our study such as climate, geology and land use is presented here.

The Sava is a river in southeast Europe, a right-side tributary of the Danube River discharging in Belgrade. It is 990 km long, including the 45 km Sava Dolinka headwater rising in Zelenci, Slovenia, and covering 97,713 km^2 of surface area. It flows through Slovenia, Croatia, along the northern border of Bosnia and Herzegovina and through Serbia. Its central part is a natural border of Bosnia–Herzegovina and Croatia. The Sava is considered to be the northern border of the Balkan Peninsula. It belongs to the Black Sea drainage basin and, together with Sava Dolinka, is the third longest tributary of the Danube. It drains a significant portion of the Dinaric Alps region, through the tributaries of Krka, Kupa, Una, Vrbas, Bosna and Drina. The Sava River flows through a variety of landscape types, including Alpine, karstic, deep river valleys and shallow Pannonian flats.

One of the most heterogeneous parameters is climatology, by which SRB is divided into three climatic areas: Alpine, Pannonian and Continental. Mean annual precipitation and temperature vary over the length of the river. In the upper reaches (Alpine headwater), the mean annual precipitation is in the range of 2,000–3,000 mm $year^{-1}$, with a mean annual temperature of approximately 6 °C. At the confluence of the Sava with the Danube, annual precipitation decreases to around 660 mm per year and mean annual temperatures increase to about 13 °C. The average discharge of the Sava increases downstream from 84 $m^3\ s^{-1}$ at Ljubljana to 255 m^3s^{-1} at Zagreb to 1,722 $m^3\ s^{-1}$ at Belgrade. Following trends in rainfall data, maximum flows are typically recorded in spring and low flows in autumn.

The watershed of the Sava has a heterogeneous geological composition. Along its flow from the source to the confluence with the Danube, it accumulates alluvial sediments of Holocene age. The Sava watershed is composed of Permo-Carbonian shales, Jurassic and Cretaceous rocks and Paleogene, Neogene and Holocene sediments composed of clastic rocks (e.g. conglomerates, siltstones, mudstones, sandstones). A simplified geological map of the Sava watershed is presented in Fig. 1.

Land use of the SRB is diverse and complex, reflecting the differences in relief, climate and stream flow (Fig. 2). The greatest population density is located near large cities while agriculture is the dominant activity in the Croatian and Serbian parts of the watershed. The upper part of SRB in Slovenia is mainly covered by forests (more than 50 %) with mountains above the tree line (35 %). In Croatia, forests cover 25 % and agriculture more than 40 % of the basin area. The Bosnian part of the Sava basin has valleys and hills with about 30 % agriculture and 20 % forest. The majority of the watershed area in Serbia is used for agriculture. Thermo- and hydroelectric plants, oil and gas refinery stations with pipelines, metallurgical, chemical and textile industries and mining (heavy metals and salts) comprise the anthropogenic impacts in the watershed.

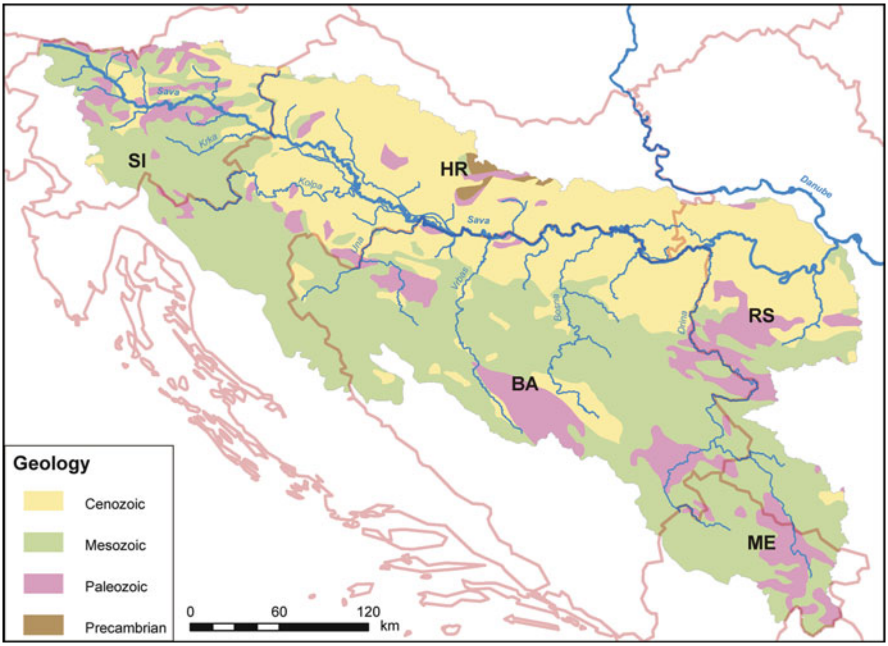

Fig. 1 Geology of the Sava River Basin (adapted from a digital map compiled by United States Geological Survey—USGS [41])

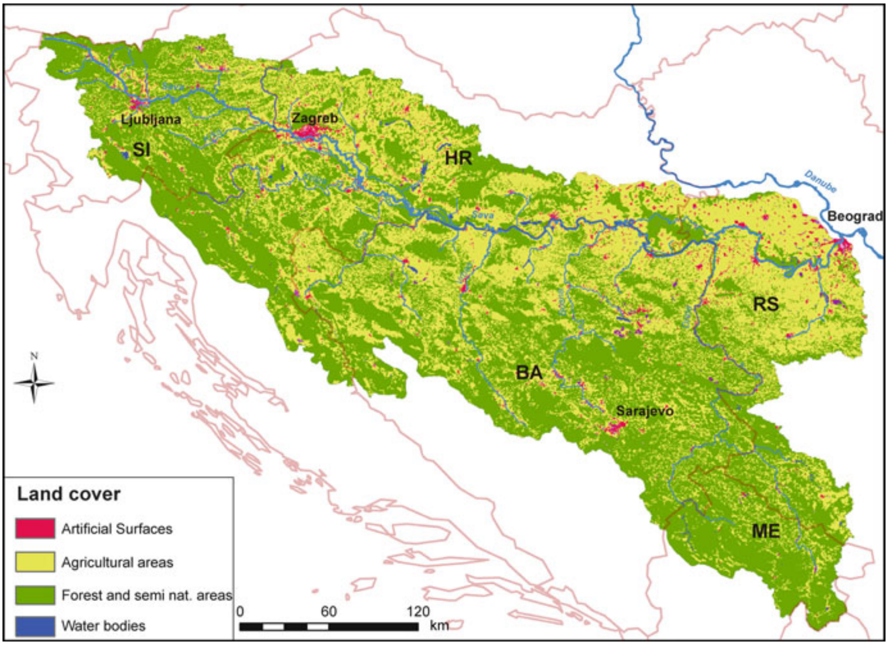

Fig. 2 Land cover of the Sava River Basin (*source*: CORINE Land Cover obtained from http://www.eea.europa.eu/data-and-maps/ [42])

3 Data Collection and Computational Methods

Discharge data were obtained from the Environmental Agency of the Republic of Slovenia and related national institutions for monitoring programmes in Croatia, Bosnia and Herzegovina (BA) and Serbia at gauging stations. Daily averaged flow rates ($m^3\ s^{-1}$) and concentrations of NO_3^- from 2001 to 2011 at two different locations (Jesenice na Dolenjskem in Slovenia—location 15 and Županja in Croatia—location 27, Fig. 3) were provided by the Slovenian Environment Agency (ARSO; URL: http://www.arso.gov.si/en/) and Hrvatske vode, respectively. Other long-term data were obtained from the European Environment Information and Observation Network (EIONET) [43].

Between 2003 and 2006 a new study was developed in the framework of Slovenian-American cooperation with the University of Michigan and the European Project SARIB (six EU Framework Programme) in order to generate a large database on physico-chemical parameters and chemical contaminants spread into the SRB. The first investigation was based on the mineral weathering study in the upper Slovenian part of the SRB between 2003 and 2005. Sampling locations

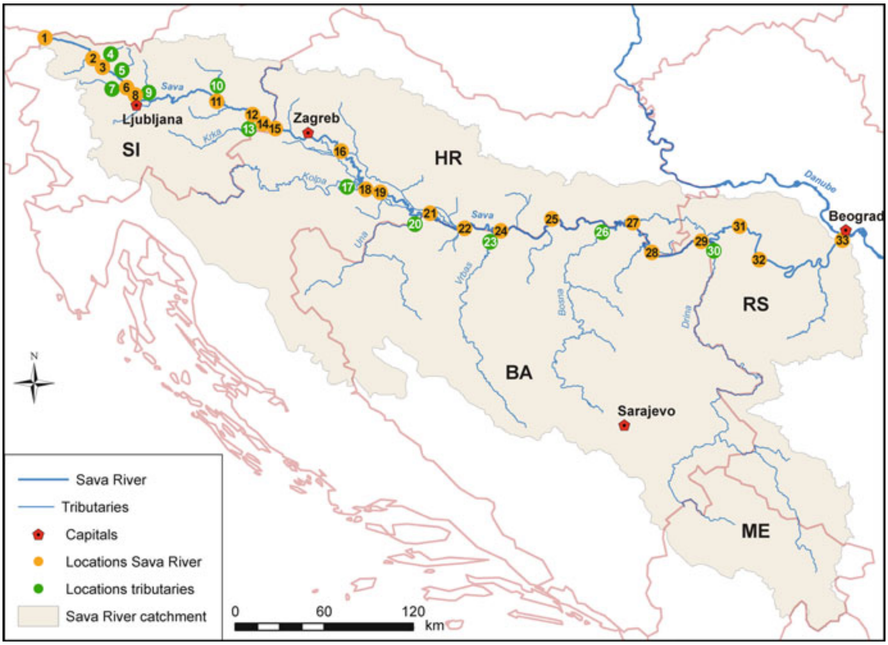

Fig. 3 Map of sampling locations in the SRB. The sampled locations on the Sava were as follows: 1. Zelenci (Sava Dolinka source), 2. Šobec, 3. Otoče, 6. Smlednik, 8. Tacen, 11. Radeče, 12. Brežice, 14. Mostec, 15. Jesenice na Dolenjskem, 16. Oborovo, 18. Črnac, 19. Lukavec, 21. Košutarica, 22. Gradiška, 24. Srbac, 25. Slavonski Brod, 27. Županja, 28. Brčko, 29. Bosanska Rača, 31. Sremska Mitrovica, 32. Šabac, 33. Beograd. The following tributaries were sampled: 4. Tržiška Bistrica, 5. Kokra, 7. Sora, 9. Kamniška Bistrica, 10. Savinja, 13. Krka, 17. Kolpa, 20. Una, 23. Vrbas, 26. Bosna, 30. Drina

were established along the Sava to capture the influence of the tributary streams and at discharge gauge locations. Tributary streams were selected based on their contribution of discharge or drainage area to the main Sava and were sampled near the discharge point to the Sava together with upstream sampling. Sampling from the sources of the Sava to Belgrade at its confluence with the Danube was performed at 33 selected locations, 22 on the main river and 11 on the Sava tributaries (Fig. 3), during autumn 2005, spring and autumn 2006 to capture seasonal variations in discharge.

The detailed procedures of field sampling and analyses are described in related studies. Szramek et al. [44] contrast the geochemistry and weathering fluxes from bedrock-dominated, geologically distinct watersheds in Slovenia with those in the glaciated mid-continent of the St. Lawrence, while a more precise weathering study in Slovenia was published recently [45]. Stable isotope analysis of dissolved inorganic carbon and particulate organic carbon and total nitrogen was used to describe their sources in more detail in Slovenia [30, 46, 47] and the whole SRB [48]. Isotopes of sulphur were used to identify sulphur sources and transformations along flow pathways in SRB, and hence stable isotope sulphur analysis, as described in [49]. Trace elements in suspended particulate matter, comprising Cd, Pb, Zn, Cu, Ni, Cr and As, were measured using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7500ce, Tokyo, Japan) under optimized measurement conditions, following microwave digestion of samples in a mixture of nitric, hydrofluoric and hydrochloric acids [50, 51].

For thermodynamic modelling, the PHREEQC for Windows program was used to calculate partial pressures (pCO_2) and saturation indexes (SI) of minerals. The available datasets were evaluated statistically to yield objective information about the various complex processes occurring in the SRB. Principal component analysis (PCA) and factor analysis (FA) were applied to the complete dataset using STATISTICA v. 7.0, StatSoft, Inc. (2001) [40].

4 Chemical Dynamics, Weathering Fluxes and Sources of Carbon in the Sava River Basin

Chemical weathering is an important process controlling atmospheric CO_2 sequestration in the terrestrial environment [7] and has a prominent effect on the geochemical composition of inland waters. While silicate weathering is considered to be the principal process for removing CO_2 from the atmosphere on a long-term scale, carbonate weathering plays a more important role on carbon cycling on a short-term scale [52]. It was shown that, due to their higher dissolution rate, carbonates are more sensitive to environmental and climatic changes, the rate being closely correlated with precipitation, temperature, soil thickness and vegetation [53]. Thus, watershed adjustments, in response to climate change, will probably be most evident in the smaller headwater streams such as the upper Slovenian part of the SRB.

4.1 Chemical Dynamics and Weathering Fluxes

The major solute composition of the Sava and its tributaries is dominated by HCO_3^-, Ca^{2+} and Mg^{2+}. In the upper Slovenian part of the SRB, the data indicate that the total ion contribution from pollution sources such as agriculture, industry and atmospheric depositions is minimal and, except in rare cases, can be considered as negligible. Therefore, these areas represent ideal locations at which to examine watershed scale mineral weathering.

Dissolved Ca^{2+} and Mg^{2+} are supplied largely by the weathering of carbonate minerals, with smaller contributions from silicate weathering. Within the SRB, silicate mineral weathering is limited, comprising less than 5 % of HCO_3^-. It was found that only the Savinja watershed in Slovenia could be influenced by both silicate and carbonate weathering [45]. A low silicate weathering contribution of 7 % was also observed on the headwaters of the Danube [54].

The chemistry in the Slovenian part of the SRB falls into two distinct groups that are close to the ideal stoichiometry of carbonate dissolution. The regions of the watershed draining, predominantly Alpine areas, have compositions between 0.6 and 1.8 mmol l^{-1} $Ca^{2+} + Mg^{2+}$ and 1.2 and 3.6 mmol l^{-1} HCO_3^-, while lower parts have higher ionic loads, with $Ca^{2+} + Mg^{2+}$ concentrations ranging between 1.9 and 2.8 mmol l^{-1} and HCO_3^- concentrations between 3.5 and 5.5 mmol l^{-1}. Slovenian streams exhibit a wide range of Mg^{2+}/Ca^{2+} ratios, ranging from 0.2 to nearly 0.8, indicating that dolomite weathering contributes, on average, about 60 % of the HCO_3^- [44]. The HCO_3^- concentrations were observed to increase downstream of the main channel and the tributary watershed, indicating the influence of increased soil thickness and alluvium as the watershed changes from Alpine to Dinaric-karst regions. The SRB streams are saturated or supersaturated with calcite, and increase in calcite saturation, resulting from degassing, can lead to possible carbonate precipitation. However such potential removal of carbonate was minimal and did not affect the total HCO_3^- flux. The discharge ratios between low and high flow in the Alpine region are 1:100, while downstream in the Dinaric-karst watershed a smaller range of discharges was observed, typically less than 15 times the mean discharge (data from 1961–2011 [55]). The increased range of discharges for the Alpine watersheds also influences the carbonate weathering intensity (mmol km^{-2} s^{-1}), which is about two times higher in Alpine regions than in the downstream areas. Importantly, the Alpine regions are also able to maintain consistent HCO_3^- and Mg^{2+} concentrations over a wide range of discharges due to high specific runoff in the Alpine region and rapid dissolution of carbonate minerals. Typically, in these settings carbonate mineral solubility is limited by water volume rather than mineral contact time. The Sava streams in Slovenia have carbonate weathering intensities 7–18 times greater than the world average of 7 mmol HCO_3^- km^{-2} s^{-1} and Mg^{2+} intensities 6–15 times greater than the world average of 2.2 mmol Mg^{2+} km^{-2} s^{-1}. A high carbonate weathering intensity of 13 mmol HCO_3^- km^{-2} s^{-1} was also observed at the mouth of the Sava in Serbia (Sava Belgrade), constituting nearly 50 % of the carbonate weathering intensity of the Danube (25 mmol HCO_3^- km^{-2} s^{-1}). These data demonstrate the importance of the contributions of temperate landscapes to the global integrated riverine fluxes of Ca^{2+}, Mg^{2+} and HCO_3^-.

4.2 Carbon Cycling in the Sava River Basin

Further chemical and stable isotope approaches were used to study the carbon dynamics in the SRB downstream to the Danube. Riverine CO_2 concentrations were up to forty times supersaturated with respect to atmospheric equilibrium, resulting in a large CO_2 emission into the atmosphere. The total CO_2 efflux for the Sava at Belgrade ranged between 4.97×10^7 and 3.1×10^8 mol day^{-1} in spring 2006 and between 3.18×10^7 and 1.98×10^8 mol day^{-1} in autumn 2006, representing between 6 and 19 % of the river's DIC transport. The overall annual DIC flux was estimated to be 1.9×10^{11} mol C. Thus the Sava contributes ~0.7 % of the global river carbon flux of 2.67×10^{13} mol C day^{-1} [56] and 23 % of the annual DIC flux of the Danube [48]. The isotope composition of DIC reflects biogeochemical processes (degradation of organic matter, exchange with atmosphere and dissolution of carbonates) in the river system. The isotope mass balance calculation was first used to quantify sources of DIC at the Sava mouth in Slovenia [30]. The process of photosynthesis was considered insignificant and therefore excluded from the mass balance calculation. The major inputs to the DIC flux originate from tributaries, degradation of organic matter, exchange with the atmosphere and dissolution of carbonates. It was calculated that the most important biogeochemical process at the Sava in Slovenia was the dissolution of carbonates, followed by degradation of organic matter, exchange with the atmosphere being less significant. According to the isotope mass balance at the Sava mouth at Belgrade, tributaries constituted up to 60 %, and thus the major input, to DIC flux. Other processes influencing the production of DIC include carbonate dissolution, contributing between 32 and 42 % of DIC, and respiratory CO_2 from degradation of organic material, contributing between 20 and 23 % [48].

The annual organic carbon flux was lower than the DIC flux and divided equally between dissolved organic carbon (DOC; 2.1×10^{10} mol C) and particulate organic carbon (POC; 4.1×10^9 mol C). In Slovenia, DOC concentrations were typically between 0.4 and 1.15 mmol l^{-1}, characteristic for unpolluted rivers [57]. Higher DOC values in the river and its tributaries were observed in late summer at lower discharges, probably due to higher production of organic matter and consequent decomposition in the terrestrial environment. DOC concentrations in the SRB increased downstream from the relatively pristine Alpine headwaters, reflecting the greater ecosystem productivity along its flow resulting from climatic, ecological and anthropogenic influences such as agricultural activity and sewage discharge. The highest concentrations of DOC were observed at agricultural locations and correlated with NO_3^- concentrations [40]. POC also came from different sources in agricultural locations. While soil is the major source of POC in 59 % of sampling sites, in agricultural part in 18 % of sampling sites, phytoplankton was the main source of POC [48].

Thus, the molar proportions of DIC:DOC:POC were 89:9:2, markedly different from the mean proportions for world rivers draining to the oceans, where DIC: DOC:POC is 45:37:18 [58], but similar to those in the Yangtze River (DIC:DOC: POC = 71:4:8 [59]). The higher DOC:POC ratios (>2) observed in the SRB are typical of European and North American rivers. These data underline the importance of DIC flux from carbonate-rich landscape regions in global C transport. Global climate models indicate that global precipitation patterns will change so that, while a drainage basin may receive the same amount of precipitation, its distribution may change [60]. SRB was observed to respond quickly to precipitation, as is reflected in the low mean residence time (MRT) of 1.32 years in the river [61]. The shortest MRT was observed in Alpine regions and is connected mainly to the greater precipitation and runoff. Increased precipitation may increase both the flux of HCO_3^- and DIC and the dolomite sourced riverine Mg^{2+} flux, since carbonate mineral weathering is controlled primarily by solubility. Thus global climate change is likely to increase the continental weathering fluxes from carbonates to the surface oceans.

5 Occurrence of Anthropogenic Pollutants in the Sava River Basin

While, in the upper part of the SRB, ion distribution is controlled by weathering of minerals, the lower part of the SRB in Croatia and Serbia is subject to anthropogenic pollution, mainly from agricultural activities. Nevertheless, even in the lower parts of the SRB, 80 % of ions are still derived from natural weathering processes in the main stream [40]. Major anthropogenic influences on water quality are nutrients and inorganic salts (e.g. Na^+, K^+, Cl^-, NO_3^- and SO_4^{2-}). Both Cl^- and SO_4^{2-} can be naturally abundant within the watershed in evaporate minerals (NaCl and $CaSO_4$) and in meteoric precipitation. Two different trends were observed with Na^+ and Cl^- departure from a 1:1 molar ratio, indicative of natural inputs. Excess of Na^+ was observed at Kamniška Bistrica (location 9) and Savinja (location 10), probably due to the dissolution of NaCl or infiltration of Na-based fertilizers such as $NaNO_3$. The excess of both ions determined in Bosna (location 26) at all sampling seasons was due to inputs from the salt mine at Tuzla. This influence could still be seen in the SRB downstream at Županja (location 27) during lower river discharges.

Sulphate is derived mainly from natural sources such as mineral weathering of gypsum or sulphide minerals, volcanism and rainout of biogenic emissions, while anthropogenic SO_4^{2-} is typically present in the watershed from air pollution, addition of fertilizers, mining, smelting of sulphide ore, refining of petroleum and from other chemical industries [62, 63]. Elevated sulphate concentrations within the SRB could also be explained by anthropogenic input via acidic rain, which is commonly reported in Central European rivers. The highest sulphate concentrations of 0.48, 0.43, 0.36 and 0.40 mmol l^{-1} were observed in Tržiška Bistrica (location 4), Savinja (location 10), Bosna (location 26) and Vrbas (location 23) during low water discharge in autumn 2006. Similar concentrations were also observed in Bosna during high water discharge, indicating the continuous input of sulphate to the river. The high SO_4^{2-} concentration observed in the Savinja watershed was in conjunction with productive agricultural and industrial regions [64]. On the other hand, the high $\delta^{34}S_{SO4}$ value of +14.9‰ and the relation between Ca^{2+} and SO_4^{2-} suggested that the SO_4^{2-} in Tržiška Bistrica is a result of gypsum and/or anhydrite weathering. The downstream samples at Košutarica (location 21) and Bosna (location 26), with higher $\delta\ ^{34}S_{SO4}$ values of +9.5‰ and +11.7‰, provide evidence of the influence of anthropogenic pollution. The isotope mass balance performed at the mouth of the Sava at Belgrade (location 33) showed that industrial activities were the major source of sulphate in the SRB, accounting for up to 64 % [49]. In the upper Slovenian part of the Sava, however, the results indicated that the major sources were tributaries (52 %), other sources, including industry, contributing only 40 % [64]. Precipitation input was estimated to be 9.6×10^8 mol S $year^{-1}$, contributing around 8 % to the total S budget.

5.1 Nitrate: Its Origin and Distribution in the Sava River Basin

It is estimated that human activities have enhanced the global cycle of nitrogen (N) and phosphorus (P) by, on average, 100 and 400 % [65]. This contribution, comprising around 160 Tg of N annually, greatly exceeds that supplied naturally by biological N fixation on land and in the ocean [66]. Increases in river nutrient loads generally lead to increased production of algae and aquatic plants and loss of biodiversity and are, at the same time, associated with water quality problems (e.g. [67]). It is clear that nutrient sources operate through both point and diffuse pathways linking land to water, but source assignment remains a challenge. Stable isotopes have been shown to be useful tools in identifying nitrate sources in water systems and were also used in our study [34–39].

NO_3^- concentrations exhibited a trend of increasing along the river path flow during the sampling periods in 2006. The concentrations in the upper part of the SRB were low (<0.08 mmol l^{-1}), while in areas draining more agricultural land they ranged from 0.04 to 0.22 mmol l^{-1} (Fig. 4).

Considerably higher concentrations were observed during low river discharges in autumn than in spring 2006. $\delta^{15}N_{NO3}$ values did not correlate with NO_3^- concentrations, indicating that concentrations alone are insufficient to describe sources of N. Further, not all locations in a catchment, even if they have the same land use, contribute equally to the delivery of nitrate to receiving waters. A positive correlation ($r^2 = 0.41$, $n = 22$) was observed between $\delta^{15}N_{NO3}$ values and the

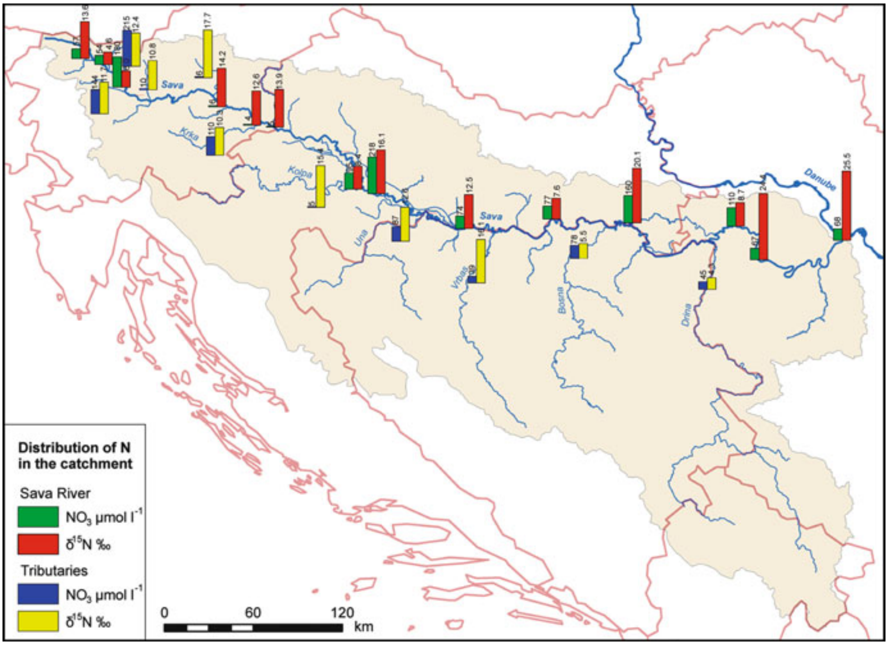

Fig. 4 Distribution of nitrate and $\delta^{15}N_{NO3}$ in the Sava River Basin

proportion of agricultural land in the catchment (Fig. 5), in agreement with other reports [37–39].

Some of the data fall near the regression line when annual load-weighted $\delta^{15}N_{NO3}$ is plotted against the % of agricultural and urban land use [39]. On the other hand, $\delta^{15}N_{NO3}$ values at some locations during low discharge in autumn 2006 fall well above the line, showing that hydrological regimes are important and influence stable isotope composition of nitrate in this watershed. Previous studies indicated that point sources might be relatively important at low flows when the contribution from diffuse catchment sources is lower [68, 69]. This was also observed in our study. Only a few points (locations 27, 32), including Belgrade (location 33), with elevated $\delta^{15}N_{NO3}$ values up to +25.5‰, fell within the range of animal waste and sewage [70] and were only found in autumn 2006. During high discharge in spring 2006, $\delta^{15}N_{NO3}$ values of ~7‰ did not show such a predominance of sewage-derived N. Further low discharges, higher temperature and nutrient enrichment observed in autumn 2006 could also promote the eutrophication of the river. These conditions were observed at Košutarica (location 21), where scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDS) microscopy of filters showed the presence of diatom alga *Stephanodiscus hantzschii* [48]. At the same time, higher concentrations of Fe (1.4, 1.1, 1.5 and 1.3 μmol l^{-1}) were observed in agriculturally active areas in Croatia at sampling locations Črnac (location 18), Lukovac (location 19), Košutarica (location 21) and Gradiška (location 22), respectively, probably due to the reductive conditions observed at low-flow discharge and higher temperatures. The negative correlation between

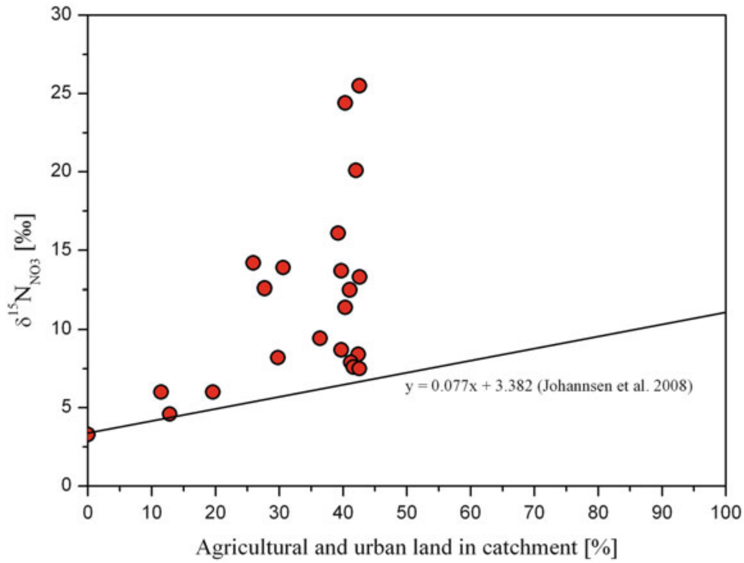

Fig. 5 The relation between land-use and $\delta^{15}N_{NO3}$ values in the Sava River Basin. The proportion of agricultural land in the upstream contributing area for each of the sampling points was calculated based on the CORINE Land Cover data (*source*: http://www.eea.europa.eu/publications/COR0-landcover)

the concentration of dissolved Fe and water level was also observed in a short-term study of the Sava [71]. The prevalence of reductive conditions was further supported by the observed presence of framboidal pyrite on suspended particulate matter (SPM) [51].

Thus at least two main sources of NO_3^- can be distinguished in the SRB. $\delta^{15}N_{NO3}$ values <6‰ were found in predominantly forested watersheds, while $\delta^{15}N_{NO3}$ values between 6 and 9‰ were observed in areas with a higher percentage of agricultural and/or urban land use (Fig. 4) and are caused by nitrate from sewage and/or manure.

The highest concentration of NO_3^- was found in Kamniška Bistrica tributary (location 9). A systematic study of this watershed was therefore performed during 2010 and 2011. $\delta^{15}N_{NO3}$ values ranged from −5.2‰ at the headwater spring to 9.8‰ in the lower reaches. Higher $\delta^{15}N_{NO3}$ values in the lower reaches suggest anthropogenic pollution from agricultural activity [47]. Higher concentrations of up to 0.69 mmol l^{-1}, in parallel with higher $\delta^{15}N_{NO3}$ values (up to +16.7‰) determined at the mouth of the river, indicate an organic fertilizer source of N or the influence of N derived from animal manure from the large pig farm (Ihan).

The concentration and flux of nutrients at different parts of rivers can vary significantly in response to short-term variations in hydrology and long-term changes of land use and population. We therefore investigated changes in NO_3^- concentrations at two locations, Jesenice na Dolenjskem (location 15) in the period from 2006–2012 and Županja (location 27) in the period from 2000 to 2012. No statistically significant trend of decreasing nitrate concentrations was found over

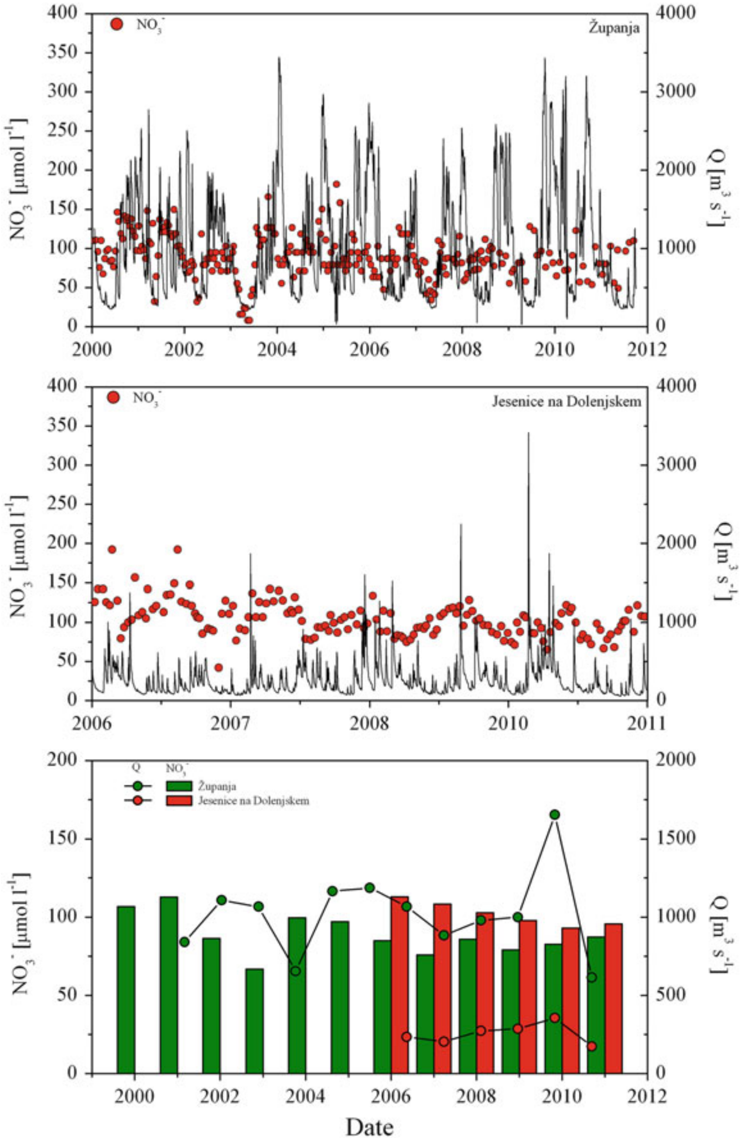

Fig. 6 The annual mean discharges and nitrate concentrations since 2003 at two locations: Jesenice na Dolenjskem (location 15) and Županja (location 27) in the Sava River Basin. The *upper graphs* show the raw data at both locations

this period (Fig. 6). However, there are substantial intra-annual variations in nitrate concentration, which could be described by fitting simple harmonic curves at Jesenice na Dolenjskem. We noted a very strong seasonal pattern, with the maximum occurring in the winter months from December to March, at Jesenice na Dolenjskem (location 15).

The timing of this maximum reflects factors such as the leaching of nitrate, which accumulated in the soil in the summer and autumn; the high rates of soil water movement in the winter months; and the absence of nitrogen uptake by plants due to low temperatures in the winter period. In contrast, diminished soil water movement and increased plant uptake of nitrogen during low-flow conditions cause NO_3^- concentrations to decrease to a minimum in July and August. The annual variation in concentration at Županja is not symmetrical and no real pattern was observed in nitrate concentration changes. At this location nitrate concentrations increased with increasing flow (positive slope). In contrast, a negative slope, signifying a dilution effect, was observed at Jesenice na Dolenjskem. It was found that variations in rainfall and temperature contribute greatly to the monthly variation in nitrate concentration.

In summary it is evident that nutrient management calls for integrated environmental assessments (IEA), which requires not only the identification and quantification of nutrient sources but also an understanding of all relevant natural and social processes and their interactions in the river basins [72].

5.2 *Trace Elements in Suspended Particulate Matter in the Sava River Basin*

Statistical evaluation of the data indicates that organic and inorganic (Al and Fe) pollution loads of anthropogenic origin were related to areas of SRB in Slovenia, Croatia and Serbia [40]. These sources of pollutants were more evident during low water discharge. The distribution of major industrial sources is presented in Fig. 7. The highest concentrations of dissolved Al (48.7 μmol l^{-1}) and Fe (44.7 μmol l^{-1}) were observed in Savinja (location 10) in autumn 2006. Concentrations (mg kg^{-1}) of trace elements in SPM in the main stream of the Sava varied over a wide range: Cd, 0.30–11.3; As, 3.28–37.8; Cu, 6.75–140; Cr, 9.21–132; Pb, 8.75–163; Ni, 10.4–359; and Zn, 25.0–1,219. Broader ranges and greater variations were observed in tributaries (mg kg^{-1}): Cd, 0.67–7.18; As, 8.73–124; Cu, 24.9–326; Cr, 19.3–479; Pb, 24.4–510; Ni, 22.5–923; and Zn, 77.5–14,670. When compared with the world average concentrations of trace elements in SPM, those in the Sava were lower for Cr, Cu, A and Pb; similar for Ni; and higher for Cd and Zn [75]. In the Danube, mean concentrations of Cu, Ni and Pb were higher than in the Sava, but lower for Cd and Zn [76].

According to LAWA classification [77], Sava SPM samples were predominately of classes I and II in both sampling seasons [51]. In contrast, Sava tributaries Una (location 20), Vrbas (location 23), Bosna (location 26) and Drina (location 30) were contaminated with Cu, Ni, Zn, Cd and Pb. These rivers drain watersheds where industrial and/or mining activities are still present and constitute sources of pollution with trace elements (Fig. 7).

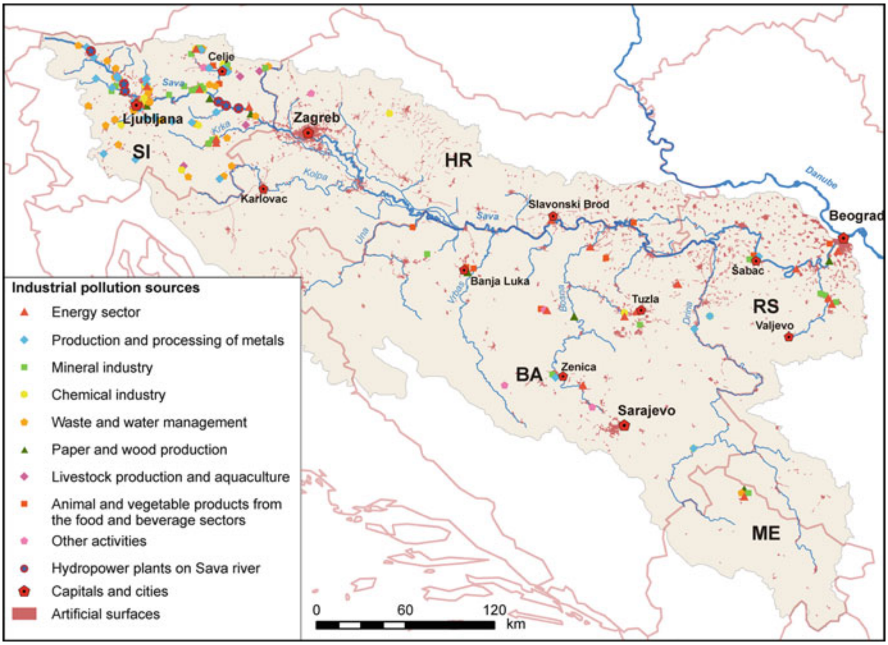

Fig. 7 Sources of industrial pollution in the Sava River Basin (*source*: [73, 74])

Details on sediment contamination of the Sava, including inorganic and persistent organic pollutants at 20 selected sampling sites, are presented in [78]. According to the Water Framework Directive, the following elements were investigated in sediments: Cd, Pb, Ni, Hg, Cu, Zn, Cr, As and P. A comparison of the range of data in SPM [51] and sediments [78] leads to the conclusion that concentrations of Zn are ten times higher in SPM, while the concentrations of other elements were comparable.

Only Cd and Zn were present in SPM at higher concentrations at Belgrade (location 33). Since they exhibited limited exchange between the sediments and overlaying water due to the short residence times of the particles, Cd and Zn were transported further into the Danube. Their impact is seen on the Danube SPM along a 1,000 km reach downstream of the confluence with the Sava [79].

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Elements and Persistent Organic Pollutants in the Sediments of the Sava River

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Abstract Among various stressors, aquatic ecosystems are exposed also to different inorganic and organic pollutants. The pollution of the Sava River is related mainly to the release of industrial wastes, untreated effluents from municipalities, and contaminants arising from agricultural activities. To assess the geographical distribution of sediment pollution, sediments were analysed at selected sites along the Sava River. Total element concentrations were determined and mobile element fractions and anthropogenic inputs of elements assessed. Selected persistent organic pollutants: polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and chlorinated pesticides were also determined. In industrially exposed sites, Hg, Cr, and Ni were found in moderate concentrations (up to 0.6, 380 and 210 mg kg^{-1}, respectively). Since Cr and Ni exist in sparingly soluble forms, they do not represent an environmental burden. Elevated P concentrations up to 1,000 mg kg^{-1} were found at agricultural areas and big cities. Regarding elements, the environmental status of sediments of the Sava River is comparable to other moderately polluted rivers in Europe, if rivers impacted by mining are not considered. Among the organic pollutants PAH were present in moderate concentrations (sum of 16 PAH up to 2,000 ng g^{-1} with two exceptions with elevated PAH concentrations up to 4,000 ng g^{-1} located downstream the oil fields) and their concentrations increased downstream the river. Concentrations of PCB were low (the sum of 7 indicator PCB was below 4 ng g^{-1}). Among selected pesticides, p,p'-DDT were found in moderate concentrations in sediments at two sampling sites in Croatia (up to 3 ng g^{-1}) and HCB in high concentration in the city of Belgrade (91 ng g^{-1}), although the use of these persistent pesticides has been banned for many years. Considering the organic pollutants, Sava is a moderately polluted river. The results of this study contribute to knowledge on the extent of pollution of sediments of European rivers and are important for water management institutes

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and local authorities, which may use these data for sustainable use, management, and protection of the Sava River water resources.

Keywords Sava River • Sediments • Elements • Polyaromatic hydrocarbons • Polychlorinated biphenyls • Organochlorine pesticides

List of Abbreviations

Al	Aluminium
As	Arsenic
CCME	Canadian Environmental Quality Guidelines
Cd	Cadmium
Cr	Chromium
Cu	Copper
DDT	Dichlorodiphenyltrichloroethane
ECD	Electron capture detector
ERL	Effects range median
ERM	Effects range low
GC-MS	Gas chromatography–mass spectrometry
HCB	Hexachlorobenzene
Hg	Mercury
ICPDR	International Commission for the Protection of the Danube River
ICP–MS	Inductively coupled plasma mass spectrometry
ISQG	Interim Sediment Quality Guidelines
Ni	Nickel
OCP	Organochlorine pesticides
P	Phosphorous
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PCB	Polychlorinated biphenyls
PEL	Probable effect levels
SARIB	Sava River Basin: Sustainable Use, Management and Protection of Resources
SQGs	Sediment quality guidelines
SRB	Sava River Basin
WFD	Water Framework Directive
Zn	Zinc

1 Introduction

The drinking water supply in the Sava basin relies mainly on the rich high-quality groundwater resources, which are directly influenced by the Sava River. Sediments are essential for the functioning of aquatic ecosystems. They serve as a source of nutrients for microorganisms and importantly contribute to the food web. On the other hand sediments represent also a sink for a variety of toxic inorganic and organic contaminants, nutrients, and pathogens. The accumulated contaminants may be remobilised to overlying waters and disrupt the ecosystem, acting as stressors, which can have harmful effects on freshwater habitat [1]. Therefore, sediment quality is of crucial importance to protect surface water quality and to maintain benthic ecosystem health. The quality of sediment is important also in preparing the management plans related to dredging activities for restoring waterways or for the removal of sediments accumulated before dams of hydroelectric power plant accumulation basins.

Chemical analysis of sediments are commonly applied since they reflect spatial and temporal variation of contaminant concentrations of elements [2–6] and organic pollutants [7–9]. Among elements, Cd, Pb, Cu, Zn, Cr, Ni, Hg, and As are often accumulated in sediments as a consequence of industrial [10–12], municipal [3], and mining activities [13–15], while intensive rural land use and domestic sewage are a major source of P deposition in sediments [16, 17]. The most frequently investigated organic contaminants in sediments are polycyclic aromatic hydrocarbons (PAH) [7, 9, 18, 19], polychlorinated biphenyls (PCB) [8, 20], and pesticides [8, 9]. PAH, formed during the pyrolysis of virtually all organic matter, are ubiquitous organic contaminants in aquatic sediment. Their acute toxicity and sublethal effects on aquatic organisms, including the mutagenic and genotoxic potential of certain PAH once in the food chain, have generated interest in studying their composition and distribution in the environment and more specifically in river sediments [7, 9, 18, 19, 21–28]. PCB were once extensively used in industrial applications including transformers, electrical equipment, and ship painting. They have been banned in Europe for over three decades; however, being chemically stable, it is likely that their presence in sediments derives from their former use. Similarly, organochlorine pesticides are chlorinated hydrocarbons used extensively from the 1940s to the 1960s in agriculture and mosquito control. Representative compounds in this group include DDT, methoxychlor, dieldrin, chlordane, toxaphene, mirex, kepone, lindane, and benzene hexachloride. Organochlorine pesticides are now banned in the developed world, but due to their physicochemical properties and long range transport, they could be still entering our environment.

Once the concentration of the chemical contaminant reaches a point at which it causes adverse effects to the biota, the chemical contaminant is considered as a pollutant [29]. In past decades numerous sediment quality guidelines (SQGs) have been developed to estimate the environmental status of sediments and to determine management options for dredged material disposal [29–31]. Sediment quality was assessed using chemical quality criteria [29], while biological effect-based assessment approaches have gained more interest. A group of Canadian researchers derived information on concentrations of selected chemicals that maintain healthy aquatic life associated with bed sediments, which is provided in the Canadian Environmental Quality Guidelines [32]. The Interim Sediment Quality Guidelines (ISQG) correspond to the threshold level effects below which adverse biological effects are not expected, while probable effect levels (PEL) characterise concentrations of pollutants that may affect the aquatic life [32]. For improving sediment quality assessment and sediment management alternatives, expert groups are still developing sediment quality criteria [1, 33, 34]. Recently, the European Commission (EC) has provided technical guidance for the derivation of SQGs as a part of common implementation strategy for the Water Framework Directive (WFD) [35].

In many cases the extent of pollution cannot be estimated solely on the basis of the determination of the total content of chemical substances in sediments because bioavailability and toxicity to organisms depend on their chemical forms. The use of fractionation procedures provide useful information on the partitioning of elements between easily and sparingly soluble fractions of sediments and enable assessment on the proportion of the potentially mobile and bioavailable element fractions.

Different fractionation procedures are applied in sediment and soil analysis, most frequently following Tessier's [36] or BCR [37] sequential extraction procedures [13, 38–40]. The leaching protocol of the first step of these sequential extraction procedures is also used to investigate the easily soluble elements fraction, applying aqueous solutions of ammonium chloride [36] or acetic acid [37] as extracting agents, respectively. Data on mobility and potential bioavailability of elements in sediments are useful for the estimation of the environmental burden [13, 38, 39] and represent a good basis for the management of dredged sediments [41] as well as remediation of polluted sediment sites. In order to estimate the natural and anthropogenic input of elements in sediments, normalisation approaches are commonly used based on correlations between the concentration of trace elements and the element that is naturally present in the environment investigated. For this purpose Al as a major constituent of aluminosilicates and Fe as a clay mineral indicator element have usually been applied [2, 42, 43]. However, these elements cannot be used in normalisation if they are present in sediments as a consequence of external contamination, e.g., mining or industrial activities [13].

In the present chapter ecological status of the Sava River sediments is assessed. Data on total element concentrations and highly mobile element fractions (extraction in 0.11 mol L^{-1} acetic acid) are given. Anthropogenic inputs of pollutants to sediments are identified by normalisation of total element concentrations to Al. In addition, information on occurrence of persistent organic pollutants, 16 PAH, 7 PCB, and selected chlorinated pesticides, in sediments is provided. The choice of pollutants investigated followed recommendations of the WFD [44], list of the priority substances and certain other pollutants from the WFD [45], as well as specific pollutants which have been recently listed in support to maintain physicochemical conditions in water that would prevent diverse biological effects on aquatic life [46]. Most data were obtained from results of the 6th FW EC project: Sava River Basin: Sustainable Use, Management and Protection of Resources (SARIB).

2 Sampling of Sediments: The Sava River Profile and Grain-Size Distribution

Sampling was performed in April 2005, October 2005, and May 2006. Twenty sampling locations (Fig. 1, Table 1) were selected along the Sava River considering the sample accessibility and representativeness in terms of different anthropogenic sources of pollution like the industry, agriculture, urban activities, and traffic.

In the Slovenian part of the basin, the riverbed is relatively steep and formed from solid rock. So, samples were taken from the reaches where sediment deposition occurs, a few meters from the riverbank. At the locations of hydroelectric power plants Moste and Vrhovo, sediments were sampled just before the hydroelectric dams. Downstream from the Slovenian–Croatian border, the Sava River turns into a flatland river with fine-grained sediments covering the riverbed. From each location, about 3 kg of the top 15 cm sediment layer was collected using the piston corer with plastic core liners (Fig. 2).

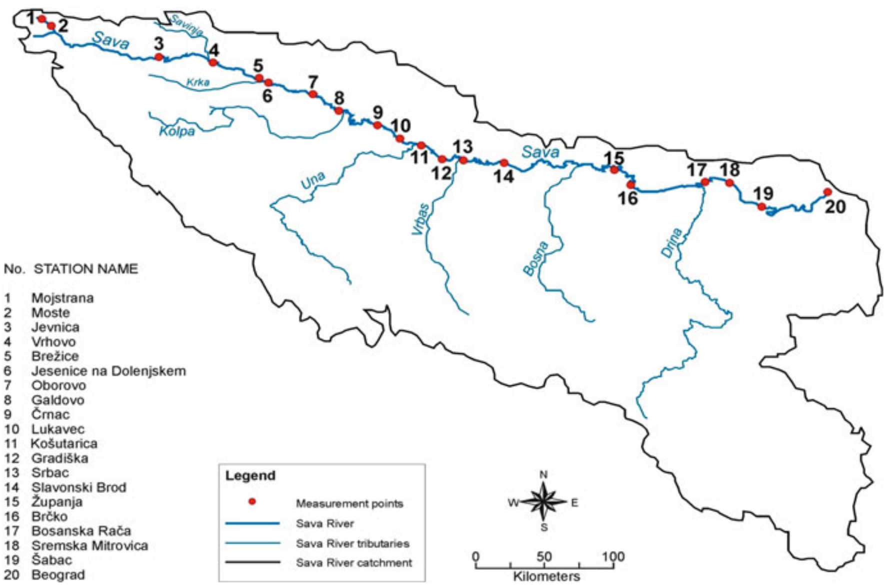

Fig. 1 Sava River basin: sediment sampling sites along the Sava River

Table 1 GPS data for sediment sampling sites along the Sava River

Sample no.	Sampling site	GPS
1	Mojstrana	N46.0644°; E13.959822°
2	Moste	N46.415057°; E14.105334°
3	Jevnica	N46.105710°; E14.787503°
4	Vrhovo	N46.045294°; E15.215272°
5	Brežice	N45.897421°; E15.591798°
6	Jesenice na Dolenjskem	N45.861740°; E15.683890°
7	Oborovo	N45.41217°; E16.14.810°
8	Galdovo	N45.28922°; E16.23155°
9	Črnac	N45.26304°; E16.25520°
10	Lukavec	N45.24087°; E16.32339°
11	Košutarica	N45.15062°; E16.57157°
12	Gradiška	N45.09°; E17.15°
13	Srbac	N45.10876°; E17.51570°
14	Slavonski Brod	N45.08380°; E18.04441°
15	Županja	N45.02389°; E18.41924°
16	Brčko	N44.88220°; E18.80366°
17	Bosanska Rača	N44.90960°; E19.29548°
18	Sremska Mitrovica	N44.97481°; E19.59324°
19	Šabac	N44.76057°; E19.70745°
20	Beograd	N44.81456°; E20.44646°

Sampling and sample preservation followed the recommendations of the Guidance Document No. 25 on chemical monitoring of sediment and biota under the WFD [46]. For comparability of analytical data to other river basins, wet sieving through 63 μm sieve was applied [46–48].

From the Sava River profile and grain-size distribution of sediments (Fig. 3), it can be seen that in Slovenia the Sava River is a mountain river containing between 40 and 60 % of fine particles in the sediment (<63 μm). At the Slovenian–Croatian border, the Sava River turns into a flatland river and the percentage of the fine particles in sediments (<63 μm) is gradually increased, reaching up to 90 % of the total sediment content.

3 Elements in the Sediments of the Sava River

3.1 Total Element Concentration of Sediments in the Sava River and Normalisation to Aluminium

Total element concentrations were determined after microwave-assisted digestion of sediments by ICP–MS and total Hg by oxidative combustion using DMA-80

Fig. 2 Sediment sampling and piston corer used for sampling of sediments

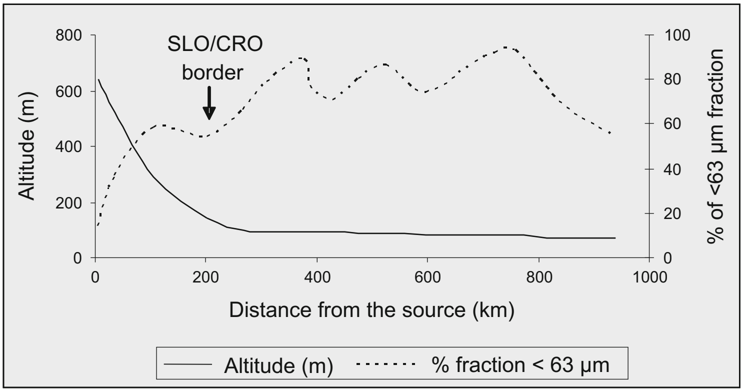

Fig. 3 The Sava River profile and grain-size distribution of sediments

Direct Mercury Analyzer. The sampling protocol, sample preparation, and analytical procedures are described in detail in the study of Milačič et al. [49]. In the sediment of the Vrhovo impoundment, Hg speciation was also performed. Total Hg (THg) in solid part was determined by acid digestion, oxidation, reduction, gold amalgamation, and cold vapour atomic absorption spectrometry (CV AFS). MeHg was determined by CH_2Cl_2 extraction, ethylation, and detection by cold vapour atomic fluorescence spectrometry (CV AFS) [50, 51]. Methylation and reduction potential of inorganic Hg were determined using spikes of inorganic radioactive isotope ^{197}Hg. After incubation MeHg was extracted. Radioactive decay of the isotope ^{197}Hg was measured [52]. The data on total element concentrations in the sediments of the Sava River are presented in Table 2. To evaluate the quality of the Sava River sediments, ISQG and PEL values are also given, considering the Canadian Quality Guidelines [32].

In order to account for geochemical variations along the Sava River, the normalisation by a conservative element Al, as a major constituent of aluminosilicates, was applied (Fig. 4). Significant deviations from the linear relationship may be used to differentiate between natural against anthropogenic inputs [42, 43, 49].

From the data of Table 2, it can be seen that the lowest concentrations of elements, which represent a natural background, were observed at the mountain village Mojstrana, an unpolluted site close to the Sava Dolinka River spring. Ščančar et al. [53] reported similar low concentrations of elements along the Sava Dolinka River from Mojstrana up to Jesenice, an industrial city with well-developed steelmaking industry. As a consequence of dredging of sediments, low element concentrations were found also at Galdovo. So this sampling site may not represent the actual ecological status of sediments. In general, the concentrations of elements in sediments of the Sava River gradually increase from the Sava River spring to its outflow to the Danube River.

Among metals, Hg concentrations in sediments in general ranged from 0.2 to 0.6 mg kg^{-1} and in most sampling sites exceeded the ISQG value (0.17 mg kg^{-1} Hg). In Košutarica, Gradiška, and Šabac, Hg concentrations were around 0.6 mg kg^{-1} and exceeded also the PEL value (0.486 mg kg^{-1} Hg). Normalisation to Al (Fig. 4) also indicated that higher Hg concentrations in Košutarica and Gradiška are most probably related to the oil refinery activities, while in Šabac the Hg input is most likely associated to pollution from the chemical industry. Slightly elevated Hg levels in Vrhovo (the Slovenian part of the river) were associated to former industrial pollution from a chemical plant in Hrastnik (the use of Hg cells in chlor-alkali production until 1997), while in Jevnica the impact of Ljubljana city is evident. In our previous work [42], comparable Hg concentrations at the same sampling sites in Slovenia were also determined. A similar concentration of Hg as in Jevnica was determined in Oborovo (Croatia), a sampling site, which reflects the pollution of the Zagreb city. In general lower concentrations than in the Sava River (between 0.1 and 0.3 mg kg^{-1} Hg) were reported by Sakan et al. [12, 54] for the canal sediments from the Danube alluvial formation and the Tisa River in Serbia. The extent of pollution of Hg in sediments from the Sava River is comparable to the majority of sampling sites in the Danube River (around 0.4 mg kg^{-1} Hg) [48, 55, 56] and is similar to the concentrations reported in Odiel River in Spain (from 0.1 to 0.7 mg kg^{-1} Hg) [57]. Hg concentrations from the Sava sediments are lower than reported by Meybeck et al. [5] for the Seine River, France (around 1 mg kg^{-1} Hg), and for the polluted Bílina River, Czech Republic (1–3 mg kg^{-1} Hg) [58], and are much lower than those determined in contaminated sediments of the Soča River (10–20 mg kg^{-1} Hg) due to former mercury mining activities in Idrija, Slovenia [14, 59].

Table 2 Determination of total element concentrations in sediments of the Sava River by ICP–MS and Hg by DMA-80 Direct Mercury Analyzer. Concentrations are expressed on a dry mass basis. Results represent average of two parallel samples ± standard deviation

Sampling site	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Ni (mg kg^{-1})	Cr (mg kg^{-1})	Cd (mg kg^{-1})	As (mg kg^{-1})	Hg (mg kg^{-1})	P (mg kg^{-1})
1. Mojstrana	11.5 ± 0.1	55.4 ± 0.7	14.4 ± 0.4	12.6 ± 3.9	23.1 ± 1.3	0.28 ± 0.01	6.9 ± 0.6	0.035 ± 0.001	170 ± 5
2. Moste	40.9 ± 0.6	118 ± 1	33.1 ± 0.4	70.1 ± 1.1	184 ± 4	0.58 ± 0.02	14.7 ± 0.1	0.134 ± 0.004	275 ± 10
3. Jevnica	41.7 ± 3.7	142 ± 1	34.4 ± 3.0	27.3 ± 0.7	73 ± 1	0.42 ± 0.08	10.5 ± 0.8	0.388 ± 0.043	625 ± 20
4. Vrhovo	47.8 ± 3.4	223 ± 2	45.9 ± 1.0	36.9 ± 0.6	94 ± 2	0.73 ± 0.03	9.0 ± 1.3	0.397 ± 0.011	1,190 ± 30
5. Brežice	27.3 ± 2.7	113 ± 1	18.6 ± 3.0	17.6 ± 1.9	48.2 ± 0.6	0.24 ± 0.01	9.6 ± 2.1	0.247 ± 0.006	360 ± 10
6. Jesenice na Dol.	29.2 ± 6.6	106 ± 13	13.6 ± 1.9	12.3 ± 0.1	50.3 ± 0.8	0.22 ± 0.01	8.4 ± 0.8	0.235 ± 0.042	315 ± 10
7. Oborovo	41.1 ± 1.2	172 ± 5	39.1 ± 1.2	36.7 ± 1.1	88 ± 3	0.62 ± 0.02	11.3 ± 0.3	0.390 ± 0.007	950 ± 30
8. Galdovo	13.8 ± 0.4	77 ± 2	10.9 ± 0.3	16.3 ± 0.5	43 ± 1	0.40 ± 0.01	2.9 ± 0.1	0.091 ± 0.005	380 ± 10
9. Črnac	32.5 ± 1.0	155 ± 5	27.1 ± 0.8	30.8 ± 0.9	85 ± 3	0.55 ± 0.02	7.7 ± 0.2	0.366 ± 0.004	750 ± 20
10. Lukavec	32.7 ± 1.0	163 ± 5	35.5 ± 1.1	44.4 ± 1.3	99 ± 3	0.64 ± 0.02	11.4 ± 0.3	0.282 ± 0.010	930 ± 30
11. Košutarica	41.5 ± 1.2	224 ± 7	30.3 ± 0.9	39.5 ± 1.2	127 ± 4	0.83 ± 0.02	20.1 ± 0.6	0.585 ± 0.015	670 ± 20
12. Gradiška	36.0 ± 1.1	220 ± 7	29.8 ± 0.9	41.9 ± 1.3	167 ± 5	1.02 ± 0.03	12.1 ± 0.4	0.629 ± 0.025	910 ± 30
13. Srbac	25.5 ± 0.5	84 ± 2	27.1 ± 0.5	79 ± 2	236 ± 5	0.43 ± 0.01	11.2 ± 0.2	0.376 ± 0.009	400 ± 10
14. Slavonski Brod	25.5 ± 0.8	118 ± 4	31.9 ± 1.0	102 ± 3	186 ± 6	0.58 ± 0.02	16.5 ± 0.5	0.347 ± 0.012	860 ± 25
15. Županja	33.9 ± 1.0	134 ± 4	31.1 ± 0.9	212 ± 7	381 ± 11	0.47 ± 0.01	19.8 ± 0.6	0.269 ± 0.010	690 ± 20
16. Brčko	52 ± 1	165 ± 3	43.4 ± 0.8	185 ± 4	312 ± 6	0.62 ± 0.01	16.7 ± 0.3	0.297 ± 0.031	730 ± 20
17. Bosanska Rača	122 ± 1	184 ± 4	47.1 ± 0.9	186 ± 4	273 ± 6	0.66 ± 0.01	17.9 ± 0.4	0.374 ± 0.004	760 ± 20
18. Sremska Mitrovica	79 ± 1	275 ± 6	44.9 ± 0.9	177 ± 4	276 ± 6	0.84 ± 0.02	23.6 ± 0.5	0.444 ± 0.091	800 ± 25
19. Šabac	117 ± 1	361 ± 7	49.6 ± 1.0	163 ± 3	232 ± 5	1.40 ± 0.03	25.1 ± 0.6	0.624 ± 0.012	930 ± 30
20. Beograd	97 ± 1	173 ± 4	46.2 ± 0.9	82 ± 2	151 ± 3	0.84 ± 0.02	15.7 ± 0.3	0.275 ± 0.030	1,060 ± 30
ISQG	30.2	124	18.7	/	52.3	0.7	7.24	0.17	/
PEL	112	271	108	/	160	4.2	41.6	0.486	/

Canadian Sediment Quality Guidelines for the Protection of Aquatic Life [32]
Interim Sediment Quality Guidelines (ISQG) corresponds to the threshold level effects below which diverse biological effects are not expected
Probable effect levels (PEL) characterize concentrations of pollutants that may affect the aquatic life
/ data not provided

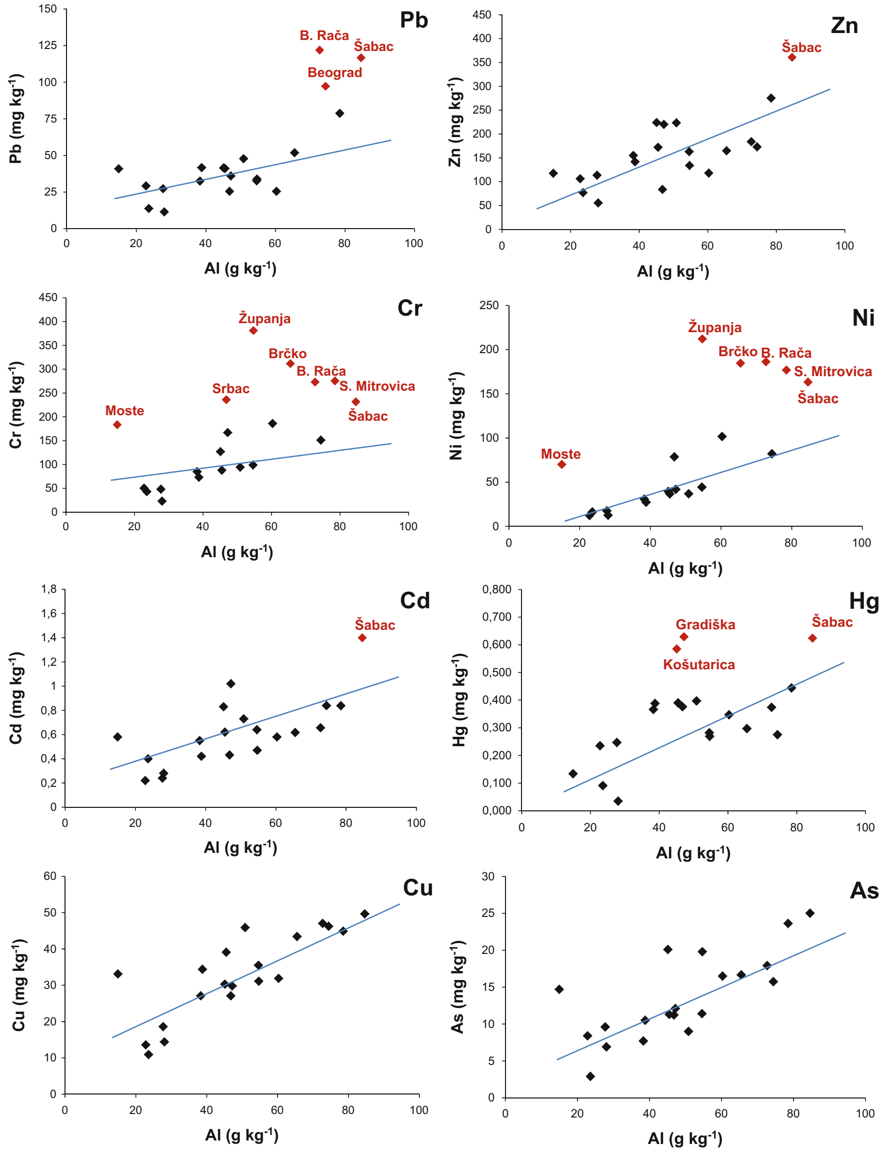

Fig. 4 Relationship of Pb, Zn, Cr, Cd, Ni, Cu, Hg, and As with Al in the sediments from the Sava River

It is well known that Hg can be transformed from inorganic to more toxic organic form—monomethylmercury (MeHg), which is bioaccumulative form of Hg. Moreover, mobility of mercury can also be enhanced due to oxidation/reduction processes in the aquatic environment [60], particularly in impoundments. In order to assess the potential for these transformations speciation of mercury was

Table 3 Some parameters measured in sediment core sample from accumulation basin of HP Vrhovo

Parameter	Value
Redox potential (mV)	−170
THg (ng g^{-1}), dry weight	283 ± 14
MeHg (ng g^{-1}), dry weight	7.5 ± 0.6
Methylation Hg (ng g^{-1} day^{-1})	6.78
Reduction Hg (ng g^{-1} day^{-1})	0.021

done. In addition, experiments using radioactive tracer ^{197}Hg were performed to estimate reduction and methylation potential of the impoundment sediment.

THg in the impoundment sediment core from hydropower plant (HP) Vrhovo (Table 3) was slightly higher in comparison to the Slovenian background [61, 62]. The percentage of Hg present as MeHg exceeded 2.5 % of THg. That proportion is relatively high compared to the literature data where less than 1 % of Hg as MeHg is normally reported [63, 64].

Tracer experiments also confirmed very high ability for methylation of Hg in sediment of the HP Vrhovo. For instance, in one day per gram of sediment 6.8 ng of MeHg can be formed, which is 200 higher, than in 1 L of river water from same sampling location [65]. This indicates extreme sensitivity of the impoundment sediment of the system for free Hg(II) loads.

For comparison, the sediment of the Idrijca River, which is heavily impacted by the past mercury mining, has shown much lower methylation capacity (about 10 times lower) [52, 66]. Consequently, MeHg in water and fish of the HP Vrhovo were also elevated [65]. These results indicate that for the river systems, total mercury is not a good indicator, but the speciation is of paramount importance to understand the potential risk of Hg.

Data from Table 2 also demonstrate that the Sava River is moderately polluted with Cr and Ni at sampling site Moste in Slovenia (steelworks Acroni) and at sampling sites in Croatia, Bosnia and Herzegovina, and Serbia, from Gradiška up till Šabac. At these sampling locations the concentrations of Cr are higher than those of PEL values (160 mg kg^{-1} Cr) and, at most sampling sites investigated, higher also from ISQG values (52.3 mg kg^{-1} Cr). For Ni there are no data on Canadian sediment quality standards. Normalisation to Al exhibited the same pattern of Cr and Ni inputs to sediments. At the sampling site in Moste, the Cr and Ni contamination arises from steelworks Acroni, while sampling sites from Gradiška up till Šabac indicate the influence of the heavy metal and chemical industry activities along the Sava River in this region. Cr concentrations in industrial-impacted sites range from 180 up to 380 mg kg^{-1} Cr and of Ni from 70 up to 200 mg kg^{-1} Ni. Similar concentrations were found in the sediments of the Po River, Italy (from 120 to 230 mg kg^{-1} of Cr and from 100 to 240 mg kg^{-1} of Ni) [67]. However, the concentrations of Cr and Ni in the Sava River sediments influenced by the industrial activities are higher than most of Cr and Ni values reported for the Danube River [48, 55, 56, 68] and Tisa River [69] sediments, in which concentrations of Ni in general did not exceed 100 mg kg^{-1} and Cr 150 mg kg^{-1}. Concentration levels of Cr and Ni in the Sava sediments are also higher from those determined in sediments of the Odiel River, Spain (30–150 mg kg^{-1} Cr and 15–40 mg kg^{-1} Ni) [57].

From the data on Table 2, it can be further seen that most of Pb concentrations in the Sava sediments exceeded ISQG value (30.2 mg kg^{-1}) and at Bosanska Rača and Šabac sampling sites, the sediments exceeded also PEL value (112 mg kg^{-1} Pb). From data on normalisation to Al (Fig. 4), the anthropogenic input of Pb in Belgrade arises presumably due to heavy city traffic, while in Bosanska Rača high Pb concentrations in sediments are related to heavy traffic on the border between Bosnia and Herzegovina and Serbia. In Šabac anthropogenic input of Pb in sediments is most probably related to the activities of the chemical industry. By comparing the amount of Pb in the sediment from the Sava River at Šabac (sampling on May 2006, 117 mg kg^{-1}) with that reported by Vuković et al. [70] for the same location (around 30 mg kg^{-1}), a significant decrease in Pb concentration is evident most likely due to reduced emissions from the chemical plants. Pb concentrations in the Sava River are in general comparable to concentrations of Pb (40–70 mg kg^{-1}) in River Po, Italy [67], and Tisa River, Serbia [69], and are similar to Pb concentrations reported for the Danube River (30–100 mg kg^{-1} Pb) [48, 54–56, 68] as well as for the Bílina River, Czech Republic [58], but are much lower than those reported for the mining area (100–9,000 mg kg^{-1} Pb [13] and 500–5,000 mg kg^{-1} Pb [57]).

Cd concentrations in sediments of the Sava River (Table 2) in general did not exceed ISQG value (0.7 mg kg^{-1}). Anthropogenic input (data on normalisation to Al, Fig. 4) in Šabac is most probably related to the emissions from the chemical industry. Cd concentrations in the sediments of the Sava River are comparable to River Po, Italy (0.4–1.4 mg kg^{-1}) [67], and are lower than those reported for the Tisa River, Serbia (around 3 mg kg^{-1}) [69]. Slightly higher Cd concentrations than in the Sava River sediments were found in the Seine River, France (1–2 mg kg^{-1}) [5], and Bílina River, Czech Republic (around 3 mg kg^{-1}) [58], but appreciably higher Cd concentrations were determined in the sediments of the Danube River (around 2–3 mg kg^{-1} Cd) [48, 56]. However, recent reports on Cd concentrations in the Danube River indicate on the reduced pollution with Cd (concentrations between 1 and 10 mg kg^{-1}) [54, 55, 68]. In comparison to mining area sites (2–130 mg kg^{-1} Cd [13] and 2–9 mg kg^{-1} Cd [57]), the concentrations of Cd in the Sava River sediments are significantly lower.

Zn concentrations in sediments of the Sava River (Table 2) in general exceeded ISQG value (124 mg kg^{-1} Zn) and at two sampling sites also PEL value (271 mg kg^{-1} Zn). Anthropogenic input of Zn in Šabac (normalisation data on Al concentration, Fig. 4) most probably arise from activities of the chemical industry. Zn concentrations in the sediments of the Sava River are comparable to most data reported for the Danube River (200–500 mg kg^{-1} Zn) [47, 54–56, 68]; Tisa River, Serbia [69]; and River Po, Italy [57] and are slightly lower than Zn concentrations in the sediments of the Seine River in France [5] and Bílina River, Czech Republic [58] (600–800 mg kg^{-1} Zn). These concentrations are much lower than those determined in the sediments at the mining areas of the Mežica valley, Slovenia (400–16,000 mg kg^{-1} Zn) [13], and Odiel River, Spain (1,000–8,000 mg kg^{-1} Zn) [57].

Concentrations of Cu in the Sava River sediments in general ranged from 30 to 50 mg kg^{-1} and exceeded ISQG value (18.7 mg kg^{-1} Cu) but were lower than the PEL value (108 mg kg^{-1} Cu). Concentrations of Cu in sediments of the Sava River do not represent anthropogenic inputs (see normalisation to Al, Fig. 4) and are lower than reported for sediments of the Danube River [47, 55, 56, 68]; River Po, Italy [67], Seine River, France [5]; and Tisa River, Serbia [69] (50–200 mg kg^{-1} Cu). Cu concentration levels in the Sava River sediments are much lower than at contaminated sites (Cu exploiting) in the Odiel River, Spain (200–2,800 mg kg^{-1} Cu) [57].

Concentrations of As in the sediments of the Sava River range from 7 to 25 mg kg^{-1} As (Table 2) and in general exceeded the ISQG value (7.24 mg kg^{-1} As). However, these As concentrations do not reflect anthropogenic inputs (see normalisation data in Fig. 4) but are characterised by its natural background. As concentrations in the sediments of the Sava River are lower than most of those reported for the Danube River [47, 55, 56] and Bílina River, Czech Republic [58] (40–80 mg kg^{-1} As).

Finally, data from Table 2 indicate that concentrations of total P in the sediments along the Sava River tend to increase from spring toward the inflow into the Danube River. The highest concentrations (around 1,000 mg kg^{-1} of total P) were found mainly due to the use of P-containing fertilisers in rural areas and also due to the influence of the municipal sewage outflow (use of P-containing detergents in household) in big cities, e.g., sampling site in Oborovo (outflow of the municipal sewage system in Zagreb, Croatia) and sampling site in the city of Belgrade before the Sava River merges with the Danube River. The influence of municipal sewage system on the quality of river sediments was observed also by House and Denison [16], who reported that sewage outflows at the Blackwater River in United Kingdom importantly contributed to P input to the river sediments. Concentrations of P downstream the sewage outflows reached concentrations up to 4,000 mg kg^{-1}of the total P. Similar P concentrations to those in the Sava River sediments (around 1,000 mg kg^{-1} of total P) were found in the sediments of the Danube River [47].

3.2 Partitioning of Elements in Sediments from the Sava Dolinka River

In sequential extraction procedures various extractants are applied successively to the sediment or soil for selective leaching of the particular chemical forms of elements from samples analysed. Due to its simplicity, short time of analysis, and the amount of information obtained, the BCR sequential extraction scheme [37] is most commonly applied. In step I (extraction in 0.11 mol L^{-1} acetic acid, pH 2.8) of the BCR scheme, metals present in ionic form bound to carbonates and the exchangeable fraction is released. In step II (extraction in 0.1 mol L^{-1} hydroxylamine hydrochloride, pH 2), metals bound to amorphous Fe and Mn oxides and hydroxides are leached. In step III (oxidation in acid-stabilised 30 % hydrogen peroxide and extraction in 1 mol L^{-1} ammonium acetate, pH 2, adjusted with nitric acid) metals bound to organic matter and sulphides are separated. For the determination of the metal fraction associated with aluminosilicates to the original BCR scheme, step IV is added in which the residue is digested with nitric, perchloric, and hydrofluoric acids.

To estimate the applicability of the BCR extraction procedure to the sediments of the Sava River, two representative sediments from the Sava Dolinka River—contaminated sediment from the basin of the hydroelectric power plant Moste (impacted also by the steelmaking industry) and non-contaminated sediment from Mojstrana site (close to Sava Dolinka spring)—were analysed. The results of the partitioning of elements by applying the modified BCR extraction scheme are presented in Fig. 5.

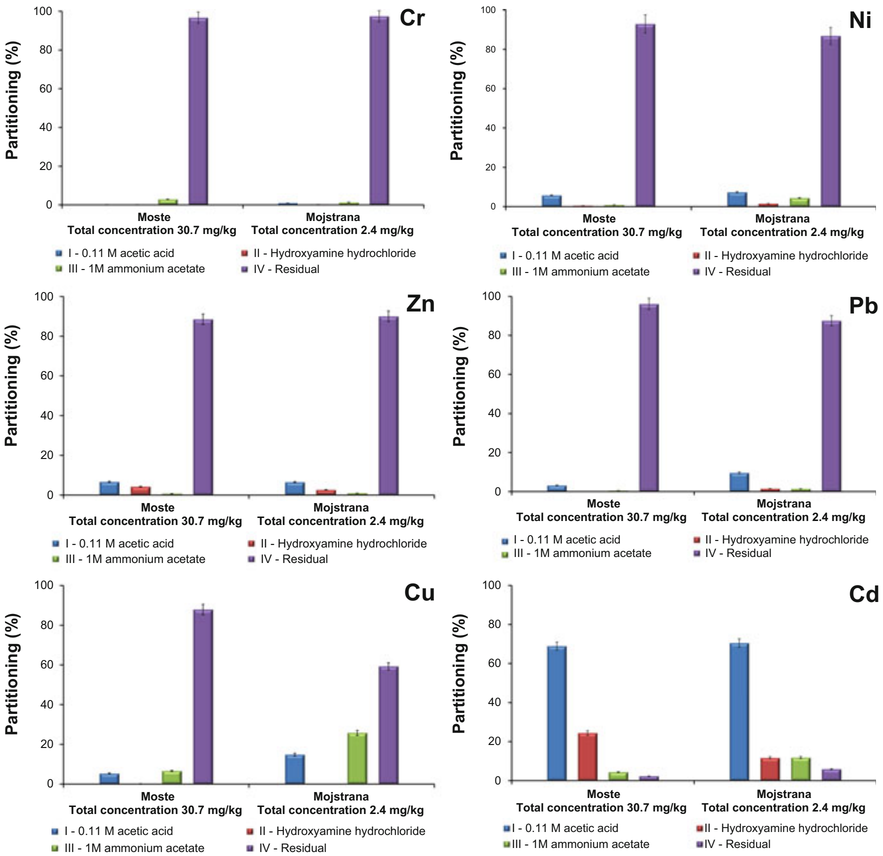

Fig. 5 Partitioning of Cr, Ni, Zn, Pb, Cu, and Cd in sediments from Moste and Mojstrana sampling sites of the Sava River by applying the modified BCR extraction scheme

The data from Fig. 5 indicate that Cr, Ni, Zn, Pb, and Cu from contaminated site are incorporated mainly into the silicate lattice, while the easily soluble fraction of the elements is negligible, meaning low hazard of these elements for the biota and environment. Cu from non-contaminated site is distributed between easily soluble fraction (about 15 %), bound to amorphous Fe and Mn oxides and hydroxides (about 25 %), and incorporated into the silicate lattice (about 60 %). Different distribution of Cu between sediment compartments in comparison to contaminated site represents its different association with sediment minerals and larger extent of Cu mobility in non-contaminated sediment. A completely different distribution pattern is evident for Cd, which is in contaminated and non-contaminated sediments present in about 70 % in the form of carbonates. This fraction is easily soluble and highly mobile in the environment and represents also the potential environmental burden, if the total Cd concentration is high.

Therefore, for the estimation of the environmental burden, the most important is the highly mobile and bioavailable metal fraction, which was further examined in sediments along the Sava River.

3.3 Assessment of Element Mobility in Sediments from the Sava River

For the investigation of the potential bioavailability of elements in sediments of the Sava River, the leaching protocol of the first step of the BCR sequential extraction procedure was used. By applying 0.11 mol L^{-1} acetic acid as an extracting agent, it is possible to estimate the easily soluble elements fraction, which has the highest impact on the environment. The portions of elements in sediments extractable in acetic acid, together with the total element concentrations, are presented in Fig. 6.

The data from Fig. 6 demonstrate extremely low mobility of Cr and low mobility of Ni. The proportions of the easily soluble metal fractions were below 0.3 % for Cr and 16 % for Ni. Since these two elements exist primarily in the sparingly soluble forms, it can be assumed that total Cr and Ni concentrations in sediments at industrially exposed sites (Moste in Slovenia and sites along the Sava River from Košutarica to Šabac) do not represent an environmental burden.

The mobile fractions of Cu in the sediments of the Sava River represent less than 2 % of its total contents, of As less than 6 %, and of Pb (with exception of Brežice site) less than 4 %, indicating their low mobility into the aquatic environment and low environmental burden.

On the contrary, high proportions of the mobile fractions in sediments were found for Cd (30–50 %) and in about half of the sampling sites also for Zn (20–40 %). However, despite the high proportion of the easily soluble Cd content, these concentrations do not represent an environmental hazard, since total Cd concentrations were low. The extractable, easily soluble Zn fraction in the sampling sites

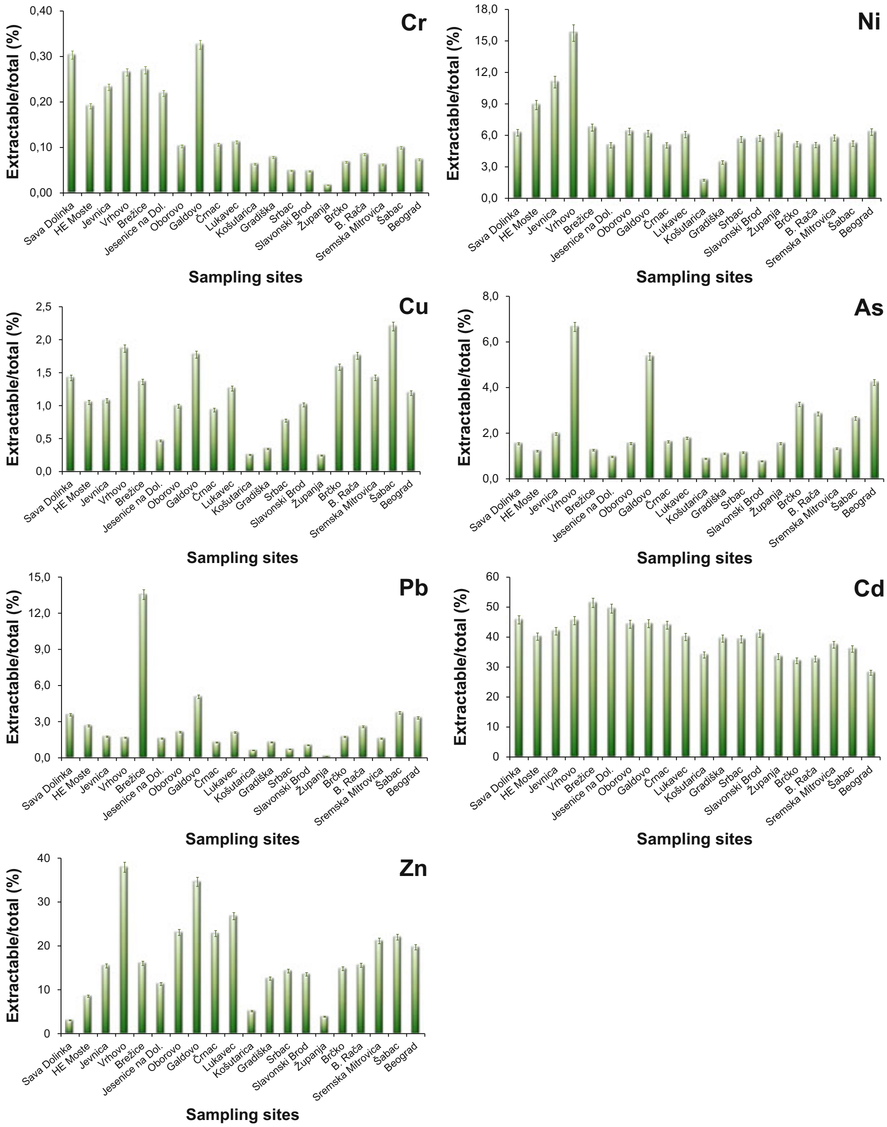

Fig. 6 Percentages of Cr, Cu, As, Pb, Ni, Zn, and Cd from sediments of the Sava River extracted with 0.11 mol L^{-1} acetic acid

investigated represented less than 90 mg kg^{-1} of Zn. This concentration is below ISQG level (124 mg kg^{-1} Zn). Therefore, regarding mobile concentrations of Zn, the potential environmental hazard and threat for the aquatic life is estimated to be low.

4 Organic Pollutants in the Sediments of Sava River

Between organic pollutants, the presence of PAH, PCB, and OCP was determined in surface Sava River sediments (Table 4).

4.1 PAH in Sava River Sediment

In this study 16 EPA priority list PAH were determined in the Sava River sediment samples. They included naphthalene, acenaphthene, acenaphthylene, anthracene, phenanthrene, fluorene, fluoranthene, benzo(*a*)anthracene, chrysene, pyrene, benzo (*a*)pyrene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, dibenzo(*a*,*h*)anthracene benzo(*g*,*h*,*i*)perylene, and indeno(1,2,3 *cd*)pyrene. Selected PAH were determined by GC-MSD (Hewlett-Packard model 6890 GC and 5972A MSD) after extraction by an ISCO supercritical fluid extractor (SFX2-10, Lincoln, NE, USA). The sample preparation and analytical procedures including quality control are described in detail in the study by Heath et al. [71, 72].

Sava River sediments contained total PAH concentrations of between 51 and 1,963 ng g^{-1}. The two exceptions were Županja and Brčko (≤3,965 ng g^{-1}) (Table 4, Fig. 7). Table 4 also reveals increasing PAH values downstream from Črnac with four sites having significantly higher PAH (the sum of 16 PAH) levels, e.g., Županja, Brčko (≤4,000 ng g^{-1}), and Bosanska Rača, Gradiška (approx. 2,000 ng g^{-1}). All four are situated downstream of the Črnac and Lukavec oil fields.

Liu et al. [25] studied the distribution and sources in surface sediments of the rivers in Shanghai, China, and found the total PAH concentration to be between 107 and 1,707 ng g^{-1}. Surface sediments from the Yellow River, China [28], revealed slightly higher total PAH concentrations (≤2,621 ng g^{-1}), while Taiwanese research found 9.8 μg g^{-1} of total PAH concentrations in the surface sediments of the Susquehanna River [19]. Total PAH content in the sediments downstream of the Kishon River in Israel [26] were ≤299 ng g^{-1}, which is comparable to Ebro River PAH sediment levels (1.07–224 ng g^{-1} [24]). In the Danube samples the total amount of PAH was 130–1,850 ng g^{-1} with the highest amount of total PAH in the bottom sediment layers in the Morava tributary (5,150 ng g^{-1} [48]).

A comparison of Sava River sediment's PAH content with reported values [19, 24–26, 28, 48] shows that the PAH pollution levels of sediment top layers are comparable (Table 4). By vertical profiling of river sediments Götz et al. [22] found that the highest concentration of the 16 EPA PAH occurred in the 1960s (43,580 ng g^{-1} in 1964) which confirms better sediment quality status in recent years.

The Canadian Environmental Quality Guidelines [32] for separate PAH in sediments quotes ISQG (Interim Freshwater Sediment Quality, dry weight) 6.71–111 ng g^{-1} and PEL (probable effect level, dry weight) 88.9–2,355 ng g^{-1}. With the exception of Županja and Brčko, pollution with PAH in sediments can be considered moderate along the Sava River.

Table 4 Concentrations of PAH (sum of 16 EPA PAH), PCB (7 indicator congeners), DDTs (the sum of *p-p* DDT, *p-p* DDE, and *p-p* DDD), and selected organochlorine pesticides (HCB, heptachlor, aldrin, lindane, dieldrin, and endrin) in Sava River sediments. All concentrations are expressed as ng g^{-1} on a dry mass basis

Sampling site	Sum 16 PAH	Sum 7 PCB	Sum DDT	HCB	Heptachlor	Aldrin	Lindane	Dieldrin	Endrin
1. Mojstrana	56	0.31	0.007	0.007	0.014	0.0050	0.021	0.0040	0.092
2. Moste	470	1.9	0.27	0.076	0.089	0.11	0.017	0.081	0.60
3. Jevnica	180	1.2	0.13	0.20	0.028	0.057	0.0020	0.099	0.0030
4. Vrhovo	290	0.77	0.19	0.035	0.044	0.058	0.066	0.011	0.26
5. Brežice	51	0.55	0.36	0.096	0.056	0.059	0.012	0.0010	0.29
6. Jesenice na Dol.	58	0.63	0.081	0.051	0.072	0.050	0.070	0.0010	0.059
7. Oborovo	450	3.9	0.61	0.011	0.41	0.042	0.057	0.11	0.21
8. Galdovo	260	2.1	2.8	0.0020	0.15	0.012	0.016	0.027	0.20
9. Črnac	1,200	2.2	0.31	0.008	0.16	0.023	0.039	0.064	0.18
10. Lukavec	1,000	0.26	0.70	0.13	0.30	0.039	0.068	0.17	0.29
11. Košutarica	1,300	5.5	1.8	0.86	0.10	0.12	0.032	0.060	0.25
12. Gradiška	1,900	2.3	0.42	0.50	0.064	0.020	0.020	0.058	0.071
13. Srbac	720	0.84	0.074	0.16	0.021	0.0050	0.0090	0.041	0.021
14. Slavonski Brod	750	2.0	0.34	0.34	0.059	0.016	0.077	0.049	0.47
15. Županja	4,000	1.6	0.62	0.10	0.16	0.011	0.058	0.087	0.98
16. Brčko	3,600	2.1	0.51	0.16	0.0010	0.0070	0.034	0.12	0.093
17. Bosanska Rača	2,000	3.4	0.88	0.11	0.0010	0.014	0.030	0.10	0.089
18. Sremska Mitrovica	1,000	3.3	0.50	0.61	0.0010	0.070	0.096	0.068	0.044
19. Šabac	1,000	2.8	1.0	1.1	0.0050	0.0060	0.069	0.074	0.049
20. Beograd	330	3.4	1.5	91	0.041	0.014	0.094	0.32	0.029

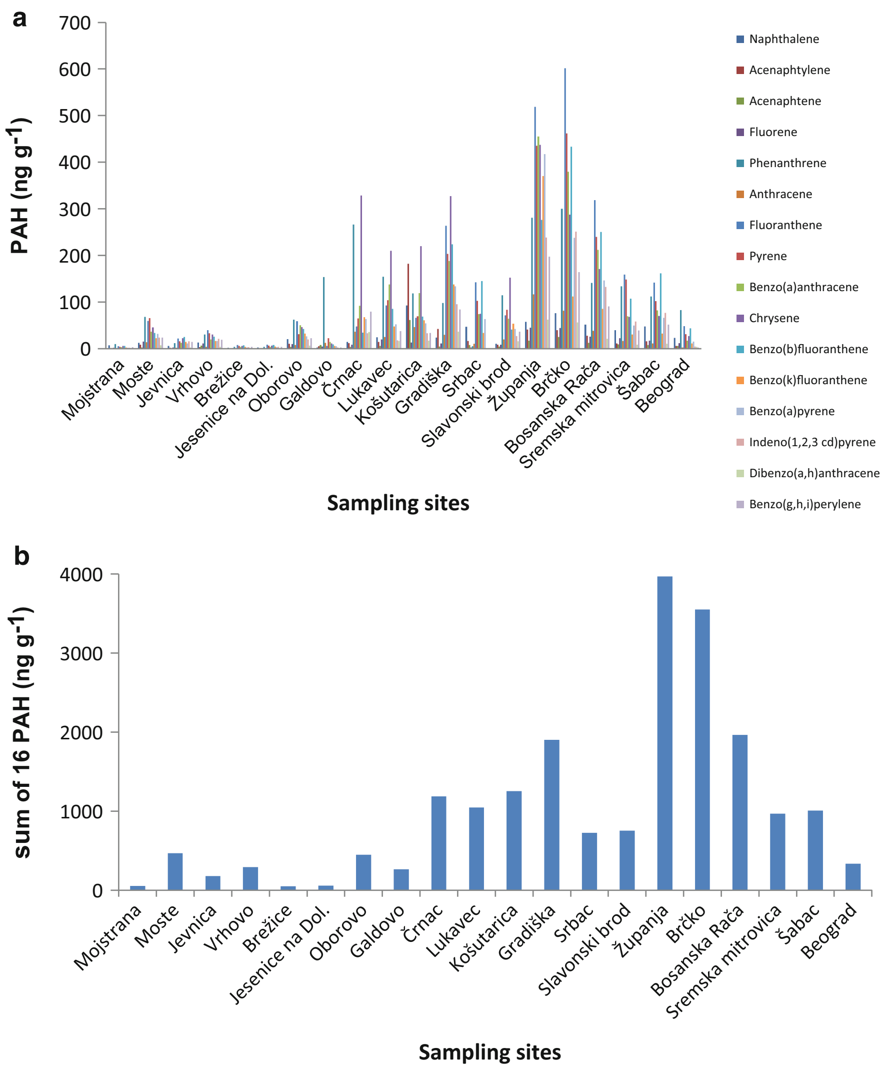

Fig. 7 The concentration of single PAH (**a**) and their sum (**b**) in sediment samples from the Sava River

Based on the sediment quality guideline of effects [19], the contents of total PAH are below the effect range median (ERM) of 44.8 μg g^{-1}, while some exceed the effect range low (ERL) of 4.02 μg g^{-1} [19]. According to the literature [19, 22, 24–26, 28], we believe that except at those locations, where levels exceed the ERL [19], PAH should not cause adverse ecological effects.

An attempt was also made to estimate the source of PAH pollution by calculating the ratio of alkylated PAH to its parent PAH (methylphenanthrene/phenanthrene and methylpyrene/pyrene, [72]). Results suggest that the main pollution in the northern part of the Sava River (Slovenia) is the direct result of combustion processes from local coal and wood heating. There was no data to show that fossil fuels were the source of PAH, which is surprising since a heavy petrochemical industry is located around Sisak (Croatia). The presence of retene is indicative of forest fires, and its elevated concentrations indicate sites possibly polluted by PAH resulting from forest fires [72].

4.2 PCB in Sava River Sediments

Polychlorinated biphenyls exist in 209 congeners sharing the same chemical skeleton but varying in the number and position of the chlorine atoms. Environmental monitoring usually concentrates on a set of seven marker or indicator PCB. These congeners were selected because they are ubiquitous in all environmental compartments [73] and cover the range of toxicological properties of the group [74]. The seven indicator PCB are 28: 2,4,4′-trichlorobiphenyl, 52: 2,2′5,5-′-tetrachlorobiphelyl, 101: 2,2′,4,5,5′-pentachlorobiphenyl, 118: 2,3′,4,4′5-pentachlorobiphenyl, 138: 2,2,3,4,4′,5′-hexachlorobiphenyl, 153: 2,2′,4,4′,5,5-′-hexachlorobiphenyl, and 180: 2,2′,3,4,4′,5,5′-heptachlorobiphenyl. Selected PCB were determined in Sava River sediments by GC-ECD (Hewlett-Packard 6890) after Soxhlet extraction with Lab-line® multi-unit extraction heater (Barnstead/Lab-line, Dubuque, IA, USA). The sample preparation and analytical procedures including quality control are described in detail in the study of Heath et al. [72].

The presence of each of the seven indicator PCB determined along the Sava River catchment is presented in Fig. 8a, while their sum is shown in Fig. 8b and Table 4. Results show no elevated concentrations in the sediments at the sampling sites downstream of the Sava River (≤ 6 ng g^{-1}). Among the samples, elevated values occur at Moste (≤ 2 ng g^{-1})—a likely result of historical steel industry pollution from Jesenice and at Košutarica (≤ 6 ng g^{-1}), resulting from local industrial activities.

When the content of PCB in Sava sediments is compared to sediments from the Danube (average concentration 4.3 ng g^{-1}, maximal concentration 46 ng g^{-1} [48]), Rhine (≤ 200 ng g^{-1} [75]), Volga (≤ 40 ng g^{-1} [75]), and Niagara (≤ 124 ng g^{-1} [76]), the Sava river is clearly less polluted. Only at Košutarica does the amount exceed 5 ng g^{-1}. With closer look at Danube river basin as a whole, of which the Sava River Basin is part of, we find that the Danube river basin is less polluted compared to values reported in the literature [8, 22, 75–77].

Vertical profiling of river sediments by Götz et al. [22] found that the highest PCB concentration correlates to the year 1980 (sum PCB: 322 ng g^{-1}) and in the period 1964–1970 (sum PCB: 224 ng g^{-1}) at two different locations on the Elbe River in Germany [22] confirms the intensive use of these compounds before 1980.

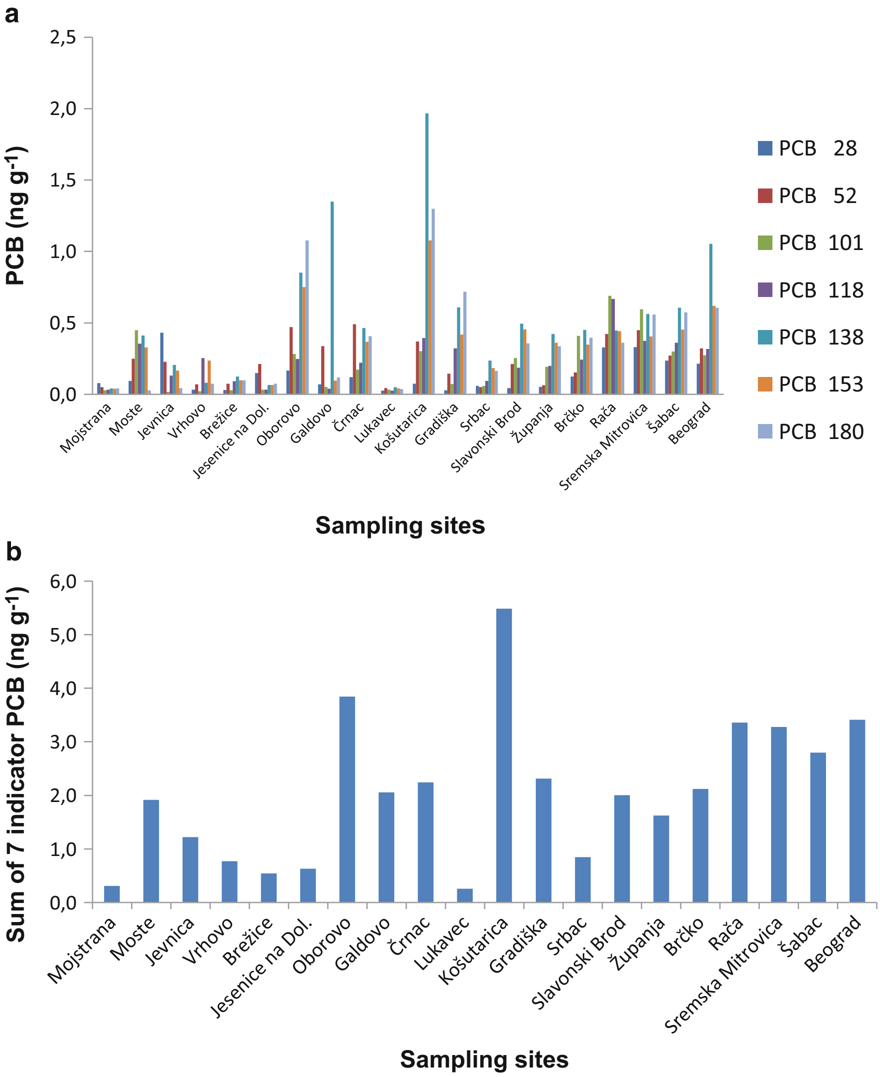

Fig. 8 The concentration of seven indicator PCB (**a**) and their sum (**b**)

According to the Canadian Environmental Quality Guidelines [32], the total PCB Interim Freshwater Sediment Quality (ISQG, dry weight) is 34.1 ng g^{-1}, while probable effect level (PEL, dry weight) is 277 ng g^{-1} [32]. According to the Sava River PCB contents determined within this study (Table 4), we can conclude that PCB pollution is not significant downstream the Sava River.

4.3 OCP in Sava River Sediments

The presence of a representative group of halogenated pesticides (hexachlorobenzene, heptachlor, aldrin, *p*,*p*′ DDE, lindane, *p*,*p*′-DDD, *p*,*p*′-DDT, dieldrin, and endrin) was evaluated in Sava River sediments (Table 4, Fig. 9). Selected organochlorine pesticides (OCP) were determined by GC-ECD (Hewlett-Packard 6890) after Soxhlet extraction with Lab-line® multi-unit extraction heater (Barnstead/Lab-line, Dubuque, IA, USA). The sample preparation and analytical procedures including quality control are described in detail in the study by Heath et al. [72].

The concentrations of OCP have different spatial distribution, resulting from different inputs, rates of degradation, and sediment texture [77]. Residues of hexachlorobenzene (HCB) occur in the environment as a result of past manufacture and as a by-product during the production of certain chlorinated compounds and is used as a pesticide and in ammunition [72, 77]. Our results are presented in Table 4. Other than in the one sample from near Belgrade, where HCB was 90.8 ng g^{-1}, we found no elevated concentrations of individual pesticides. The high HCB content in the sediment from Belgrade could be a result of the recent military conflict [72]. For most of the OCP, their levels are below 1 ng g^{-1}. Exceptions include DDT at Galdovo (2.845 ng g^{-1}) and Košutarica (1.82 ng g^{-1}) and endrin at Županja (0.98 ng g^{-1}), which is a likely consequence of intensive farming activities.

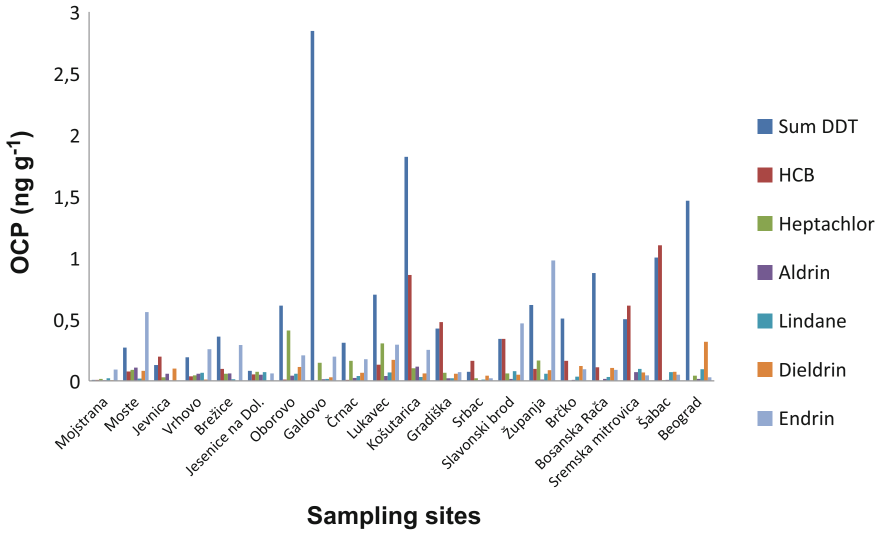

Fig. 9 The presence of selected organochlorine pesticides in the Sava River sediments. The highest OCP concentration (HCB; 90.823 ng g^{-1}) determined in a sediment sample from Belgrade is not shown on this figure

When looking at OCP levels in the Danube catchment as a whole [47], elevated levels of HCB occur near Belgrade (90.8 ng g^{-1}) on the Sava and near Budapest (23 ng g^{-1}) on the Danube with both values exceeding the Canadian "Lowest effect Level" for HCB [47] for sediments. Repeat sampling of the Sava sediments found significantly lower HCB levels and that the original high value was due to a point source of pollution [72]. When compared with other reported values, the levels of identified OCP in the Sava and Danube [48] are in the same order as the lower values found in the Buffalo River in South Africa [78], the Elbe in Spain [9, 24], and the Daliaohe River in China [20]. Overall, the levels of OCP determined in the Sava and Danube sediments shows they are not significant pollutants for the Danube catchment as whole [48].

The Canadian Environmental Quality Guidelines [32] for separate OCP are reported to be 0.6–3.54 ng g^{-1} (ISQG) and 2.74–62.4 ng g^{-1} (PEL). This confirms that there is no significant pollution by OCP in the Sava River Basin.

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Metal Bioavailability in the Sava River Water

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Abstract Metals present one of the major contamination problems for freshwater systems, such as the Sava River, due to their high toxicity, persistence, and tendency to accumulate in sediment and living organisms. The comprehensive assessment of the metal bioavailability in the Sava River encompassed the analyses of dissolved and DGT-labile metal species of nine metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the river water, as well as the evaluation of the accumulation of five metals (Cd, Cu, Fe, Mn, and Zn) in three organs (liver, gills, and gastrointestinal tissue) of the bioindicator organism, fish species European chub (*Squalius cephalus* L.). This survey was conducted mainly during the year 2006, in two sampling campaigns, in April/May and September, as periods representative for chub spawning and post-spawning. Additionally, metal concentrations were determined in the intestinal parasites acanthocephalans, which are known for their high affinity for metal accumulation. Metallothionein concentrations were also determined in three chub organs, as a commonly applied biomarker of metal exposure. Based on the metal concentrations in the river water, the Sava River was defined as weakly contaminated and mainly comparable with unpolluted rivers, which enabled the analyses of physiological variability of metal and metallothionein concentrations in the chub organs, as well as the establishment of their constitutive levels.

Keywords Acanthocephalans • DGT • European chub • Metals • Metallothioneins

1 Introduction

In the aquatic environment, metals present one of the major contamination problems and a permanent threat to health of both aquatic organisms and eventually humans, due to their high toxicity, persistence, and tendency to accumulate in sediment and living organisms [1]. Metals in aquatic ecosystem originate from

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natural and anthropogenic sources, such as industrial and domestic run-off, agricultural sources, mining, natural leaching, and geological weathering. Some metals, like Zn, Cu, Fe, and Mn, are required by aquatic organisms for their normal growth and physiological functions. For the majority of aquatic organisms, the uptake of these metals occurs from a combination of water and food, including sediment [2]. However, together with essential metals, toxic metals, like Cd, Hg, and Ag, for which no clear biological function was established, are also accumulated from the surrounding media [3]. Therefore, for comprehensive assessment of aquatic systems contamination with metals, the information on their levels in both water and aquatic organisms is needed.

Among freshwater organisms, fish are often used in the environmental biomonitoring due to their role in the biotic communities and their sensitivity to low concentrations of environmental pollutants [4]. Metals are taken up by fish through the skin, gills, and gastrointestinal tract, and therefore, common indicator organs for the assessment of metal bioavailability in the river water are the gills and gastrointestinal tissue, as metal uptake organs, and the liver and kidney, as metal detoxification organs. Specific and direct response to elevated intracellular metal concentrations is the induction of the synthesis of metallothioneins (MTs), a family of low-molecular, cysteine-rich proteins, known as biomarkers of metal exposure [4].

Metal exposure assessment of the Sava River involved evaluation of the dissolved and labile metal concentrations in the river water, as well as cytosolic concentrations of metals and MTs in three organs (liver, gills, and gastrointestinal tissue) of European chub (*Squalius cephalus* L.), which was selected as the representative indicator species among fish communities inhabiting the Sava River. Metal concentrations in the chub intestinal parasites, acanthocephalans, were additionally assessed. Acanthocephalans are potentially sensitive biological indicators which accumulate metals more effectively than their host organisms—the fishes [5].

The samplings were conducted at the 150 km long section of the Sava River in Croatia, starting at the Croatian–Slovenian state border (Otok Samoborski) and ending at the state border between Croatia and Bosnia and Herzegovina (Jasenovac). The following five sampling sites were selected (Fig. 1):

1. Otok Samoborski—low-polluted reference location (10 km upstream of Zagreb)
2. Zagreb—located within the Zagreb city area, but 20 km upstream of the main household and industrial wastewater outlets
3. Oborovo (Fig. 2)—located 15 km downstream of the industrial and municipal effluents of Zagreb (one million inhabitants and heavily industrialised) and 5 km downstream of the wastewater outlet of the city of Velika Gorica (35,000 inhabitants)
4. Lukavec Posavski—15 km downstream of Sisak city (55,000 inhabitants, oil refinery, pesticide production facility, ironworks)
5. Jasenovac—50 km downstream of Sisak city, close to the confluence of the Una River (the Sava River right tributary) [6]

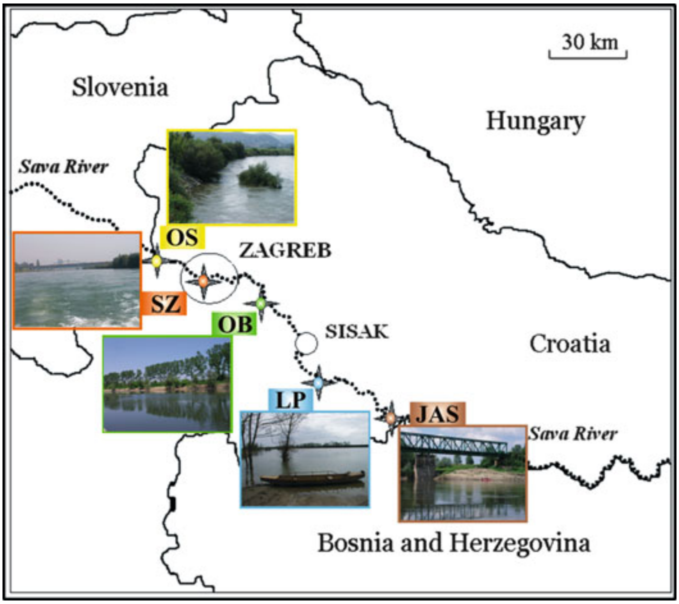

Fig. 1 The map of the 150 km long sampling section of the Sava River in Croatia, with marked sampling sites (*OS* Otok Samoborski, *SZ* Sava in Zagreb, *OB* Oborovo, *LP* Lukavec Posavski, *JAS* Jasenovac)

Fig. 2 Sunset at the sampling site Oborovo in July 2006

Table 1 Fish sampling sites, the coordinates recorded with GPSMAP 76CS (Garmin International, USA), and the basic physico-chemical parameters of the Sava River water in April/May and September of 2006

Fish sampling sites	April/May 2006				September 2006			
	WT	pH	O_2	Con.	WT	pH	O_2	Con.
Otok Samoborski N 45° 50.543′ E 15° 43.497′	12.8	7.87	97.9	465	18.4	8.26	82.1	433
Sava in Zagreb N 45° 46.572′ E 15° 56.524′	11.5	7.86	93.7	473	14.8	8.14	74.8	477
Oborovo[a] N 45° 41.286′ E 16° 14.875′	12.1	7.76	84.7	507	16.1	7.81	62.4	408
	–	7.82	–	486				
Lukavec Posavski[a] N 45° 24.081′ E 16° 32.337′	17.1	7.85	82.9	491	16.7	7.78	63.5	395
	11.7	7.68	80.5	415				
	14.8	7.59	80.9	495				
Jasenovac N 45° 15.825′ E 16° 53.658′	19.5	7.59	76.0	403	19.5	8.29	62.7	432

WT water temperature (°C), O_2 dissolved oxygen (%), *Con.* water conductivity (μS cm^{-1})
[a]In the spring period, the chub sampling was performed at more than one occasion at two sites: two times at Oborovo and three times at Lukavec Posavski; the data are presented for each of these samplings separately

Geographic coordinates and basic physico-chemical parameters of the selected locations are shown in Table 1 [7]. Two sampling campaigns were performed during 2006, in April/May, coinciding with the chub spawning and presumably higher water filtration through gills, as well as more intense feeding, and in September, coinciding with the chub post-spawning period and presumably lower metabolic activity.

2 Dissolved Metal Concentrations in the Sava River Water

The metal fraction obtained after filtration of the river water through 0.45 μm filter is defined as dissolved metal fraction. It comprises free metal ions as well as labile inorganic and organic complexes which could be easily introduced in the organs of aquatic organisms and therefore are considered as bioavailable. However, the dissolved fraction is not regarded as fully bioavailable, since it also comprises inert high-molecular organic metal complexes and colloids. Still, the measurement of dissolved metal fraction enables closer estimation of metal bioavailability in the water than determination of total metal concentrations which further comprise particulate metal fraction, collectable by 0.45 μm filter [8].

The concentrations of nine dissolved metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in the Sava River water were determined during the spring of 2006. Several samplings (8–11, depending on the sampling site) were carried out in the period from March 28 to June 16 at three river sections under different anthropogenic impact: Zagreb, Oborovo, and Lukavec Posavski (Fig. 1, Table 1). In the immediate vicinity of the sampling site Oborovo, i.e. 5 km upstream, a municipal sewage outlet of the city of Velika Gorica effuses wastewater into the Sava River. To examine direct influence of the point source of pollution on the river water quality, two additional sampling points were selected in the Oborovo area: 0.5 km upstream (N 45°43.09′ E 16°12.75′) and 0.5 km downstream (N 45°42.49′ E 16°13.58′) of the sewage outlet. The measurements were performed in the filtered and acidified (0.65 % HNO_3, *suprapur*) samples of the river water using high-resolution inductively coupled plasma-mass spectrometry (HR ICP-MS, Element 2, Thermo Finnigan, Germany) [9].

Dissolved metal concentrations in the surface water of the Sava River in spring 2006 showed the following increasing order: Cd (0.003–0.020 μg L^{-1}) < Co (0.023–0.136 μg L^{-1}) < Pb (0.003–0.234 μg L^{-1}) < Cr (0.068–0.426 μg L^{-1}) < Cu (0.055–0.881 μg L^{-1}) < Ni (0.307–1.07 μg L^{-1}) < Zn (0.089–8.74 μg L^{-1}) < Mn (0.352–14.72 μg L^{-1}) < Fe (0.646–44.52 μg L^{-1}) [9]. The analysis of spatial variability indicated increased concentrations of Co, Fe, and Mn at the sites influenced by point sources of pollution (municipal and industrial wastewater outlets of the cities of Zagreb, Velika Gorica, and Sisak) (Fig. 3b, e, f). The previous investigations indicated that the untreated wastewater of Zagreb city presents a significant source of metal input into the Sava River [10, 11]. The highest concentrations of Mn and Co, as well as increased Fe concentrations, were found immediately after the sewage outlet of the city of Velika Gorica. Dissolved concentrations of these three metals decreased with the distance from the point source of pollution. Due to their adsorption on suspended particles and subsequent precipitation, the increased concentrations of several metals can be expected rather in the river sediment than in the water [12]. The highest dissolved Fe concentrations (Fig. 3e), on the other hand, were found at Lukavec Posavski, downstream from the industrial centre of the city of Sisak, contrary to dissolved Cd (Fig. 3a) and Cr (Fig. 3c) which concentrations were the lowest at that site. The concentrations of Cu, Ni, Pb, and Zn (Fig. 3d, g–i) have not differed notably between sites [9].

The above-presented concentrations were comparable with the previously reported dissolved metal concentrations for the same section of the Sava River (in January 2005, in μg L^{-1}: Cd 0.015, Co 0.068, Pb 0.045, Cr 0.590, Cu 1.27, Ni 0.56, Zn 2.77, Mn 8.72, Fe 14.10; [11, 13]). However, they were generally slightly above the concentrations reported for some unpolluted rivers in Croatia, such as Krka (in μg L^{-1}: Cd 0.005, Pb 0.017, Cu 0.11, Ni 0.15, Fe 1.35 [14]) and Una (in μg L^{-1}: Cd 0.005, Co 0.016, Pb 0.077, Cr 0.15, Cu 0.10, Ni 0.14, Zn 0.22, Mn 1.64, Fe 1.63 [11, 13]), indicating certain level of anthropogenic impact on the Sava River. On the other hand, comparison with the environmental quality standards (EQS) set by European Water Framework Directive [15] revealed that the concentrations of several dissolved metals were still below recommended levels for inland

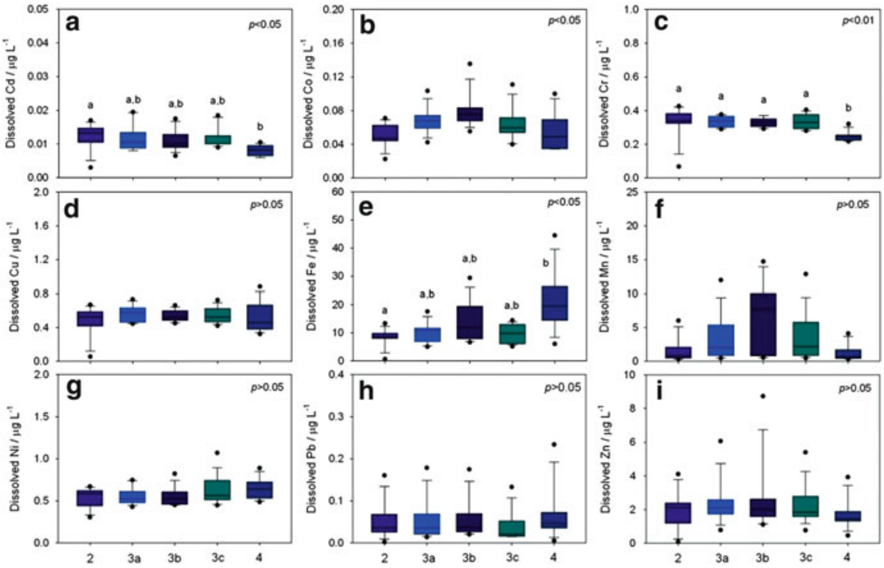

Fig. 3 Spatial distribution of the concentrations of nine dissolved trace elements in the Sava River water, in the period from March 28 to June 16, 2006, at five sampling sites (2—Zagreb ($n = 8$); 3a—0.5 km upstream from the sewage outlet of the city of Velika Gorica ($n = 9$); 3b—0.5 km downstream from the sewage outlet of the city of Velika Gorica ($n = 9$); 3c—Oborovo ($n = 11$); 4—Lukavec Posavski ($n = 8$). The results are presented as *box plots* which boundaries indicate 25th and 75th percentiles; a *line* within the *box* marks the median value; *whiskers* above and below the *box* indicate 10th and 90th percentiles, whereas *dots* indicate outliers. Differences among sites are indicated with *different letters* (a, b), based on Kruskal–Wallis one-way analysis of variance on ranks (p-values indicated within the figures) and post hoc Dunn's test ($p < 0.05$)

surface waters. The highest dissolved Cd concentration in the Sava River was approximately 4 times lower than the strictest EQS defined for Cd ($\leq$0.080 µg L^{-1}). The highest dissolved Pb and Ni concentrations were 30 and 18.5 times lower than their respective EQSs (Pb 7.2 µg L^{-1}; Ni 20.0 µg L^{-1}). In addition, the comparison was made with the Canadian water quality guidelines for the protection of aquatic life, which are derived based on a goal of no observable adverse effects on aquatic ecosystems over the long term (calculated taking into consideration the concentration of $CaCO_3$ in the water: Cd 0.067 µg L^{-1}, Pb 7.0 µg L^{-1}, Cu 4.0 µg L^{-1}, Ni 150 µg L^{-1}, Zn 30.0 µg L^{-1}, Fe 300 µg L^{-1}; http://st-ts.ccme.ca/). These recommendations were higher from 3 (for Cd and Zn) to 140 times (for Ni) than dissolved metal concentrations in the Sava River water. And finally, the average levels of dissolved Cr concentrations were lower than limits defined for unpolluted freshwaters (<2 µg L^{-1} [16]). Based on the presented data, the water of the studied section of the Sava River could be considered as only weakly contaminated with metals and still environmentally acceptable.

3 Labile Metal Concentrations in the Sava River Water Measured by Diffusive Gradient in Thin Films

Although the dissolved metal concentrations provide valuable information on water quality, the fact that they are commonly based on grab water sampling with a frequency of once or twice a month presents a serious problem for a reliable assessment of water contamination, because some elements are characterised by high short-term temporal variability. This was observed for several metals measured in the Sava River water, with the highest average relative standard deviation within a site obtained for Mn (104 %, Fig. 3f), then Pb, Zn, and Fe (94 %, 65 %, 45 %, respectively; Fig. 3e, h, i [9]).

This problem could be overcome by application of passive samplers for metals, i.e. diffusive gradient in thin films (DGT). They facilitate determination of the time-integrated average metal concentrations after long-term deployment in natural waters [17]. They also provide an advantage of determining exclusively the labile metal species in natural waters [18] which are very often associated with the biological response in aquatic organisms [19]. DGT method is based on the diffusion of dissolved metal species through a polyacrylamide gel and their immobilisation in a chelating (Chelex) resin [17]. The kinetically inert organic species are excluded by this method, as well as large colloids, because the pore size of 2–5 nm in diffusive gel does not enable their diffusion [17].

The measurement of labile metal species in the Sava River water was performed during autumn of 2005, at the same sites as measurement of the dissolved metals. The commercially available DGTs (diffusive gel thicknesses either 0.76 or 0.84 mm; DGT Research Ltd., UK [20]) were deployed 1–2 times per site, for few weeks (in total 22–33 days), in the period from October 10 to November 11 [21]. During the entire deployment period, temperature was recorded continuously using temperature data loggers StowAway® Tidbit® (Onset Computer Corporation) which enabled the precise determination of the average water temperature and thereby also of the diffusion coefficients for each metal in each deployment period.

The concentrations of nine metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were determined in the eluent acid (1 M HNO_3) obtained after 24 h elution of Chelex resin taken from DGTs, which were retrieved from the river water. The measurements were performed by two methods: (1) atomic absorption spectrometry (AAS, Varian SpectrAA 220, Australia), using flame technique for Fe, Mn, and Zn and electrothermal technique for Cd, Co, Cr, Cu, and Ni, and (2) HR ICP-MS (Element 2, Thermo Finnigan, Germany) for Pb and for the lowest Cr concentrations. Based on the metal concentrations determined in the eluent acid (C_e), the masses of the metals accumulated on the ion-exchange resin (M) were calculated according to Eq. (1), in which V_r stands for the resin volume, V_e for the eluent volume, and f_e for the elution factor. The concentrations of the labile metal species in the river water (C_{DGT}) were then calculated using Eq. (2), in which Δg represents the joint thickness of the diffusive gel and the membrane filter, δ represents the diffusive boundary layer, D is the diffusion coefficient of the metal in the gel at the defined temperature, A is the effective diffusion area, and t is the deployment time [17]. The diffusive boundary layer is a layer of water adjacent to all solid surfaces where flow velocity approaches zero [22]. It is presumably negligible in the fast flowing waters, i.e. above a low-threshold water flow of 0.02 m s^{-1} [23]. Since the Sava River is a fast-flowing river (~0.5 m s^{-1} at low water level [24]), the thickness of the diffusive layer in our calculations was equal to Δg value [21].

$$M = \frac{C_e \times (V_r + V_e)}{f_e} \tag{1}$$

$$C_{DGT} = \frac{M \times (\Delta g + \delta)}{D \times A \times t} \tag{2}$$

The DGT-labile metal concentrations measured in the Sava River during October/November of 2005 were considerably lower than the dissolved metal concentrations but showed similar increasing order: Cd (0.0001–0.0032 μg L^{-1}) < Co (0.0001–0.037 μg L^{-1}) < Pb (0.009–0.044 μg L^{-1}) < Cr (0.019–0.071 μg L^{-1}) < Cu (0.017–0.276 μg L^{-1}) ≤ Ni (0.187–0.257 μg L^{-1}) < Zn (1.28–3.80 μg L^{-1}) < Mn (1.74–42.01 μg L^{-1}) < Fe (1.21–90.01 μg L^{-1}) (Fig. 4) [21]. The increased labile concentrations of Co, Cr, Fe, and Mn (Fig. 4b, c, e, f) were found downstream of the sewage outlet of the city of Velika Gorica. At

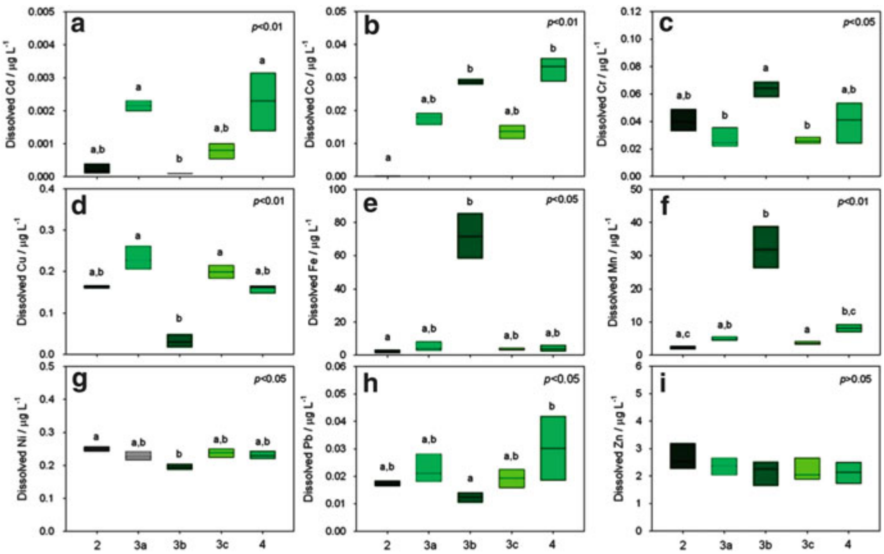

Fig. 4 Spatial distribution of the concentrations of the labile species of nine dissolved trace elements in the Sava River water measured by DGT (diffusive gradient in thin films), in the period from October 10 to November 11, 2005, at five sampling sites. The site legend and the results are presented as indicated in Fig. 3. Each *box plot* is based on the results obtained by deployment of four DGTs

that site, DGTs were deployed in the river water during the prolonged period of low water level, and the concentration increase was probably a consequence of the sewage material preconcentration combined with oxygen depletion (oxygen saturation, 22 ± 27 %; [21]). Low oxygen level usually accompanies the increase of the content of dissolved organic matter in the water due to enhanced oxygen consumption by bacteria in the process of organic matter biodegradation [12, 25]. It consequently leads to the reduction of Mn and Fe oxides, which could explain increase of their labile forms, as well as the labile forms of associated metals in the river water [26].

Contrarily, the labile concentrations of Cd, Cu, Ni, and Pb were decreased at that same site (Fig. 4a, d, g, h), which could be a consequence of the formation of inert complexes with dissolved organic matter in the river water. Such occurrence is especially characteristic for Cd and Cu. Copper complexes with organic matter, for example, are more inert compared to the complexes formed by the majority of other bivalent metal ions [27]. Similar to our study, low levels of labile species of several metals were also reported for the Lambro River, at the highly contaminated site near Milan, due to high concentration of organic material [28]. Somewhat increased concentrations of the labile species of Cd, Co, and Pb were observed at the site impacted by industrial wastewaters, Lukavec Posavski (Fig. 4a, b, h). Nevertheless, with the exception of the labile concentrations of some metals downstream of the sewage outlet (e.g. Fe and Mn) during the dry mid-autumn season, the average DGT-labile concentrations of nine analysed metals in the surface water of the selected section of the Sava River were comparable to the concentrations previously reported for the rivers regarded as unpolluted (e.g. River Wyre [21, 29]).

4 Metal Bioaccumulation in Three Tissues of Bioindicator Organism

For the assessment of the metal bioavailability, it is not enough to measure the metal concentrations in the river water, but it is also important to define the level of metal bioaccumulation in the tissues of aquatic organisms caused by determined level of exposure. Among aquatic organisms, fish are often used as bioindicators. They are one of the most indicative species in freshwater systems, for the estimation of trace metal pollution and possible risk to human health. From the ecological point of view, they are at the top of the aquatic food chain and therefore mirror the combination of the biotic and abiotic conditions in the particular aquatic environment. In addition, their size and mass of their organs enable numerous analyses, while their long life span results in a pronounced metal accumulation [30]. Among fish communities inhabiting the sampled section of the Sava River, European chub (*S. cephalus* L.; Fig. 5) was selected as an indicator species for the assessment of metal bioavailability. It is a fish species from the family of carps (Cyprinidae),

Fig. 5 European chub (*Squalius cephalus* L.) from the Sava River

Table 2 The biometric data (length, total mass, Fulton condition index (FCI), gonadosomatic index (GSI), percentage of females (F), and age) for the chub (*S. cephalus*) sampled in the Sava River at five sites (1, Otok Samoborski, $n = 15$; 2, Zagreb, $n = 18$; 3, Oborovo, $n = 13$; 4, Lukavec Posavski, $n = 15$; 5, Jasenovac, $n = 15$) in April/May of 2006

	Length (cm)	Total mass (g)	FCI (g cm^{-3})	GSI (%)	F (%)	Age (year)
1	17.41 ± 2.24	55.62 ± 25.07	0.99 ± 0.07	1.57 ± 1.94	53.3	2.4 ± 0.5
2	18.66 ± 2.82	72.54 ± 40.59	1.03 ± 0.07	0.60 ± 0.58	66.7	2.6 ± 0.7
3	19.99 ± 3.11	92.13 ± 42.44	1.07 ± 0.09	0.97 ± 1.22	84.6	2.8 ± 0.8
4	17.85 ± 1.75	68.29 ± 23.64	1.16 ± 0.12	0.80 ± 1.22	46.7	2.7 ± 0.5
5	20.49 ± 1.63	95.95 ± 26.75	1.08 ± 0.07	0.54 ± 0.15	53.3	2.8 ± 0.6

widespread in the European freshwater and tolerant to chemical and physical pollution [31]. European chub is an omnivorous fish species, which feeds on algae, plants, and various seeds [32], as well as worms, molluscs, crayfish, and insect larvae, whereas larger chub specimens also eat different species of small fish [33]. Therefore, metal analyses in the chub organs can reflect the combined metal uptake from water, as well as both plant and animal food sources.

Based on the fact that gonad development in the fish is related to increments in the daylight period, water temperature, and food supply [34], the exact period of *S. cephalus* spawning depends on the climate and in the Sava River occurs from April to June [35]. Sampling campaigns at five sampling sites along the Sava River (Fig. 1) were, therefore, conducted in April/May and September 2006, as representative periods of the chub spawning and post-spawning, respectively. The biometric data for the sampled chub are presented separately for each sampling site in Table 2 (April/May) and Table 3 (September). In the April/May campaign, 76 chub specimens of the following biometric characteristics were sampled: length 14.7–27.0 cm, total mass 29.6–205.1 g, Fulton condition index 0.88–1.35 g cm^{-3} and gonadosomatic index 0.22–6.92 %. In the September campaign, 59 chub specimens of the following biometric characteristics were sampled: length 13.5–31.5 cm, total mass 20.1–312.7 g, Fulton condition index 0.79–1.12 g cm^{-3}, and gonadosomatic index 0.15–1.09 %. In both sampling periods, 2- and 3-year-old chub were predominant in the sampled group, although in April/May, 2–5-year-old specimens and in September 1–5-year-old specimens were collected. Representation of females was 60.5 % in April/May sampling and 66.7 % in September sampling.

Table 3 The biometric data (length, total mass, Fulton condition index (FCI), gonadosomatic index (GSI), percentage of females (F), and age) for the chub (*S. cephalus*) sampled in the Sava River at five sites (1, Otok Samoborski, $n = 15$; 2, Zagreb, $n = 14$; 3, Oborovo, $n = 10$; 4, Lukavec Posavski, $n = 10$; 5, Jasenovac, $n = 10$) in September of 2006

	Length (cm)	Total mass (g)	FCI (g cm^{-3})	GSI (%)	F (%)	Age (years)
1	23.90 ± 2.98	144.84 ± 58.23	1.01 ± 0.06	0.66 ± 0.23	76.9	3.0 ± 0.7
2	17.86 ± 3.12	57.99 ± 33.49	0.92 ± 0.08	0.54 ± 0.18	91.7	2.0 ± 0.8
3	18.65 ± 4.00	68.59 ± 50.13	0.92 ± 0.07	0.59 ± 0.12	55.6	2.6 ± 0.7
4	20.60 ± 4.04	92.27 ± 55.96	0.94 ± 0.09	0.55 ± 0.23	40.0	3.3 ± 0.7
5	26.85 ± 2.43	194.92 ± 58.46	0.98 ± 0.05	0.47 ± 0.17	60.0	3.9 ± 0.7

Metals are taken up in the fish through the skin, gills, and gastrointestinal tract and, consequently, the pattern of metal distribution among fish organs is dependent on the route of metal uptake. Three chub organs were, accordingly, selected for metal analyses: the liver [36, 37], the gills [7, 38], and the gastrointestinal tissue [39, 40]. The liver is the main detoxification and storage organ, which could reflect metal accumulation caused by chronic exposure. The gills and gastrointestinal tissue, on the other hand, present main uptake sites for metals in freshwater fish, through water filtration and food consumption, respectively [41]. These two organs are in direct contact with the ambient water and ingested food, and therefore they are expected to respond quickly to changes in the metal exposure [42]. The concentrations of essential metals Cu, Fe, Mn, and Zn and nonessential metal Cd were measured in the soluble tissue fractions and not in the whole digested tissues, as common in the environmental studies. The aim of such approach was to obtain the information on the portion of metal which is presumably available for the interactions with vital cell components and consequently could cause toxic effects [43]. The soluble tissue fractions were obtained by tissue homogenisation in the cold homogenising buffer (100 mM Tris-HCl/Base buffer; pH 8.1 at 4 °C), followed by centrifugation at 50,000 × g for 2 h at 4 °C. The resulting supernatant corresponded to the cytosolic fraction, in which metal measurements were performed by electrothermal and flame AAS (Varian, SpectrAA 220, Australia [7, 36–40]).

4.1 Metal Bioaccumulation in the Chub Liver

The fish liver was shown to be the main target organ for accumulation of some metals, such as Cu and Cd [44–47]. However, since both dissolved and labile metal concentrations within the selected section of the Sava River were reported as comparable with the levels characteristic for unpolluted rivers [7, 9, 21], fish from the Sava River were subjected to relatively low metal exposure. The concentrations of trace elements in the hepatic cytosol of chub from this river were, therefore, regarded as constitutive for specific periods, April/May as a representative of spawning period, and September as a representative of post-spawning period. In both sampling periods, trace elements had the same decreasing order: Fe > Zn > Cu > Mn > Cd [36, 37].

The following concentration ranges were determined in April/May and September, respectively: Fe (2.04–14.16 μg mL^{-1} and 2.10–7.16 μg mL^{-1}), Zn (2.88–11.83 μg mL^{-1} and 2.72–6.90 μg mL^{-1}), Cu (0.295–3.66 μg mL^{-1} and 0.435–5.13 μg mL^{-1}), Mn (157.5–405.0 ng mL^{-1} and 100.0–337.5 ng mL^{-1}), and Cd (1.17–20.86 ng mL^{-1} and 2.30–25.10 ng mL^{-1}). Although three elements, Fe, Zn, and Mn, had reached higher maximal values in the spring period, statistically significant difference between two sampling periods was obtained only for Mn ($p < 0.001$, Mann–Whitney rank sum test). Elevated concentrations of some essential metals, such as Fe, Mn, and Zn, in the chub hepatic cytosol in the spring period could be ascribed to the processes connected to the reproductive cycle, as reported for red mullet (*Mullus barbatus* [48, 49]). General increase in the fish metabolism before and during the reproductive period is reflected in increased hepatic metal levels since essential metals form active parts of proteins/enzymes [48]. Karadede and Ünlü [50] also observed higher Mn concentrations in the spring in the whole liver tissue of freshwater fish *Silurus triostegus*, as well as higher concentrations of both Mn and Zn in the freshwater fish *Mastacembelus simacks*.

Analysis of spatial distribution pointed to slight accumulation in the cytosol of chub liver only for Cd (Fig. 6a, b) and Cu (Fig. 6c, d) at the most downstream site, Jasenovac, and it was more prominent in September than in April/May sampling. Similarly, Kraemer et al. [42] reported more pronounced accumulation of Cd and Cu than Zn in the liver tissue of yellow perch from metal-contaminated Lake Dufault (Canada). Andres et al. [51] observed increased Cd accumulation in the chub liver at the site close to the zinc ore treatment facility, whereas Zn concentrations varied only slightly. Since dissolved Cd and Cu concentrations in the Sava River water were comparable at all sampling sites [9], dietary and not only waterborne metal uptake should be considered as a possible source of slight, but statistically significant, increase of hepatic Cd and Cu concentrations at Jasenovac. It could be associated with the specific impacts of the industrial facility, possibly with the input of pyrolytic and petrogenic hydrocarbons from the oil refinery situated in the city of Sisak. However, it should be also emphasised that in September, the oldest and the biggest fish were caught at Jasenovac (Table 3), and, therefore, higher Cd and Cu accumulation compared to the other sites could partly be a reflection of longer exposure period.

Contrarily, essential elements Fe, Mn, and Zn were slightly increased at three upstream sites, but only in the spring sampling (Fig. 6e, g, i). In September, their concentrations were comparable at all sites (Fig. 6f, h, j). Although fish in this study were young and mostly not sexually mature, several of them had increased GSIs in the spring period, which was especially evident at the upstream sites (Table 2). The cause of the increase of essential elements at upstream sites, therefore, could be their role in metabolic processes and gonad development, and not necessarily the increased exposure in the river water.

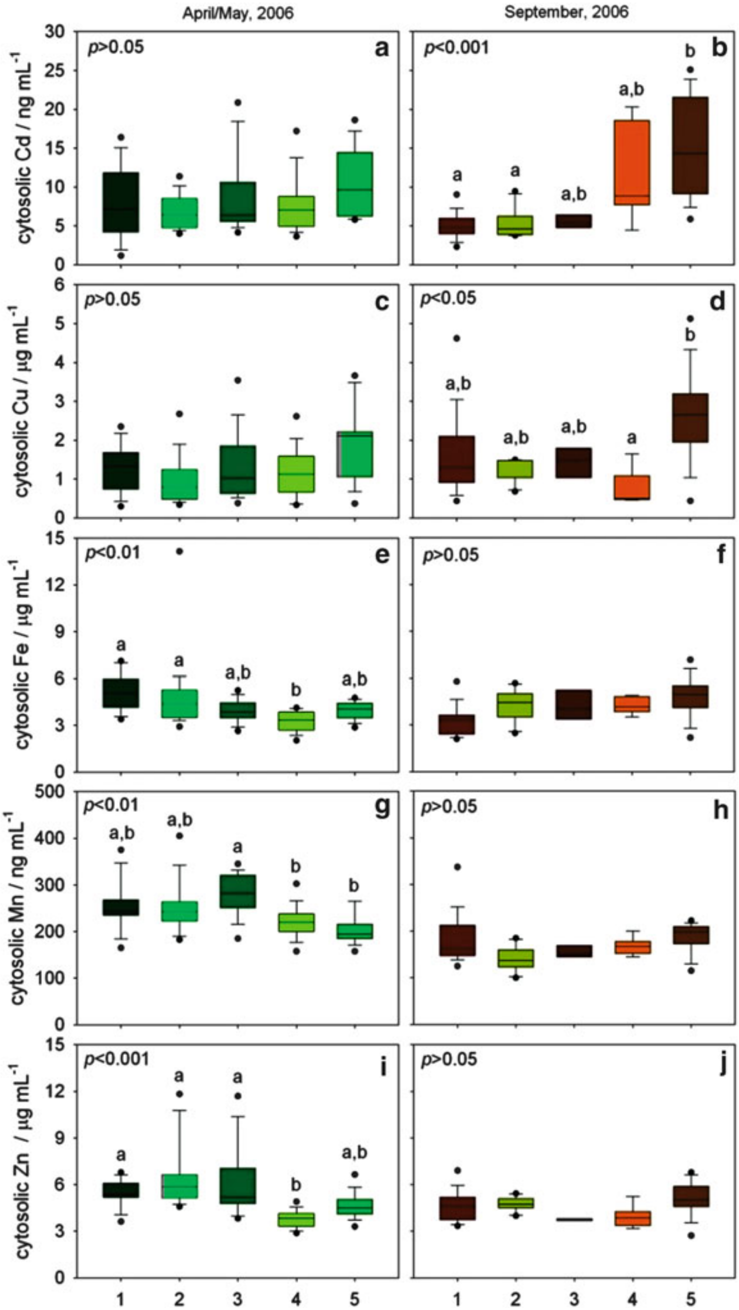

Fig. 6 Spatial distribution of the cytosolic concentrations of five trace elements in the liver of the chub caught in the Sava River in two periods, April/May and September 2006, at five sampling sites (1, Otok Samoborski ($n = 8$ and 14, respectively); 2, Zagreb ($n = 15$ and 6, respectively); 3, Oborovo ($n = 11$ and 4, respectively); 4, Lukavec Posavski ($n = 12$ and 5, respectively); 5, Jasenovac ($n = 11$ and 10, respectively)). The results are presented as indicated in Fig. 3

4.2 Metal Bioaccumulation in the Chub Gills

Contrary to metal concentrations in the liver, which represent long-term storage of metals, metal concentrations in the gills are expected to reflect short-term metal exposure in the water [52]. Therefore, they should be a good indicator of the sudden changes in the metal exposure [42]. In the chub gills, cytosolic concentrations of trace elements had the following decreasing order in both sampling periods: Zn $\geq$ Fe > Cu = Mn > Cd [7, 38]. The following concentration ranges were determined in April/May and September, respectively: Zn (5.30–16.19 μg mL^{-1} and 3.60–14.67 μg mL^{-1}), Fe (2.28–16.61 μg mL^{-1} and 3.14–8.36 μg mL^{-1}), Cu (40.42–181.97 ng mL^{-1} and 19.58–56.91 ng mL^{-1}), Mn (33.87–103.59 ng mL^{-1} and 28.23–82.49 ng mL^{-1}), and Cd (1.30–26.60 ng mL^{-1} and 0.83–2.12 ng mL^{-1}). All five elements had statistically significantly higher values in the spring period than autumn ($p < 0.001$, Mann–Whitney rank sum test). Cadmium and Cu were twice higher, whereas Fe, Mn, and Zn were 90 %, 50 %, and 40 % higher in the spring, respectively. The seasonal changes of metal concentrations in the fish tissues can arise due to the changes of the feeding and growth rate, as well as the result of the changes in the fish condition [53, 54]. The metal concentrations, especially for essential metals like Zn, increase following the increase of the metabolic activity [51]. Therefore, it can be hypothesised that the observed increase of all measured metals in the gill cytosol of chub in April/May sampling was the consequence of higher metabolic and feeding rates in the spring period than autumn [38]. It could be further supported by generally higher spring than autumn Fulton condition indices (Tables 2 and 3), which reflect the energy reserves and give the information about the recent feeding activity [55].

The analysis of the spatial distribution of cytosolic metal concentrations in the chub gills indicated generally more pronounced differences between sites in the spring period (Fig. 7). It could be possibly associated with implied increase of water filtration and feeding rates in the spring period, which could further lead to increased uptake of metals, and finally to easier identification of metal-contaminated sites.

Increase towards the downstream sites was observed for three metals: Cd, Cu, and Fe [7]. The concentrations of Cd were the highest at Oborovo and Lukavec Posavski in the spring (Fig. 7a), whereas the increase was shifted towards more downstream sites in September (Lukavec Posavski and Jasenovac; Fig. 7b). For nonessential metals, such as Cd, the concentration gradient in the water can be also expected in the fish organs [51] because Cd tissue concentrations are independent of strict physiological control, which is characteristic for the majority of essential metals [56]. Therefore, it can be assumed that higher Cd concentrations measured in the fish gills at the specific sites were the reflection of higher Cd bioavailability in the ambient water. However, dissolved and labile Cd concentrations were very low in the Sava River water ($\leq$20 ng L^{-1} and $\leq$3 ng L^{-1}, respectively) at all analysed sampling sites (Figs. 3 and 4). Although Cd, as a nonessential metal, tends to accumulate in the tissues of aquatic organisms even at relatively low water

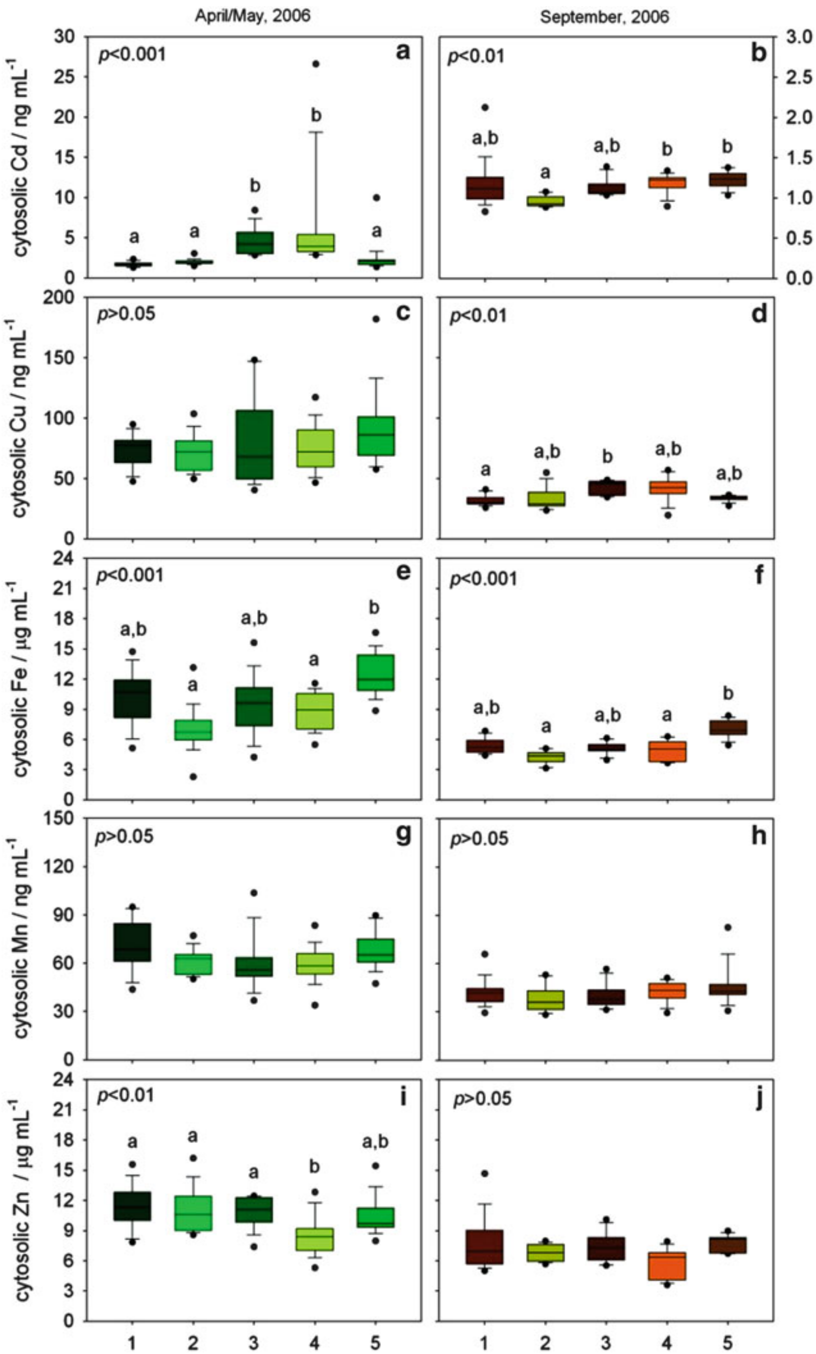

Fig. 7 Spatial distribution of the cytosolic concentrations of five trace elements in the gills of the chub caught in the Sava River in two periods, April/May and September 2006, at five sampling sites (1, Otok Samoborski ($n = 10$ and 13, respectively); 2, Zagreb ($n = 14$ and 10, respectively); 3, Oborovo ($n = 12$ and 7, respectively); 4, Lukavec Posavski ($n = 15$ and 9, respectively);

concentrations, in water with low metal levels, metal uptake from the food prevails [57]. This is also a possible explanation for increased cytosolic Cd at downstream sites observed in our study.

The differences in Cu concentrations between sites were less prominent, but slight increase was observed at Oborovo (Fig. 7c, d). Slight increase of Fe concentrations was also recorded at Oborovo, but the highest values were measured at the most downstream site Jasenovac in both samplings (Fig. 7e, f). The dissolved and labile Fe concentrations in the river water were also increased at Oborovo compared to Zagreb (Figs. 3e and 4e), whereas they were not determined in the river water at Jasenovac, thus disabling the comparison between the metal exposure and the highest cytosolic Fe measured at that site. In addition, the significant age dependence was previously observed for Fe, with the 4-year-old fish having significantly higher Fe concentrations compared to juvenile, 2- to 3-year-old fish [38]. The increase of Fe concentrations, therefore, could be partially attributed to the chub age at Jasenovac, since the sampled fish at that site were on average older and bigger compared to the remaining sampling sites (Tables 2 and 3). The concentrations of Mn (Fig. 7g, h) and Zn (Fig. 7i, j) varied less between sites, and only noticeable difference referred to the lowest Zn concentrations at Lukavec Posavski in both seasons. This can be explained by the fact that the concentrations of essential elements, such as Cu, Mn, and Zn, are generally efficiently regulated in the fish tissues by homoeostatic processes, except at highly polluted sites [51, 56]. For example, Andres et al. [51] observed increased Zn concentrations in the chub gills after exposure to extremely high concentrations of dissolved Zn in River Lot water (890 μg L^{-1}), but the increase was still not observed at a water Zn concentration of 45 μg L^{-1}, which is still much higher than dissolved Zn concentrations in the Sava River water (<5 μg L^{-1} [9]).

4.3 *Metal Bioaccumulation in the Chub Intestine*

There is a growing concern that dietborne metal uptake may be of equal or greater importance than the waterborne metal uptake for native fish [58]. In distinct contrast to seawater fish, freshwater fish drink negligible amounts of water. Consequently, in the gastrointestinal tract of freshwater fish, primarily dietborne metals accumulate [59], which enables the application of the digestive tract as an indicator organ for dietary metal exposure. In both sampling seasons, metal levels in the gastrointestinal cytosolic fraction of European chub followed the order: Zn > Fe > Cu ≥ Mn > Cd (Fig. 8). The following concentration ranges were determined in April/May and September, respectively: Cd (3.94–244.9 ng mL^{-1} and

Fig. 7 (continued) 5, Jasenovac ($n = 14$ and 10, respectively)). The results are presented the same as indicated in Fig. 3

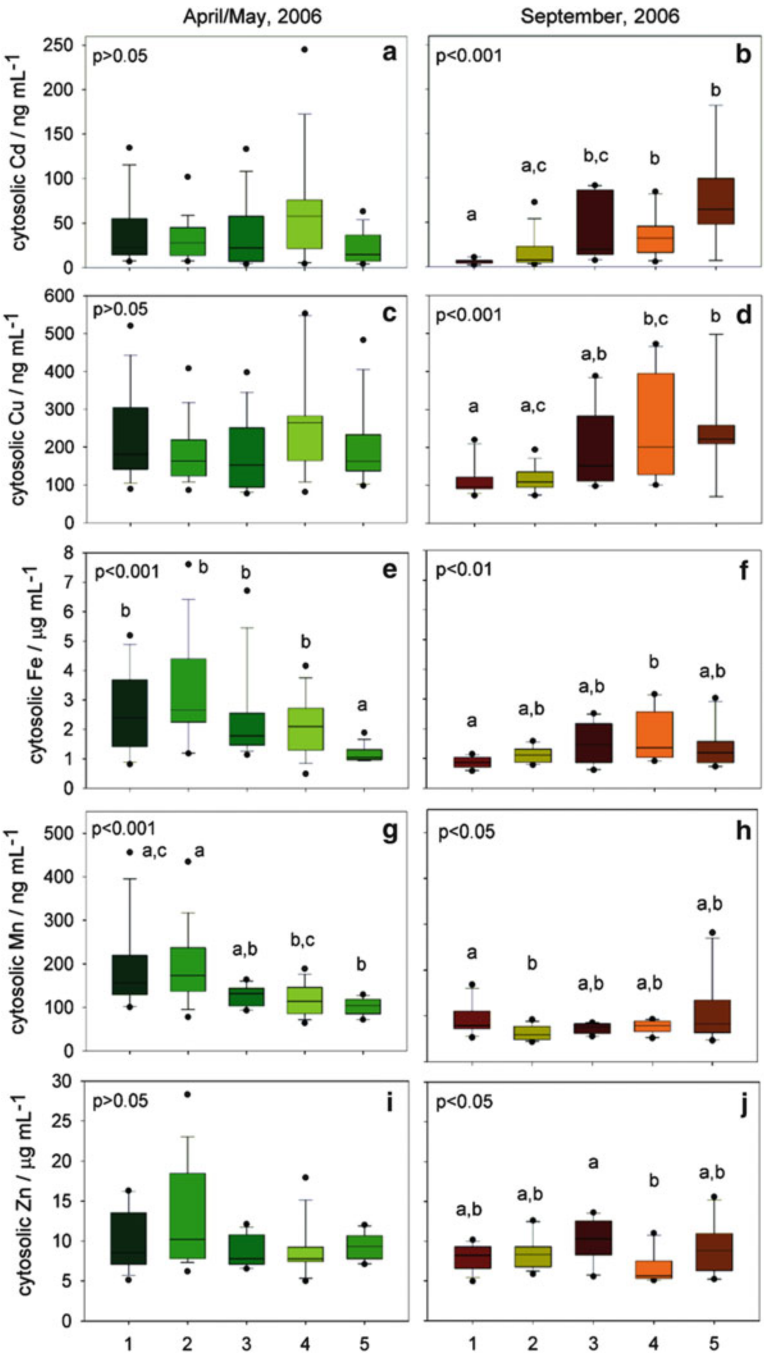

Fig. 8 Spatial distribution of the concentrations of five trace elements in the gastrointestinal cytosol of chub caught in the Sava River in two periods, April/May and September 2006, at five sampling sites (1, Otok Samoborski ($n = 13$ and 15, respectively); 2, Zagreb ($n = 18$ and 14, respectively); 3, Oborovo ($n = 13$ and 10, respectively); 4, Lukavec Posavski ($n = 15$ and

1.78–181.8 ng mL^{-1}), Cu (77.6–553.5 ng mL^{-1} and 70.4–498.0 ng mL^{-1}), Fe (0.49–7.61 μg mL^{-1} and 0.58–3.16 μg mL^{-1}), Mn (64.0–456.2 ng mL^{-1} and 43.6–281.8 ng mL^{-1}), and Zn (5.0–28.32 μg mL^{-1} and 4.96–15.58 μg mL^{-1}).

Comparison of the cytosolic metal concentrations between two sampling seasons indicated significantly higher levels of all five measured metals during the period of fish spawning and intense feeding [39]. Iron and Mn were 80–90 % higher during the spawning period, while Cu, Zn, and Cd from 20 to 30 %. The variability of the gastrointestinal cytosolic metal concentrations between spawning (April/May) and post-spawning period (September) might reflect differences in fish nutritional processes, which arise due to increased water temperature, food availability, and reproduction-related physiological changes in April/May period [60]. As already stated, it can be supported by higher Fulton condition indices in the chub sampled in April/May (Tables 2 and 3), which reflect the energy reserves and give the information about the recent feeding activity [55].

Seasonal differences were also reflected in different spatial distributions of metal levels in the gastrointestinal cytosolic fraction in two samplings. In the spring spawning period, higher Zn, Fe, and Mn levels were found at two upstream locations compared to three downstream locations, and the difference was statistically significant for Fe and Mn (Fig. 8e, g, i). As stated in Sect. 4.1, several chub individuals might be considered as sexually mature based on the increased GSIs in the spring period, which was especially evident at the upstream sites and which might have caused increased levels of these essential metals associated with gonad development. Copper levels were slightly increased at Otok Samoborski and Lukavec Posavski (Fig. 8c), while Cd levels were the lowest at Jasenovac and comparable at other four locations (Fig. 8a). Under the conditions of low metal contamination of the river water, metal levels tend to show significant relationship with biotic factors. Such association was evident between metals in the chub gastrointestinal cytosol and hepatosomatic index. Positive association with hepatosomatic index was statistically significant for Fe and Mn in the gastrointestinal cytosol ($r = 0.32$, $p < 0.01$, and $r = 0.41$, $p < 0.01$, respectively). In addition, as seen from Table 2, enhanced fish nutrition (higher condition and hepatosomatic indices) was specific for April/May, the period characterised by chub spawning and presumably increased feeding rate and metabolic activity. Therefore, different feeding rates, metabolic activity, and spawning-related changes in fish might have influenced the spatial distribution of the gastrointestinal metal concentrations in the April/May campaign [61].

In September, spatial metal distribution followed different pattern. Cadmium and Cu tended to increase towards the downstream locations (Fig. 8b, d), while Fe and Zn were significantly higher at Oborovo compared to the remaining sites (Fig. 8f, j). Manganese concentrations were the highest at Otok Samoborski and

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Fig. 8 (continued) 10, respectively); 5, Jasenovac ($n = 14$ and 10, respectively)). The results are presented the same as indicated in Fig. 3

Jasenovac (Fig. 8h). Since dissolved metal concentrations in the Sava River water were low (Sect. 2) [9], evident increase of some metal concentrations towards the downstream locations might indicate that the gastrointestinal metal levels reflected metal exposure from food, thus highlighting the importance of considering both waterborne and dietborne metal uptake. This statement could be further confirmed by the spatial distribution of metal concentrations in the gut content, which was comparable to the spatial distribution of the gastrointestinal metal levels in the cytosolic fraction for all five measured metals in April/May [61] and for Cu, Mn, and Zn in September [40]. Besides possible impacts of the industrial facilities at downstream sites, especially of the oil refinery situated in the city of Sisak, possible impacts of biotic factors on metal levels were again analysed for the September campaign, especially having in mind that the oldest fish were found at downstream locations. The results of correlation analysis confirmed significant positive relationship with fish age for the gastrointestinal cytosolic concentrations of Mn ($r = 0.28$, $p < 0.05$) and Cd ($r = 0.46$, $p < 0.01$). Accordingly, statistically significantly higher metal concentrations in the gastrointestinal cytosol of 4–5-year-old chub compared to 1–2-year-old individuals were obtained for Cu, Mn, and Cd (Mann–Whitney rank sum test, $p < 0.05$ [40]). In addition to already reported age dependence of several elements in the liver, kidney, or gill tissue of various freshwater and marine fish [38, 62, 63], our results confirmed that accumulation of Mn and Cd also occurs with age in the gastrointestinal cytosol of European chub.

4.4 Comparison of Metal Bioaccumulation in Three Organs

Various elements show a tendency to accumulate in different fish organs, which can differ between sampling periods but also can depend on the route of metal uptake (waterborne/dietborne) and on the level of metal exposure. At low level of metal exposure, such as observed in the Sava River, the following patterns of metal distribution between three chub organs were defined for constitutive metal levels:

Cu, Mn (April/May and September)	Liver > gastrointestinal tissue > gills
Zn (April/May)	Gills > gastrointestinal tissue > liver
Zn (September)	Gastrointestinal tissue > gills > liver
Fe (April/May and September)	Gills > liver > gastrointestinal tissue
Cd (April/May and September)	Gastrointestinal tissue > liver > gills

The tendency to accumulate in the liver was observed for Cu and Mn, whereas an opposite trend was observed for Zn with the lowest accumulation in the liver, in both April/May and September samplings. High tendency of Zn to accumulate in the gastrointestinal tissue is consistent with the previous finding that intestine serves as Zn storage tissue in fish [64]. The highest Fe levels were measured in the gills, probably in association with Fe being an integral part of the oxygen binding metalloprotein haemoglobin [65], since gills are richly supplied with blood vessels in order to act as a respiratory organ. The highest accumulation of Cd was observed in the gastrointestinal tissue, thus implying the predominant uptake from the food sources, which is consistent with previous reports on prevailing dietary metal uptake in the water with low metal levels [57]. The difference in metal concentrations between three organs was especially evident for Cu. The hepatic Cu levels were on average 6–15 times higher compared to the gastrointestinal tissue and gills in the spring and as much as 10–40 times in September. Contrarily, the differences between the concentrations in different chub organs for other metals were much less pronounced.

In addition to the selection of the most appropriate organ which shows the highest tendency for specific metal accumulation, it is also important to keep in mind that metal accumulation depends on many other factors, such as the time of sampling, the physiological variability, as well as the route of metal uptake. It was observed that seasonal variability of metal accumulation was mostly governed by the reproduction-related processes, such as gonad development, as well as the concurrent increase in the water filtration and feeding rate in the spring period due to higher requirements for nutrients. Accordingly, due to the function of essential metals in the metabolic processes during the spawning period, their concentrations were increased in all three organs in the spring, while in the uptake organs (gills and intestine), even the concentrations of nonessential metal Cd.

The spatial distribution of metal concentrations in the chub organs was also influenced by the reproductive cycle. The association with gonadosomatic index, hepatosomatic index, and the sexual maturity of fish was observed in the spring period for essential elements in both the liver and the intestine, which is the reason why the post-spawning period was recommended as more appropriate for the assessment of chub metal exposure by the use of these two organs. Contrarily, the spring period seems more adequate for the assessment of metal exposure if the gills are applied as target organ, due to higher uptake of metals as a consequence of higher rate of water filtration. Finally, when evaluating chub metal exposure using any of these three organs, the chub age also has to be considered, since several elements exhibit tendency to accumulate with age.

5 Metal Bioaccumulation in the Chub Intestinal Parasites Acanthocephalans

In the past decades, the interrelation between parasites and contaminants has gained increasing interest, especially in aquatic ecotoxicology [5, 66]. Certain parasites, particularly the intestinal acanthocephalans of fish, have enormous accumulation capacity for metals, especially toxic ones, and can respond very rapidly to changes in the environmental exposure [5]. Accordingly, attempts were made at using acanthocephalans as biological indicators of metal exposure in the environmental risk assessment studies [67]. Till now, most of the papers indicated that metal

Fig. 9 Acanthocephalans, the intestinal parasites of European chub (*Squalius cephalus* L.) from the Sava River

Table 4 Basic epidemiological characteristics of acanthocephalans from the chub sampled along the Sava River: number and gender of sampled chub, number and percentage of uninfected chub, prevalence of infection for each parasite (number and percentage of infected chub), mean intensity of infection, and total number of parasite individuals in the sampled chub (in males and females)

Sampling period *n* (number of ♂/♀/ND)	Uninfected chub *n* (%)	Prevalence *n* (%)		Mean intensity of infection		Total number *n* (♂/♀)	
		PL	AA	PL	AA	PL	AA
April/May *n* = 76 (30/46/0)	20 (26 %)	40 (53 %)	36 (47 %)	4.2	3.3	167 (53/114)	120 (36/84)
September *n* = 59 (18/36/5)	25 (42 %)	31 (53 %)	11 (19 %)	3.0	1.4	93 (14/79)	15 (5/10)

n number of fish, *ND* not determined, *PL P. laevis*, *AA A. anguillae*
Total number, total number of parasite individuals in the sampled chub

accumulation in the parasites is more effective than in the tissues of their hosts or commonly used indicator organisms. The application of acanthocephalans as biological indicators in metal exposure assessment of the Sava River involved the comparison of metal concentrations and their spatial distribution in two acanthocephalan species, *Pomphorhynchus laevis* and *Acanthocephalus anguillae*, and their host, European chub (Fig. 9). For the purposes of direct comparison of metal concentrations in the fish and acanthocephalans, essential (Fe, Zn, Mn, Cu) and nonessential (Cd) trace metals were not only measured in the fish gastrointestinal cytosol, but additionally in the gastrointestinal tissue.

Basic epidemiological characteristics of acanthocephalans from the chub sampled along the Sava River are shown in Table 4. In both sampling periods, higher intensity of infection was found for *P. laevis* than for *A. anguillae*. Moreover, *P. laevis* individuals were predominant in female chub in both seasons, while *A. anguillae* only in April/May. Seasonal differences were evident in mean intensity of infection, which was higher in the spring for both acanthocephalan species [68].

5.1 Metal Concentrations in the Chub Gastrointestinal Tissue and Acanthocephalans

In both seasons, the concentrations of Cu, Mn, and Cd in acanthocephalans were significantly higher than in the chub gastrointestinal tissue, while Zn and Fe levels were significantly higher in the chub gastrointestinal tissue, with exception of comparable Fe levels in *A. anguillae* and the chub gastrointestinal tissue in September (Fig. 10).

The most pronounced difference between metal concentrations in the intestinal parasites and the gastrointestinal tissue of the chub from the Sava River was evident for Cu and Cd, whose levels were from three to five times higher in acanthocephalans than in their host in both seasons. One of the possible explanations of higher metal concentrations in acanthocephalans than in the fish tissues is based on parasite dependence on host micronutrients, since they lack the gastrointestinal system. Essential metals, as elements of physiological importance, are highly absorbed from fish intestine by acanthocephalans. It is therefore possible that the competition among parasites for essential elements may also lead to the increased absorption of nonessential elements, such as Cd [69].

Parallel analysis of metal concentrations in the intestinal parasites and their host represents a combination of short time and long time exposures, since acanthocephalan life span is relatively short and ranges from 50 to 140 days [70], compared to a much longer chub life span, which ranges from 10 to 15 years [71]. Therefore,

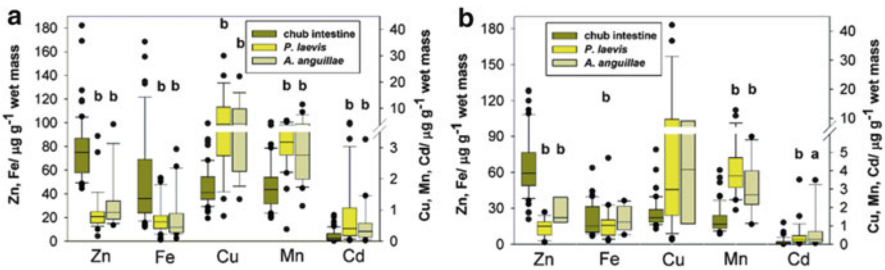

Fig. 10 Metal levels (μg g^{-1} wet mass) in the chub gastrointestinal tissue, *Pomphorhynchus laevis* and *Acanthocephalus anguillae* in (**a**) April/May and (**b**) September. The results are presented the same as indicated in Fig. 3. Statistically significant differences (Mann–Whitney rank sum test) in metal levels between the chub gastrointestinal tissue and parasites at the significance levels $p < 0.01$ (a) and $p < 0.001$ (b) are indicated

the ratio between metal concentrations in the acanthocephalans and the host tissue, which is named bioconcentration factor, could provide information on the duration of environmental exposure, as metal uptake occurs more rapidly in the parasites. Low ratio, i.e. comparably high metal levels in both fish parasites and intestine, would indicate a longer exposure time compared to high ratio, i.e. higher metal levels in the parasites than in the intestine [72]. The highest bioconcentration factors in the chub intestinal parasites were found for Cd and Cu, which ranged from 3.3 to 5.1 in both sampling seasons, followed by Mn (2–3), Fe (0.4–1), and Zn (0.2–0.4). Compared to our study, bioconcentration factors previously calculated as the ratio between metal concentrations in *Acanthocephalus lucii* and perch intestine were higher for Cu (50), Cd (20), Fe (6), and Zn (8) and comparable for Mn (2) [73]. Data related to *P. laevis*-barbel system also reported higher bioconcentration factors compared to our study, for example, for Cd (15.6), Cu (11.0), Zn (4.0), and Mn (3.9) between *P. laevis* and the intestinal tissue of barbel from the Danube River in Bulgaria [74], and for Cd (15.6), Cu (11.0), Zn (4.0), and Mn (3.9) between *P. laevis* and the intestinal tissue of barbel from the Danube River in Hungary [75]. Therefore, lower bioconcentration factors in both acanthocephalan species from chub compared to the other studies indicated that acute metal exposure did not occur in the Sava River. This finding is supported by the results on average total dissolved metal concentrations in the surface water of the Sava River, which were not significantly above the natural level (Sect. 2) [9].

5.2 *The Comparison of Spatial Metal Distribution in the Chub Gastrointestinal Tissue and Acanthocephalans*

In order to evaluate the application of acanthocephalans as bioindicators of metal exposure in the Sava River, spatial metal distribution in the parasites and chub hosts was compared. Due to the influences of fish spawning and higher feeding intensity in the spring period on the gastrointestinal cytosolic metal concentrations in fish dwelling in the low metal-contaminated river water, site-specific differences of metal levels were considered only for the post-spawning season, in September (Fig. 11). In addition, metal concentrations in the gastrointestinal tissues of uninfected chub and chub infected with *P. laevis* were compared, since it was reported that acanthocephalans might alter metal uptake and accumulation, resulting in reduced metal levels in the tissues of infected host [76]. Only the data for *P. laevis* were presented since the total number of *A. anguillae* at five sampling locations was too low to allow statistical treatment (1–7 individuals per location) [77].

As seen in Fig. 11, the spatial distribution of metals with higher accumulation in parasites than the chub gastrointestinal tissue was presented, i.e. Cu, Mn, and Cd. All three metals showed the same spatial pattern in *P. laevis* and the

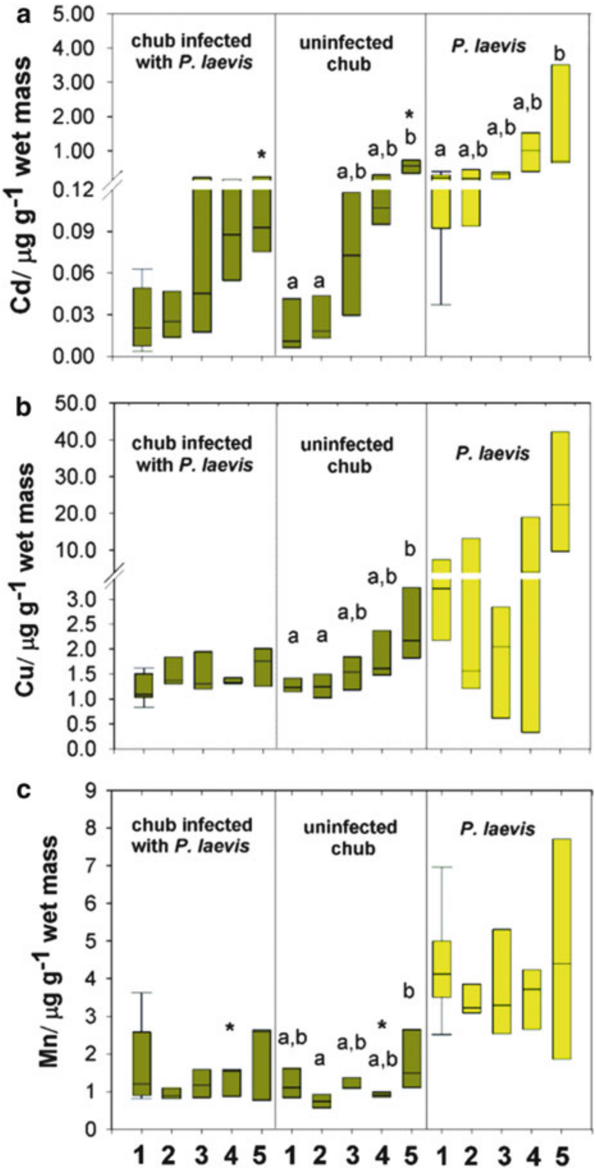

Fig. 11 Spatial distribution of (**a**) Cd, (**b**) Cu, and (**c**) Mn levels (μg g^{-1} wet mass) in *P. laevis* and the gastrointestinal tissue of uninfected chub and chub infected with *P. laevis*. The number of analysed samples per site was 9/7/4/3/3 for infected chub, 5/4/4/7/5 for uninfected chub, and 15/8/6/4/3 for *P. laevis*. The results are presented the same as indicated in Fig. 3. Statistically significant differences at the significance level $p < 0.05$ among different locations are indicated with different letters (Kruskal–Wallis one-way analysis of variance with Dunn's test for all-pairwise comparisons) and between uninfected and infected groups of chub from the same location by asterisk ($p < 0.05$, Mann–Whitney rank sum test)

gastrointestinal tissue of uninfected and infected chub. The concentrations of these metals were increased towards the downstream locations, with statistically significant difference between upstream and downstream locations for Cu and Cd in uninfected chub and Cd in *P. laevis*. The Spearman correlation analysis confirmed a significant relationship of Cd ($r = 0.75$, $p < 0.01$) in the chub gastrointestinal tissue and *P. laevis*. Increased Cd concentrations towards the downstream locations were also evident in the cytosolic fractions of the chub liver (Fig. 6b), gills (Fig. 7b), and gastrointestinal tissue (Fig. 8b) and of Cu and Mn in the chub gastrointestinal tissue (Fig. 8d, h). Therefore, our data indicate *P. laevis* as promising biological indicator of bioavailable metal concentrations. It is evident that site-specific differences were more pronounced in uninfected chub for Cu and Cd, whose levels were 6.0 and 1.5 times, respectively, higher than in the chub infected with *P. laevis*. Previous studies revealed that acanthocephalans can reduce metal levels in the tissues of their hosts [76, 78]. Bile–metal complexes formed in the fish liver pass down the bile duct into the small intestine, where in the infected fish acanthocephalans take up bile-bound metals and reduce the amount of metals available for reabsorption by the host. In uninfected fish, bile-bound metals can either be reabsorbed by the intestinal wall or, to a lesser extent, excreted with the faeces [78]. Our results indicate that even in the river water with the low metal contamination, *P. laevis* reduced Cu and Cd levels in the chub gastrointestinal tissue, and, therefore, parasites should be taken into account as a potential confounding factor in the environmental risk assessment studies [68, 77].

6 Metallothionein: Biomarker of Metal Exposure

Metallothioneins (MTs) constitute a family of low-molecular, cysteine-rich proteins functioning in the regulation of the essential metals Cu and Zn, as well as in the detoxification of both essential metals excessively present in the cells and nonessential metals with no known biological functions, such as Cd, Hg, and Ag [4]. The induction of MT synthesis is one of the best known biochemical responses to increased bioavailability of metals in the environment and, therefore, it is applied as a biomarker of metal exposure [4, 79]. The binding of metals to MT has a sequestration function that renders them unable to interact with other sensitive molecules and, thereby, produces protection against metal toxicity at the cellular level [80, 81].

MT concentrations in the liver, gills, and gastrointestinal tissue were used to evaluate biochemical response to metal exposure in the chub from the Sava River. Many factors unrelated to metal contamination can also induce MT synthesis, and their influence on MT level should also be considered and estimated [82, 83]. Therefore, next to the assessment of the spatial variation of MTs as a result of different metal exposure, MT levels were also compared between two sampling seasons, to observe their possible association with fish spawning and concurrent physiological changes.

Since MTs present heat-stable proteins, their measurement was performed in the heat-treated cytosolic fraction, which was obtained after the heat treatment at 85 °C for 10 min [84]. MT analyses were performed by differential pulse voltammetry on 797 Computrace (Metrohm, Switzerland), according to the modified Brdička procedure [85]. MTs were quantified from the calibration straight line using commercially available >95 % pure zinc-MT (I+II) from rabbit liver (MT-95-P, Ikzus Proteomics), dissolved in 0.25 M NaCl.

The comparison of MT concentrations in three chub organs indicated that MTs were always present in the highest concentrations in the gastrointestinal tissue, then in the gills, and the lowest in the liver. Since the gills and gastrointestinal epithelial tissues are involved in the uptake, detoxification, and excretion processes [86], higher MT presence in those tissues is probably associated with the important function of MTs in metal uptake, as well as their protective role against excessive uptake.

However, the concentrations in the gills exhibited the strongest seasonal dependence, with the spring concentrations being almost two times higher than in September ($p < 0.001$; Mann–Whitney rank sum test [87]). Gastrointestinal MT concentrations were comparable in both sampling periods ($p > 0.05$ [39]), whereas hepatic MT was higher in the spring ($p < 0.001$ [36]), but the difference was less pronounced than in the gills. Therefore, gill MT concentrations were close to high levels of gastrointestinal MTs in the spring period and decreased to lower hepatic MT value in September sampling (Fig. 12). The spring increase of metal and MT concentrations in the gills could be explained as a consequence of increased metabolic and feeding activity [38]. Enhanced feeding was previously suggested as a possible influential factor on MT level [53, 88], and it was confirmed in the spring period by significantly higher Fulton condition indices compared to autumn season (Tables 2 and 3 [38]). It could have caused metabolic stimulation, which subsequently causes accelerated gill ventilation, and thereby also the enhanced uptake of essential as well as toxic metals [30]. It is, therefore, possible that pronounced spring increase of MT level in the gills was an outcome of its important role in uptake of essential metals, such as Zn [89]. The seasonal variability of hepatic MT was, on the other hand, attributed to different phases of the reproductive cycle, with higher levels obtained in the pre-spawning/spawning period due to the process of vitellogenesis [36]. Less pronounced seasonal difference of hepatic MT compared to gill MT could be explained by the fact that chub in this study were mainly not sexually mature.

Although MT is a biomarker of metal exposure, any factor which is able to influence protein metabolism will be also able to influence MTs directly, whereas factors known to influence metal uptake and accumulation, such as size, sex, or sexual maturity, will be able to influence MTs indirectly [79]. For example, female chub had somewhat higher average hepatic MT level in the reproductive period (2.01 mg g^{-1}) than males (1.82 mg g^{-1} [36]), while there was no difference between sexes in gill MT level [87]. The sex differences were probably more evident in the liver than gill tissue, because the liver has an important role in the reproduction, for example, in the process of vitellogenesis [90]. In addition, the estimation of gender-related differences in the gastrointestinal tissue indicated significantly higher MT levels in males in April/May than in September ($p < 0.5$, Mann–Whitney rank sum test), probably because the sampled chub population in April/May comprised few sexually mature males from the upstream sites [39].

Gill MT levels in the spring period, on the other hand, varied depending on the chub age and mass. Approximately 10 % higher MT levels were obtained in the gills of younger fish ($p < 0.05$), and accordingly correlation between mass and gill

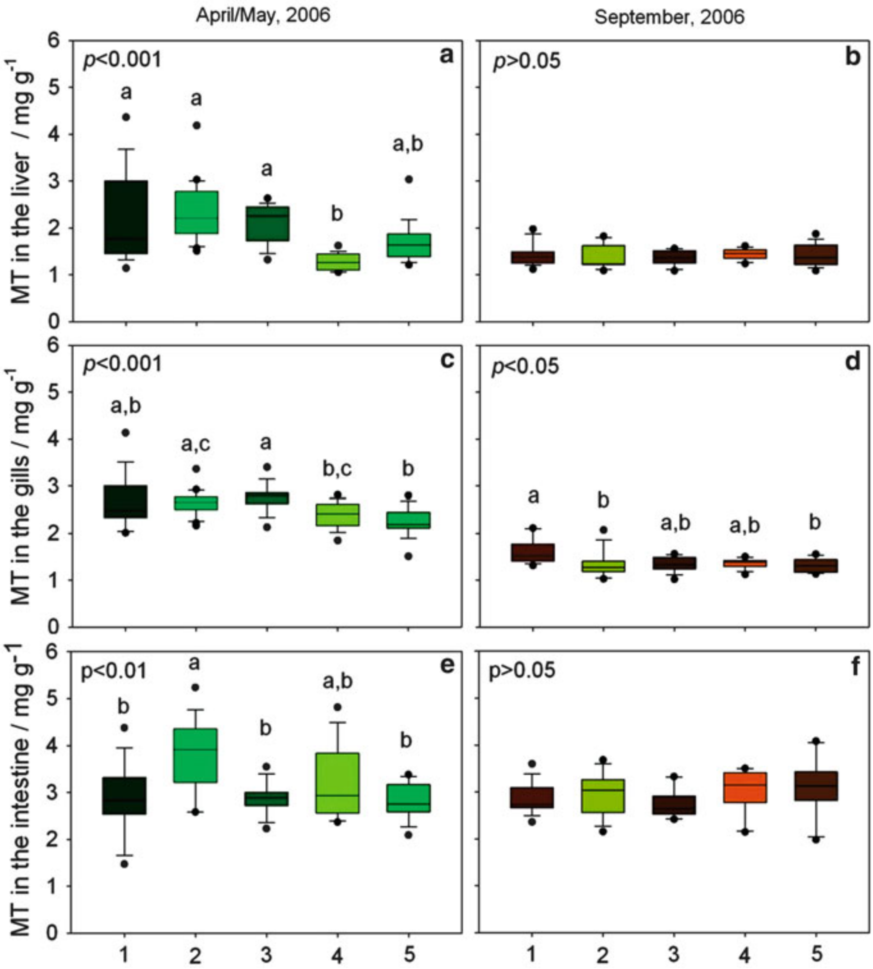

Fig. 12 Spatial distribution of metallothionein (MT) concentrations in the liver, gills, and gastrointestinal tissue of chub caught in the Sava River in two periods, April/May and September 2006, at five sampling sites. The number of analysed samples per site was 13/17/11/15/14 in April/May and 15/7/7/8/10 in September for hepatic MT, 15/18/12/15/14 in April/May and 13/14/10/10/10 in September for gill MT, and 14/18/13/15/14 in April/May and 15/14/10/10/10 in September for gastrointestinal MT. The results are presented the same as indicated in Fig. 3

MT level was negative. Younger and smaller fish are known to have faster filtration and metabolic rates, and consequently higher concentrations of proteins (e.g. metallothioneins) in fish tissues can be expected as the result of homeostatic regulation [91], especially in the period of more intense metabolic activity, such as presumably spring reproductive period [87]. Contrarily, in the September sampling campaign, positive association was obtained between MTs and the gill mass [87].

Physiological variability of MT levels has also reflected on the spatial distribution of this biomarker. In the spring period, increased MT concentrations in all three organs were found at upstream sites: in the liver and gills at the sites 1–3 (Otok Samoborski, Zagreb, Oborovo; Fig. 12a, c [36, 87]) and in the gastrointestinal tissue only at the site 2 (Zagreb; Fig. 12e [40]). Increased MT levels coincided with increased cytosolic concentrations of essential elements: Zn and Mn in the liver [36], Zn in the gills [87], and Zn, Fe, and Mn in the gastrointestinal tissue. Gastrointestinal MT additionally showed a clear association with the spatial distribution of HSI [40]. Although chub in this study were mainly not sexually mature, at upstream sampling sites, several specimens had increased GSI in the spring period, which indicated their sexual maturity. Therefore, the influence of fish spawning and concurrent increase of metabolic activity, feeding rate, and water filtration was probably reflected in MT concentration increase in all three organs. In addition, the association between the spatial distribution of MT and Zn concentrations was characteristic for all three organs, which could be attributed to a significant role of MTs in both Zn homoeostasis and detoxification. However, in our study, MT association with cytosolic Zn was more probably related to its function in Zn regulation than to the level of exposure in the water, since Zn concentrations in the Sava River water were exceptionally low (<5 μg L^{-1} [9]).

In the September campaign, MTs generally showed less variability between sites in all three organs (Fig. 12b, d, f), and only significant differences were observed in the gills (Fig. 12d), with the highest MT level measured at the sampling site 1 (Otok Samoborski). The spatial distribution of gill MTs was similar to Fulton condition indices and the gill masses, indicating to strong MT association with the chub size and condition [87]. Although in September Cd, Cu, and Fe in the gill cytosol and Cd and Cu levels in the gastrointestinal and hepatic cytosol, as well as in *P. laevis*, showed increasing trend towards the downstream locations, accumulated metal levels were probably not high enough to induce additional MT synthesis and to show significant association with MTs.

Since metal exposure in the Sava River water was defined as low and comparable to natural conditions [9, 21] and the variability of MT concentrations in all three organs was predominantly associated with reproduction- and nutrition-related changes [36, 40, 87], the constitutive MT levels were defined for each organ, separately for the spawning and post-spawning periods, as mean ± one standard deviation (encompassing 68 % of the obtained data). Constitutive MT concentrations in the gastrointestinal tissue were similar in the spawning and post-spawning period, 2.4–3.9 mg g^{-1} and 2.5–3.4 mg g^{-1}, respectively. Contrarily, constitutive gill and hepatic MT concentrations were higher in the spawning period (2.1–3.0 mg g^{-1} and 1.2–2.7 mg g^{-1}, respectively) compared to the post-spawning period (1.2–1.7 mg g^{-1} and 1.2–1.6 mg g^{-1}, respectively). At low level of dissolved metals in the river water, MTs in the chub organs reflected physiological changes to a greater extent than metal exposure from the river water.

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Potentiometric Determination of Anionic and Nonionic Surfactants in Surface Waters and Wastewaters

Milan Sak-Bosnar, Dubravka Madunić-Čačić, Zorana Grabarić, and Božidar Grabarić

Abstract Surfactants are used in almost all branches of industry, in everyday life as home and industrial cleaning compounds, in cosmetics, in pharmaceuticals, in foods, in crop protection, etc. Their waste belongs to the most widespread organic pollutants, representing a global environmental problem, giving thus a great importance for their monitoring in the environment. The existing methodology for the monitoring of anionic and nonionic surfactants in effluents is based on the time-consuming extraction-spectrophotometric procedures connected with numerous drawbacks: considerable chemicals consumption, use and disposal of toxic organic solvents, etc. Potentiometric methods with surfactant sensors (surfactant-selective electrodes) sensitive to the surfactants overcome almost all of these disadvantages offering an attractive alternative to the existing methods. The biggest challenge in surfactant analysis is the determination of low levels in environmental samples.

This review outlines the principles of response mechanisms of these sensors and their application for the determination of anionic and nonionic surfactants in surface waters and effluents. Advantages of the use of surfactant-selective electrodes vs. classical methods and their limitations are also outlined. The potentiometric methods mentioned can be used for simple determination of anionic and nonionic surfactants in the Sava River in Zagreb and downstream, at inflow of wastewater from the treatment plants.

Keywords Anionic surfactants • Nonionic surfactants • Ion-selective electrodes • Potentiometric sensors

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List of Abbreviations

AS	Anionic surfactant
ASE	Accelerated solvent extraction
AS-FET	Anionic surfactant ion-sensitive field-effect transistor
c	Concentration
CE-DAD	Capillary electrophoresis with diode array detection
CMMWCNT	Carboxyl-modified multiwall carbon nanotube
CS	Cationic surfactant
CSE	Commercial surfactant electrode
CTAB	Cetyltrimethylammonium bromide
DBS	Dodecylbenzene sulfonate
DMIC	1,3-Didecyl-2-methylimidazolium chloride
DMI-TPB	1,3-Didecyl-2-methylimidazolium-tetraphenylborate
DS	Dodecyl sulfate
ELSD	Evaporative light scattering detection
EO	Ethoxy
EONS	Polyethoxylated nonionic surfactant
FIA	FLOW-injection analysis
FL	fluorescence detection
HPLC	High-performance liquid chromatography
Hy	Hyamine
ISE	Ion-selective electrode
LAS	Linear alkylbenzene sulfonate
LC–MS	Liquid chromatography–mass spectrometry
LED	Light-emitting diode
LLE	Liquid–liquid extraction
MB	Methylene blue
MBAS	Methylene blue active substance
N	Number of determination
NaDBS	Sodium dodecylbenzene sulfonate
NPDE	*o*-Nitrophenyl decyl ether
NPDOE	*o*-Nitrophenyl dodecyl ether
NPOE	*o*-Nitrophenyl octyl ether
NPTE	*o*-Nitrophenyl tetradecyl ether
NS	Nonionic surfactant
PEG	Polyethylene glycol
PPy-DBS	Dodecylbenzene sulfonate-doped polypyrrole
PVC	Poly(vinyl chloride)
SAS	Secondary alkane sulfonate
SPE	Solid-phase extraction
TA-DS	Tetrahexadecylammonium dodecyl sulfate
TPB	Tetraphenylborate
UPLC-MS	Ultra performance liquid chromatography–mass spectrometry

UV	Ultraviolet detection
σ	Standard deviation

1 Introduction

Surfactants (surface-active agents) are substances that reduce surface tension and contain in their molecular structure hydrophobic and hydrophilic functional groups. Each surfactant molecule aligns itself with its hydrophobic part far away from the aqueous phase while the hydrophilic part remains in contact with the water. Surfactants are used in almost all branches of industry, in everyday life as home and industrial cleaning compounds, in cosmetics, in pharmaceuticals, in plating baths, in petroleum products, etc. Their waste belongs to the most widespread organic pollutants, representing a global environmental problem.

Anionic and nonionic surfactants (NSs) together account for about 90 % of the total production of synthetic surfactants.

Anionic surfactants (ASs) are the oldest and the most common type of surfactants. The hydrophobic part of the molecule is usually an alkyl chain of various lengths, alkylphenyl ether, or alkylbenzene, and the hydrophilic part is carboxylate, sulfate, sulfonate, or phosphate group.

NSs do not dissociate into ions in the water solution, so the solubility of these substances is provided by their polar head groups. A balance between hydrophobic and hydrophilic structures contained in the surfactant molecule is responsible for their surface activity.

The hydrophobic part of NSs is generally an alkylated phenol derivative, fatty acid, or long-chain linear alcohol. The hydrophilic part is mostly an ethylene oxide chain of various lengths. NSs have no charge and therefore they are compatible with both cationic surfactants (CSs) and ASs. They are widely used as emulsifiers, wetting agents, and foam stabilizers. They can be also used in various biotechnological processes, in pesticide formulations, and as solubilizers.

In aquatic environments, surfactants form a surface film reducing in this way oxygen transfer at the water surface. Some surfactants may be acutely toxic to aquatic organisms. The soil characteristics can be also altered by surfactants, enabling easier movement of contaminants through soils into groundwater. The biodegradation of surfactants is very slow resulting in carcinogenic and reproductively toxic by-products such as nonylphenol.

Excessive use of surfactants and their disposal in the environment could seriously affect the ecosystem; therefore, the amounts of all surfactants released in sewage and aquatic recipient are monitored and regulated.

The European Union continuously work on the regulation regarding the use and the monitoring of surface-active agents in order to efficiently protect the environment (*Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents*).

For this reason, it is important to have a fast, accurate, and inexpensive analytical method for the determination/detection of surfactants.

2 Definition and Classification of Surfactants

As a surface-active compound, surfactants lower the interfacial tension between two liquids, or liquid and a solid phase. Basically, they are organic chemicals that consist of hydrophobic and hydrophilic parts. Surfactants are classified as anionic, nonionic, cationic, and amphoteric.

ASs are mostly high-foaming surfactants sensitive to water hardness. Their hydrophobic part consists of a straight, branched, cyclic, or aromatic hydrocarbon group, while their hydrophilic part is a negatively charged sulfonate, sulfate, or carboxylate group. They are most effective than other surfactants in particulate soil removal from natural fabrics, easily spray dries, and thus are favored for powder detergents.

NSs do not dissociate in water. They are more tolerant to water hardness and well suited for cleaning purposes. Most of them are low foaming with good water solubility at lower temperature.

CSs adhering with their positively charged hydrophilic part on the negatively charged surfaces are useful as softeners and antimicrobial, antistatic, and anticorrosion agents. They have no wash activity effect.

In *amphoteric surfactant* molecules, the charge of the hydrophilic part is controlled by the pH of the solution.

3 Potentiometric Sensors: Definition and Principles of Operation

Potentiometry is an electrochemical technique which provides information on the composition of a sample by measuring the potential, or voltage, of an electrochemical cell. The cell consists of both an indicator and reference electrode. The potential of the reference electrode is constant, thus the potential of the indicating electrode contains information related to the analyte concentration in the sample.

The sensing part of the electrode, known as an ion-selective electrode (ISE), is an ion-specific membrane. The membrane of an ion-selective electrode is responsible for its response characteristics and selectivity. It is a continuous layer, usually consisting of a semipermeable (solid or liquid) material, with controlled permeability. The membrane separates the internal components of the ISE from the test solution. ISEs can be defined as electrochemical sensors whose potential, in combination with a reference electrode, depends on activity a_i of the ion investigated in the solution according to Nernst equation:

$$E = E^0 \pm \frac{0.059}{z_i} \log a_i, \tag{1}$$

where E^0 is the constant potential term and z_i the charge of the ion measured. The sign is positive for cations and negative for anions.

The potential generated at the membrane is the result of either an ion-exchange process or an ion transport process occurring at each interface between the membrane and solution.

One of the most important advantages of an ISE is its selectivity which depends on the composition of the membrane.

The ideal sensor responds to only one single ion. Almost all sensors, with partial exception of the glass electrode (pH sensor), suffer more or less from influence of different interfering substances, which contribute to the sensor response too.

The effect of interferents on the response of an ISE is described by the Nikolskii–Eisenman equation (2):

$$E = E^0 \pm \frac{0.059}{z_i} \log \left(a_i \pm K_{ij} \times a_j^{\frac{z_i}{z_j}} \right), \tag{2}$$

where a_i and a_j are the activities of the analyte and interfering ion, respectively, and z_i and z_j are their corresponding charge numbers.

The extent of interference is expressed in terms of the potentiometric selectivity coefficient K_{ij}. The potentiometric selectivity coefficient expresses the ratio of sensitivities of interfering vs. analyte ion and defines the ability of an ISE to distinguish a particular ion from others. It is clear that selectivity coefficients should be as small as possible for interferents in order to reduce their contribution to the overall sensor response.

Furthermore, Eq. (2) demonstrates also that the effect of interference decreases with increasing sample activity a_i. In practice the concentration of analyte is more demanded rather than its activity, and this is attained by using an empirical calibration graph.

4 Anionic Surfactant Determination

The wide use of ASs in domestic and industrial washing agents results in strong environmental pollution. Therefore there is a necessity for the development of simple, inexpensive, fast, and accurate methodologies for their determination in aqueous environment.

One field of special interest in environmental analysis is control of biodegradability, because environmental legislation does not permit marketing of surfactant products with less than 90 % biodegradability [1].

An ever-widening spectrum of techniques is available for the detection, identification, and quantitative determination of surfactants in environmental samples with a complex matrix composition [2]. In the methodologies for analyzing environmental samples, the isolation and/or preconcentration of analytes constitutes an important step. The usual techniques are liquid–liquid extraction (LLE), solid-phase extraction (SPE), and accelerated solvent extraction (ASE) [3].

Chromatographic methods are among the mostly used separation techniques intended for the quantification of particular species in complex mixtures.

A simple and simultaneous analysis method for four (anionic, amphoteric, nonionic, and cationic) classes of surfactants in shampoo and hair conditioner was developed. Analysis of the surfactants was performed using a reversed-phase high-performance liquid chromatography (HPLC) combined with evaporative light scattering detection (ELSD) without any pretreatment. The elution peaks were identified by a liquid chromatography–mass spectrometry (LC–MS) [4]. The carboxyl-modified multiwall carbon nanotubes (CMMWCNTs) can be used as adsorbents of SPE for the extraction of linear alkylbenzene sulfonate (LAS) homologues that can be determined by HPLC with ultraviolet detection (UV) [5].

Rapid methods for the determination of total LAS from sewage sludge based on microwave-assisted extraction and HPLC with fluorescence detection (FL) and capillary electrophoresis with diode array detection (CE-DAD) are proposed. The determination of total LAS is carried out in less than 5 min. The methods did not require cleanup or preconcentration steps [6].

A new methodology has been developed for the determination of secondary alkane sulfonates (SAS) in environmental matrices. Determination of SAS was carried out by HPLC or ultra performance liquid chromatography–mass spectrometry (UPLC-MS) [7].

The general analytical method for low levels of ASs in water is spectrophotometry of a methylene blue active substance (MBAS), based on the formation of ion-pair compounds between cationic dye methylene blue (MB) and the ASs [8, 9]. The blue-colored complex formed during the analysis has been extracted with chloroform and spectrophotometrically determined. Sodium dodecylbenzene sulfonate (NaDBS) has been used as a reference AS. This analytical procedure has been used for more than half a century [10].

The MBAS method has many disadvantages, such as numerous interferences, difficulty of operation, long analysis times, difficult analysis of colored samples, and the need for large volumes of chloroform and a lot of laboratory glassware, among others. The main interferences are quaternary ammonium compounds and proteins which give lower results. The higher results are obtained in the presence of organic sulfates, sulfonates, carboxylates, phenols, and inorganic anions such as cyanate, nitrate, thiocyanate, sulfide, and any substance other than AS-forming compounds with MB which are soluble in chloroform. To overcome the disadvantages of the MBAS method, a lot of variations have been developed, most of which aim to reduce the reagent consumption, especially chloroform amount, or avoid its use applying alternative cationic dyes as spectrophotometric reagents [11–14].

Flow analysis has achieved its majority as a well-established tool to solve analytical problems. Its potential to minimize reagent consumption and waste generation and the ability to implement processes unreliable in batch to replace toxic chemicals is of great importance [15]. Due to evolution in flow analysis, the automated method for the determination of MBAS substances using continuous flow analysis has been accepted as a standard method for the determination of the ASs in wastewater control [16]. Although automation of the above standard method overcomes disadvantages, such as difficulty of operation, long analysis times, use of the large volumes of chloroform, and a lot of laboratory glassware, problems caused by interferences still remain.

The flow-injection analysis (FIA) was also applied for AS determination using spectrometric detection. Lavorante et al. developed a multicommuted stop-flow system employing LED-based photometer for the sequential determination of ASs and CSs in water [17]. No significant differences were observed between the results found and those obtained using reference procedure.

An optical sensor for the detection of ASs was developed too. The optical membrane responds to ASs, such as dodecylbenzene sulfonate (DBS), dodecyl sulfate (DS), and di-2-ethylhexyl sulfosuccinate in the concentration range from 1 to 50 μM [18].

4.1 Potentiometric Sensors for Anionic Surfactants

Potentiometric titrations using surfactant-sensitive electrodes as endpoint indicator provide an attractive alternative method for the determination of ASs. This simple procedure can easily be automated. Investigations of the potentiometry with surfactant-sensitive electrodes used as sensors for surfactant determination began in the 1970s [19–21]. Further investigations were directed at improving the characteristics of the sensors.

The surfactant-sensitive potentiometric sensors are sensitive to the analyte and to the titrant. Basically, the ionic surfactant-sensitive electrodes suitable for the detection of A^- and C^+ have the same design: the electroactive part of an ISE membrane consists of an ion pair (C^+A^-), where A^- is an anion of an AS and C^+ is a positively charged counterion, usually a cation of a CS.

Electromotive force of the membrane sensor assembly dipped in the solution of AS investigated is given by the Nernst equation:

$$E = E^0 - S \cdot \log a_{\mathrm{AS}^-}. \tag{3}$$

The main application of the sensor described was for indication of the endpoint in ion-pair surfactant potentiometric titrations. During titration the AS reacts with the CS accompanied by the formation of water-insoluble (1:1) ion-pair CS^+AS^-:

$$\mathrm{CS}^+ + \mathrm{AS}^- \rightleftharpoons \mathrm{CS}^+\mathrm{AS}^-. \tag{4}$$

For the above equilibrium, the solubility product is defined as

$$K_{sp} = a(CS^+) \cdot a(AS^-), \quad (5)$$

where $a(CS^+)$ and $a(AS^-)$ are activities of the corresponding surfactant ions.

From Eq. (5), $a_{AS^-} = K_{sp}/a_{CS^+}$, and after insertion into Eq. (3), the following sensor response is obtained:

$$E = E^0 - S \cdot \log K_{sp}/a_{CS^+} \quad (6)$$

which after rearrangement yields

$$E = \text{const} + S \cdot \log a_{CS^+}, \quad (7)$$

where $\text{const} = E^0 - S \cdot \log K_{sp}$.

From Eq. (7) it follows that after the equivalence point, the sensor responds to changes in the concentration of cationic titrant (cationic response). Further addition of the cationic titrant after the equivalence point causes further increase of the sensor potential E. It can be also concluded from Eq. (7) that the magnitude of the inflection at the equivalence point is strongly dependent upon the solubility product value. Lower K_{sp} values cause a higher potential change at the equivalence point, resulting in a more sensitive surfactant determination.

Sanchez et al. presented an excellent review describing the ongoing evolution of potentiometric sensors as employed in the field of ASs, beginning with the first reports published in the 1960s. Although the 1970s saw an increased use of such devices due to the adoption of PVC [poly(vinyl chloride)] matrices, it is only relatively recently that commercial electrodes for these species have been available to industry. The latest developments, particularly the study of new polymer formulations and their application to other transducing devices, are also discussed [22].

The potentiometric behavior of coated wire electrodes based on dodecylbenzene sulfonate-doped polypyrrole (PPy-DBS), a conducting polymer with an improved electrochemical activity, and Hyamine (Hy) as ion exchanger was investigated [23]. The selectivity behavior of the PPy-DBS-based electrode revealed significant improvement in comparison to conventional Hy-DB-based ion exchanger.

The effect of different plasticizers in the sensing membrane on the performance of a surfactant ISE based on a PVC membrane with no added ion exchanger was investigated [24]. As plasticizers *o*-nitrophenyl octyl ether (NPOE), *o*-nitrophenyl decyl ether (NPDE), *o*-nitrophenyl dodecyl ether (NPDOE), and *o*-nitrophenyl tetradecyl ether (NPTE) were used. Electrodes based on NPDE, NPDOE, and NPTE produced better results than NPOE-plasticized PVC membrane electrodes in terms of low detection limits. On the other hand, the use of NPOE derivatives did not enhance the performance of surfactant-selective PVC membrane electrodes with respect to slope, sensitivity, selectivity, response time, or pH effect.

The interaction of a new ionophore aza-oxa-cycloalkane 7,13-bis(*n*-octyl)-1,4,10-trioxa-7,13 diazacyclopentadecane (L1) with ASs was studied and finally utilized as an alternative to the commonly used quaternary ammonium salts in

ASs-selective electrodes [25]. PVC electrodes made with L1 and NPOE gave a Nernstian response, a reasonable detection limit, good stability, and low selectivity coefficients.

A new DBS anion-selective electrode based on polyaniline-coated Pt electrode was prepared [26]. Sensor showed a good selectivity in an aqueous solution and Nernstian response to DBS ions and response time <20 s. The low detection limit, together with the good selectivity and sensitivity of polyaniline film to DBS ions, makes this electrode potentially useful for monitoring of NaDBS in real samples.

By functionalization of PVC used for membrane sensor, high-quality surfactant-selective electrode membranes that have a Nernstian response, short response time, and appropriate stability can be prepared [27].

The new ligand 7-methyl-7,13-di-octyl-1,4,10-trioxa-13-aza-7-azonia-cyclopentadecane has been used as an ionophore in the development of ISE for ASs [28]. PVC membrane ion-selective electrodes containing this ligand and NPOE as plasticizer displayed a Nernstian response in the presence of DS and a reasonable detection limit and response time. This new ligand might be an attractive alternative to the commonly used quaternary ammonium salts as ionophores in ISE for ASs.

The cyclam derivative 1,4,8,11-tetra(*n*-octyl)-1,4,8,11-tetraazacyclotetradecane has been used as carrier for the preparation of PVC-based membrane ISE for ASs [29]. This electrode displays a Nernstian slope of -60.0 ± 0.9 mV/decade, a clear anionic response to DS and ABS anions and a much poorer response to other ASs and to NSs. The sensor has been used for the determination of DS in water samples by titration procedures.

The new surfactant sensor based on tetrahexadecylammonium DS as sensing ion-exchange complex, incorporated in plasticized PVC membrane, has been used for potentiometric titration of low-level ASs using 1,3-didecyl-2-methylimidazolium chloride as standard cationic titrant [30]. There were no significant interferences from organic and inorganic anions, commonly used in surfactant-based industrial and household formulations at AS potentiometric determination. The sensor was used for the determination of ASs in diluted industrial detergent products and industrial wastewaters. The results obtained agree satisfactorily with standard extraction-spectrophotometric MBAS method and are comparable with those obtained using a commercial surfactant electrode (Table 1).

A new highly sensitive AS sensor based on 1,3-didecyl-2-methylimidazolium-tetraphenylborate ion pair as a sensing material incorporated into plasticized PVC membranes has been prepared [31]. The sensor showed satisfactory analytical performances within a pH range of 2–12 and excellent selectivity performances for DS over almost all organic and inorganic anions investigated. The main application of the sensor described was indication of the endpoint in ion-pair surfactant potentiometric titrations. The influence of the widely used types of NSs, polyethoxylated nonionic surfactants (EONSs), on the determination of ASs was negligible if the mass ratio EONS:AS was not greater than 5. The potentiometric titration curves of the model solution containing no surfactants, with known addition of NaDS and NaDBS, are shown in Fig. 1 [32].

Table 1 Results of potentiometric titrations of industrial wastewaters using DMIC as titrant and TA-DS sensor as indicator, compared with the results obtained using a commercial surfactant electrode (CSE) and standard MBAS method

Sample	Anionic surfactant content (μM)		
	TA-DS sensor[a]	CSE[b]	MBAS method[b]
1	2.12 ± 0.21	2.27	2.16
2	2.68 ± 0.13	2.83	2.76
3	3.05 ± 0.17	2.81	2.85
4	3.74 ± 0.21	3.97	3.56
5	4.29 ± 0.19	4.44	3.94
6	6.01 ± 0.20	5.70	6.26
7	7.71 ± 0.40	8.31	7.46
8	8.23 ± 0.23	7.73	8.51

[a]Average of 5 determinations $\pm\sigma_{N-1}$
[b]Average of 3 determinations $\pm\sigma_{N-1}$

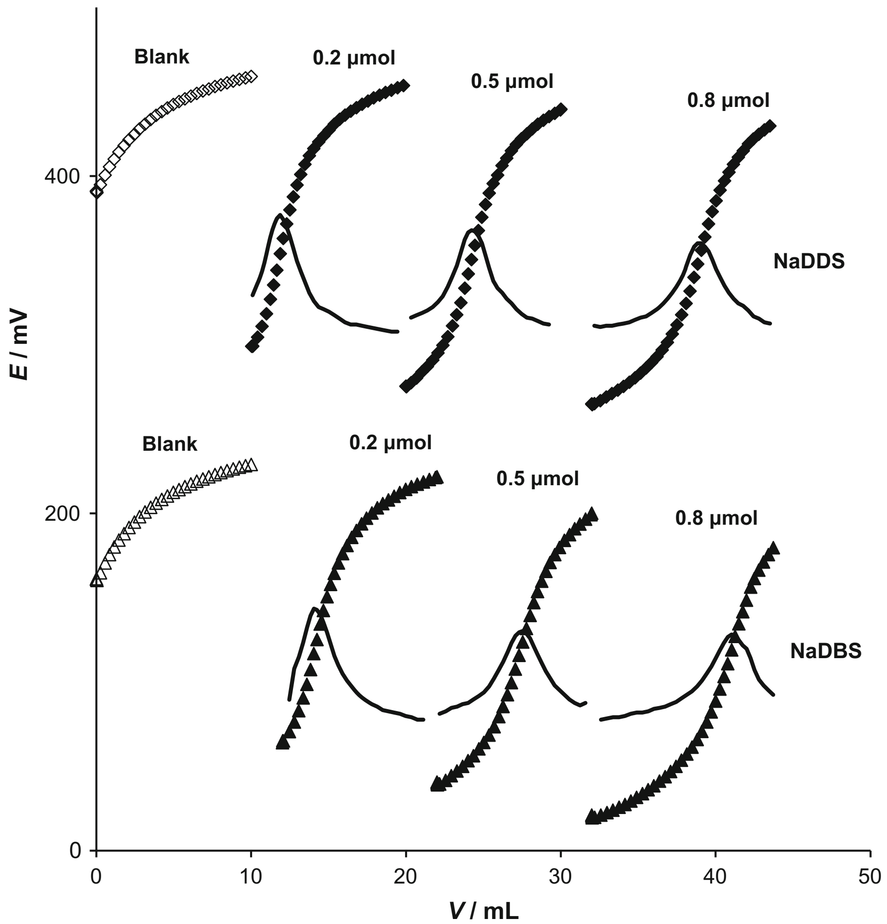

Fig. 1 Titration curves of model effluent (diluted solution of model liquid detergent without anionic surfactant) and the corresponding first derivatives with known addition (values in the graph) of NaDBS and NaDDS, using the DMI-TPB surfactant sensor as the indicator and DMIC ($c = 0.1$ mM) as the titrant

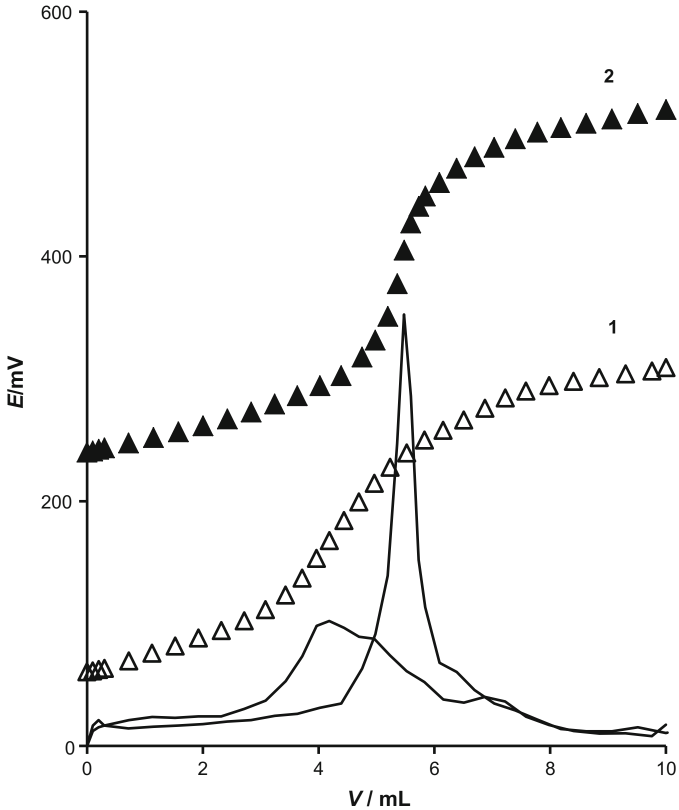

Fig. 2 Titration curves of industrial effluent (1), the same effluent with known addition of NaDBS (2) and their first derivatives, using the DMI-TPB surfactant sensor as the indicator and DMIC ($c = 0.1$ mM) as the titrant

The same sensor was used for endpoint detection during potentiometric titration of ASs in industrial effluents. The potentiometric titration curves of the model themselves and with known addition of NaDBS are shown in Fig. 2.

A satisfactory correlation has been demonstrated between the obtained results and those obtained using the standard MBAS method.

Martínez-Barrachina et al. present an automated FIA system for the determination of low levels of ASs in river water and wastewater [33]. The system uses especially constructed tubular flow-through ISEs as potentiometric sensors and on-line preconcentration techniques and was then used for the determination of total ASs in river water and wastewater.

An all-solid-state AS electrode type was developed using teflonized graphite rods coated with electrochemically prepared polypyrrole film as electric connector support [34]. The measuring membrane of the electrodes was made of ion pairs formed with the appropriate AS and CS ions incorporated into plasticized PVC film. Due to the well-defined charge transfer mechanisms at the graphite–polypyrrole membrane interfaces, the surfactant electrode showed good stability. The lower limit of detection was in the range of 0.5–1 μM in case of the different surfactants tested. Due to the relatively fast response time and the good stability of the electrode, a sample rate of 30 sample h^{-1} in flow-injection determinations could be achieved.

An anionic surfactant ion-sensitive field-effect transistor (AS-FET) based on a hydrophobic quaternary ammonium salt, tetrahexadecylammonium bromide, was developed [35]. The AS-FET exhibits an almost Nernstian response to DBS ion and reveals excellent selectivity for the DBS ion against small inorganic anions but shows the similar selectivity to other ASs such as tetradecyl sulfate and DS ions.

Another FIA system employing specifically developed tubular flow-through ISEs as detectors was used for determination of ASs [36]. The low concentration requirements needed for the environmental application are obtained with an on-line preconcentration stage embedded in the flow system enabling the unattended monitoring of ASs in surface waters. This stage performs the SPE for the enrichment and purification of the target analytes from common interfering anions. The outlined procedure improves the detection limit of a direct injection system, which is decreased from 10 to 0.25 μM. Precision was estimated as 2.9 % relative standard deviation ($n = 20$) for a 0.25 μM (0.070 mg L^{-1}) sodium dodecyl sulfate standard.

Khaled et al. [37] described a simple, reliable, rapid, and reproducible method for mass production of disposable carbon paste electrodes using screen-printing technology. The printed disposable potentiometric strips containing both working and reference electrodes are utilized as endpoint indicator electrodes for the potentiometric titration of ionic surfactants in different samples. The analytical performances of the printed electrodes are compared with those for carbon paste, coated wire, coated graphite, and PVC polymeric membrane electrodes. The proposed disposable strips have been successfully used for the potentiometric titration of CSs and ASs in their analytical grade solutions, pharmaceutical preparations, detergents, and water samples.

Cobalt(II) phthalocyanine [Co(II)Pc] is used as both an ionophore and chromogen for batch and FIA with potentiometric and spectrophotometric determination of ASs (DS), respectively [38]. The potentiometric and spectrophotometric techniques are applied to the batch and FIA of ASs in some commercial detergent products.

It can be concluded that anionic surfactants can be potentiometrically determined in surface waters of the Sava River as well as at outflows of the wastewaters into the surface water of the Sava River basin by using sensitive surfactant sensors.

5 Nonionic Surfactant Determination

NS are surface-active compounds with hydrophobic and hydrophilic moieties which are nonionized in aqueous solutions. Technical NSs are usually mixtures of homologues of different length of alkyl chain and of hydrophilic moieties differing in the number of ethylene oxide (ethoxylate), propylene oxide (propoxylate), and butylene oxide (butoxylate) units.

These compounds are second in worldwide surfactant consumption and contribute about one-third of total surfactant consumption. There is an increasing need for a fast and accurate analytical method to their determination. A lot of time-consuming and labor-intensive techniques have been used for NS determination requiring the use of sophisticated and expensive instrumentation.

Reversed-phase HPLC combined with fluorescence detection was used for the simultaneous determination of aliphatic and aromatic polyethoxylated nonionic surfactants EONSs in aqueous matrices [39]. This analytical procedure was applied to the monitoring of these two classes of surfactants in a municipal sewage treatment plant.

A boron-doped diamond anode was employed as sensor for voltammetric determination of AS, CS, and NS and their critical micellar concentration [40].

A fluorometric fullerenol sensor utilizing fluorescence quenching of a labeled protein was developed for rapid detection and quantification of ionic [cetyltrimethylammonium bromide (CTAB), NaDS] and nonionic (Tween 20 and Triton X-100) surfactants in solution [41].

Triton X-100-selective chemosensor β-cyclodextrin modified by anthracene derivative can be used [42].

The amount of polyethylene glycol (PEG) in several commercial products was determined by HPLC-MS, using the external standard calibration method [43, 44].

A colorimetric assay for determination of residual levels of octaethylene-glycol-mono(*n*-dodecyl)ether in reconstituted membrane protein preparations was developed based on the solubilization of precipitated dye by NS [45].

A simple electrochemical determination of surface-active substances by using time-dependent variation of the capacitive current in a.c. voltammetry is described [46]. The application of the method was demonstrated on freshwater samples.

5.1 Potentiometric Sensors for Nonionic Surfactants

The principle of the sensor response is based on the reaction of pseudocationic complexes of barium ion with EONS and tetraphenylborate (TPB) ion [47].

Barium ion forms pseudocationic complexes with EONS according to the following schema:

$$Ba^{2+} + x\text{EONS} \rightleftharpoons \left[Ba(\text{EONS})_x\right]^{2+}. \quad (8)$$

The "*x*" value depends on the number of ethoxy (EO) groups in the surfactant molecule. The above equation can be more simply written as follows:

$$\mathrm{Ba}^{2+} + x\mathrm{L} \rightleftharpoons \mathrm{BaL}_x^{2+}, \tag{9}$$

where L = EONS.

The corresponding formation constant is

$$K_\mathrm{f} = \frac{\left[\mathrm{BaL}_x^{2+}\right]}{\left[\mathrm{Ba}^{2+}\right][\mathrm{L}]^x}. \tag{10}$$

The sensor membrane contains slightly soluble pseudocationic tetraphenylborate ion-exchange complex as the sensing material, which is obtained by the reaction of TPB ion with pseudocationic complex:

$$\mathrm{BaL}_x^{2+} + 2\mathrm{TPB}^- \rightleftharpoons \mathrm{BaL}_x(\mathrm{TPB})_2, \tag{11}$$

whose solubility product can be defined as

$$K_\mathrm{sp} = \left[\mathrm{BaL}_x^{2+}\right][\mathrm{TPB}^-]^2. \tag{12}$$

The stoichiometry of reactions (8)–(11) depends on the chain length of the oxyethylene part (hydrophilic) of the nonionic surfactant investigated as well as on the nature of the rest of the surfactant molecule (hydrophobic part).

The sensor responds to both TPB^- and BaL_x^{2+} ions according to the Nernst equation:

$$E_{\mathrm{TPB}^-} = E^0_{\mathrm{TPB}^-} - S_{\mathrm{TPB}^-} \cdot \log[\mathrm{TPB}^-] \tag{13}$$

and

$$E_{\mathrm{BaL}_x^{2+}} = E^0_{\mathrm{BaL}_x^{2+}} + S_{\mathrm{BaL}_x^{2+}} \cdot \log\left[\mathrm{BaL}_x^{2+}\right]. \tag{14}$$

NSs in the form of pseudocationic barium complex can be titrated potentiometrically using sodium TPB as the titrant and NS sensor as the detector.

Masadome et al. [48] reported an application of the ionic surfactant-selective electrode to flow-injection analysis of NSs. This method suffers from interferences of ionic surfactants because the electrode is also highly sensitive to ionic surfactants. Therefore, ionic surfactants must be prior removed from a sample solution by means of an ion-exchange column.

The possibility of using selective membrane electrodes for monitoring of ion and NSs was described in a review of Kulapina et al. [49]. The analytical and electroanalytical characterization as well as their construction was given. The single surfactants can be estimated by using direct potentiometry, whereas their content in various commercial surfactant-based products and environmental materials can be determined by potentiometric titration using the electrodes described as endpoint detector.

It was also found [50] that electroanalytical properties of NS-selective membranes depend on the stability, composition, solubility, and degree of dissociation of a metal–polyethoxylate–tetraphenylborate complex. New fast and selective methods were developed for determination of NSs in environmental samples and industrial formulations. On-line titration of NSs in textile finishing industry wastewater treatment plants using an NS electrode was described by Feitkenhauer and Meyer [51].

Martınez-Barrachina et al. [52] reported the use of potentiometric flow-injection analysis for determination of EONS. The specially developed tubular flow-through ion-selective electrodes were developed for this purpose and are sensitive to EONS with a hydrophilic chain between 6 and 18 EO units, which are predominant species in the environment.

The system described was used for the determination of the total EONS content in environmental samples.

The use of ISEs whose surfaces are modified with molecular sieves (nylon, chitin, or PVC) has been demonstrated to be a promising solution for the separate determination of various types of surfactants in homologous series (alkyl sulfates, alkylpyridinium salts, and polyoxyethylated nonylphenols with different numbers of hydroxyethyl groups) [53]. Such modified surfactant electrodes enable the separation of the homologues of alkyl sulfates (C_{10}–C_{16}) and alkylpyridinium chlorides (C_{10}–C_{18}) and the homologues of polyoxyethylated alkylphenols that differ in the number of hydroxyethyl groups ($n = 10$–100).

The fact that NSs influence remarkably the potentiometric determination of earth-alkali metals using liquid membrane ISEs can be exploited for determination of NSs [54]. The presence of NSs reduces seriously the selectivity of these electrodes toward earth-alkali metals with regard to alkali metals. This methodology has been employed for the development of a new potentiometric analytical determination of Tegopren 5863 in synthetic seawater in the range of 0.25–5 ppm.

Lizunova et al. [55] determined NS by using liquid membrane ISEs containing pseudocationic complexes of barium with EONS and TPB anions. The range of linear response of the electrodes investigated was between 10^{-2} M and 7×10^{-6} M. The limits of quantitation were 1–2 mg L^{-1} for ethoxylated alkylphenols containing seven and ten EO groups and 0.5 mg L^{-1} for ethoxylated alcohol containing ten EO groups.

Mikhaleva and Kulapina [56, 57] carried out a comparative study of the electroanalytical properties of modified and unmodified solid-contact sensors for NSs and proposed multisensor systems of the electronic tongue type for the separate determination of the homologues of polyoxyethylated nonylphenols with different numbers of EO groups in multicomponent mixtures. Arrays of solid-contact potentiometric sensors with a high cross sensitivity for separate detection of anionic and NS homologues in multicomponent systems are designed, and their application for separate detection of homologous anionic and NSs in multicomponent model mixtures, natural waters, and technical drugs is shown.

Potentiometric titration of low concentration level of polyethoxylated NSs was determined by the use of Metrohm NIO surfactant electrode as endpoint detector

Table 2 Total nonionic surfactant recoveries found in industrial wastewater on using the potentiometric titration at a spiked level from 0.136 to 0.390 μM nonionic surfactant

Sample	*c* (surfactant content)[a] (μM)	*c* (surfactant added) (μM)	*c* (surfactant found)[a] (μM)	Recovery (%)
1	0.112 ± 0.004	0.136	0.128 ± 0.002	94.1 ± 1.5
2	0.450 ± 0.002	0.230	0.240 ± 0.004	103.4 ± 1.7
3	0.450 ± 0.002	0.390	0.370 ± 0.007	94.7 ± 1.9

[a]Average of 5 determinations $\pm\sigma_{N-1}$

[58]. The diluted solutions of NS containing 5–23 EO groups were successfully titrated in modeled formulations of widely used detergent products and industrial wastewaters. The endpoint of titration has been determined by applying extended Savitzky–Golay least-squares regression. Total nonionic surfactant recoveries found in industrial wastewater themselves with known addition of nonionic surfactants are given in Table 2.

Kokovkin et al. [59] proposed the use of the combination of potentiometric titration with sodium TPB and UV spectrophotometry for determination of an unknown NS. This enables its identification and quantitative determination without knowing its molecular mass and the stoichiometry of the reaction of pseudocationic complex of barium and EONS with TPB ion. This methodology was tested on modeled and real materials, detergent solutions, and wastewater samples.

The quantitative analysis of homologous polyoxyethylated nonylphenols in complex multicomponent model mixtures and natural water samples has been carried out by using the electronic tongue system based on the potentiometric sensor array [60].

A sensitive potentiometric surfactant sensor based on a highly lipophilic 1,3-didecyl-2-methylimidazolium cation and TPB as antagonist ion (DMI-TPB) was used as the endpoint detector in ion-pair potentiometric surfactant titrations using sodium TPB as a titrant [61]. Several analytical and technical grade CS and EONS and mixtures of both were potentiometrically titrated. The known addition methodology was used for determination of the surfactant with considerably lower concentration in the mixture.

The DMI-TPB sensor was also applied for investigation of the homologous tallow fatty alcohol and oleyl alcohol ethoxylates and for their potentiometric titration in the ppm region using TPB as a titrant [62].

The nonionic surfactants can be potentiometrically monitored at the ppm level in surface waters of Sava River and in industrial effluents at their outflows into the surface waters of Sava River basin.

6 Advantages and Limitations of Surfactant Sensors

Advantages of the use of surfactant-selective electrodes vs. classical indicators:

(a) Inexpensive and simple to use in the field as well as in the laboratory.
(b) Routine analysis can be easily automated, thus reducing analysis times.
(c) Wide concentration range: over 4–6 orders of magnitude.
(d) Short response time.
(e) Ideal for long-term monitoring of changes in ion concentration
(f) The analysis complies with good laboratory practices.
(g) Lower consumption of reagents, without the need for toxic solvents.
(h) Greater reliability in determining the endpoint (less subjective).
(i) The elimination of problems associated with the turbidity or coloring of the sample.
(j) Sample preparation is usually unnecessary, except for a possible dilution in order to create the ideal conditions for titration and make any necessary adjustments to pH.

Limitations of surfactant-selective electrodes:

(a) Limited use of direct determination (potentiometry).
(b) Precision is rarely better than 1 %.
(c) Gradual loss of their response characteristics (lifetime).
(d) Limited selectivity.
(e) Electrodes can be fouled by proteins or other organic solutes.

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Ecotoxicological Characterization of the Sava River: Biomarker Responses and Biological Assays

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Abstract Driving forces related to settlements, agriculture, and release of contaminated untreated effluents from municipalities and industrial facilities that are greatly dominated by old and environmentally unfriendly technologies have always been considered as key elements that exert significant pressure on the ecological status of the Sava River. Despite such an unfavorable situation, the biological monitoring activities and chemical identification capabilities in most of the countries of the region have been traditionally restricted to a very limited number of biological markers and potentially hazardous contaminants, respectively. Nevertheless, the biomarker approach for the detection of hazardous chemical contamination in the Sava River was applied early in the 1980s, and the research studies that followed in subsequent decades introduced various biomarkers measured in various freshwater species. The use of the small-scale or in vitro bioassays has been more frequently used only from the late 1990s and culminated more recently with the investigations carried out within the related international research projects. In this chapter we present an overview of the research that has been done so far on the ecotoxicological evaluation of the Sava River using ecotoxicological biomarkers and bioassays, summarize the described evidence, and offer a general evaluation of the present ecotoxicological status of the Sava River.

Keywords Sava River • Ecotoxicological evaluation • Biomarkers • Bioassays

List of Abbreviations

APEO	Alkylphenol polyethoxylates
B[*a*]PMO	Benzo[*a*]pyrene monooxygenase
CYP1A	Cytochrome P4501A
EDA	Effects-directed analysis
EROD	7-Ethoxyresorufin-*O*-deethylase
GC/MS	Gas chromatography/mass spectrometry
GST	Glutathione S-transferase

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HPLC	High-performance liquid chromatography
LAS	Linear alkylbenzenesulfonates
LC-QToF-MS	Liquid chromatography/quadrupole time-of-flight mass spectrometry
MXR	Multixenobiotic resistance
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
RQ	Risk quotient
WFD	European Union Water Framework Directive
WWTP	Wastewater treatment plant

1 Introduction

The Sava River basin is the major drainage basin of the Southeastern Europe covering the total area of approximately 97,700 km^2. The Sava River was the biggest national river of the former Socialist Federal Republic of Yugoslavia and was often considered as the life artery of the state. After the dissolution of former Yugoslavia in the early 1990s, it has become an international river of recognized importance. As the largest by discharge and the third longest tributary to the Danube, on its way from Slovenian Alps until its mouth to the Danube in Belgrade, the Sava River now connects the four states and the three capitals (Ljubljana, Zagreb, and Belgrade). The large complex of preserved alluvial wetlands in the middle of the basin, called Central Posavina, makes the Sava River basin unique for the outstanding biological and landscape diversity. Nevertheless, driving forces related to settlements, industry, agriculture, and waste management have always been considered as key elements that exert significant pressure on surface water bodies [1]. Furthermore, as compared to the situation in Western Europe, the key environmental problem, which is common for all transition countries in the Sava River basin, is the release of contaminated untreated effluents from municipalities and industrial facilities that are greatly dominated by old and environmentally unfriendly technologies. Since the drinking water supply in the Sava River basin relies almost exclusively on the rich resources of high-quality groundwater, which are under direct influence of the Sava River, the assessment of possible adverse effects of hazardous chemical contamination is of great importance. Despite such an unfavorable situation, the monitoring activities and identification capabilities in most of the countries of the region have been traditionally restricted to a very limited number of biological markers and potentially hazardous contaminants.

Considering the overall level of industrial activities and economy of former Yugoslavia in general, the mid-1980s represented the peak pollution pressure to the Sava River. The first comprehensive characterization of organic pollution in the Sava River was performed in 1985 by Ahel and Giger [2] using gas chromatography/mass spectrometry (GC/MS) technique. The study indicated the presence of numerous specific organic contaminants, which were not regulated by the national ordinance on the maximum allowable concentrations. It turned out that some of the compounds, identified in the analyzed samples, belonged to the compound classes that 15 years later became prominent candidates of the so-called emerging contaminants. For example, Croatia was one of the first countries that introduced water quality criteria for nonylphenol; some 15 years before it was accepted as a priority pollutant in the European Union Water Framework Directive (WFD).

Likewise, the biomarker approach for the detection of hazardous chemical contamination in the Sava River was applied very early. In order to assess the biological effects of substances being discharged in the Sava River, in the early 1980s, the ecotoxicology group from Ruđer Bošković Institute (RBI) in Zagreb performed the first large-scale biomarker studies. They measured early toxic effects, the induction of benzo[*a*]pyrene monooxygenase (B[*a*]PMO) in feral fish populations, and the late, ultimate toxic effects, the appearance of tumors in fish. In addition, the mutagenic capacity of the surface water extracts was determined by the Ames test [3–6]. The studies that followed in subsequent decades introduced additional biomarkers measured in various freshwater species. However, apart from the Ames test determinations, the use of the small-scale or in vitro bioassays as tools for the determination of ecotoxic potential of the Sava River surface water or sediments samples has been more frequently used only from the late 1990s and culminated more recently with the investigations carried out within the related EU FP6 projects EMCO and SARIB [7, 8] and the NATO Science for Peace and Security Programme [9].

In this chapter we present an overview of the research that has been done so far on the ecotoxicological evaluation of the Sava River using ecotoxicological biomarkers and bioassays. The first section is dedicated to biomarker responses in biota. The second one addresses data on the determination of the ecotoxicological potential of the Sava River complex environmental samples, obtained utilizing various bioassays and different end points. Finally, we close this chapter with an attempt to summarize the described evidence and offer a general evaluation of the present ecotoxicological status of the Sava River.

2 Biomarker Responses in the Sava River Biota

The early 1980s marked the beginning of ambitious field studies directed to the evaluation of biomarker responses in various indicator species inhabiting the Sava River. The biomarker studies that resulted with relevant publications in peer-reviewed scientific journals are chronologically enlisted in Table 1. A few important observations should be pointed out before considering the mentioned studies in more detail.

First, although the Sava River is some 990 km long, most of the studies have been carried out on the Croatian part of the Sava River or the section of the river shared between Croatia and Bosnia and Herzegovina. Furthermore, a 150 km long

Table 1 Chronological list of relevant field studies focused on biomarker responses in freshwater fish, crayfish, or plant species inhabiting the Sava River

Year	Authors	End point(s)	Species	Ref. no.
1980	Kezić et al.	Carcinogenicity (neoplasia frequency), CYP1A induction (B[*a*]PMO)	21 fish species	[3]
1981	Kurelec et al.	CYP1A induction (B[*a*]PMO), carcinogenicity (neoplasia frequency), bioactivation potential (Ames test)	21 fish species	[4]
1983	Kezić et al.	CYP1A induction (B[*a*]PMO)	European chub (*Squalius cephalus*), carp (*Cyprinus carpio*), barbel (*Barbus barbus*), nase (*Chondrostoma nasus*)	[5]
1984	Kurelec et al.	CYP1A induction (B[*a*]PMO)	European chub (*Squalius cephalus*), barbel (*Barbus barbus*), nase (*Chondrostoma nasus*)	[6]
1989	Kurelec et al.	Genotoxicity (DNA adducts)	European chub (*Squalius cephalus*), carp (*Cyprinus carpio*), barbel (*Barbus barbus*), bream (*Abramis brama*)	[10]
1993	Britvić et al.	CYP1A induction (B[*a*]PMO), bioactivation potential (Ames test), bile fluorescence	European chub (*Squalius cephalus*), carp (*Cyprinus carpio*), barbel (*Barbus barbus*), roach (*Rutilus rutilus*), *Rutilus pigus virgo*, bream (*Abramis brama*), bleak (*Alburnus alburnus*)	[11]
1999	Kolak et al.	Genotoxicity (micronucleus test)	European chub (*Squalius cephalus*)	[12]
2003	Klobučar et al.	Genotoxicity (comet assay, micronucleus test)	Zebra mussel (*Dreissena polymorpha*)	[13]
2003	Smital et al.	MXR (P-glycoprotein activity)	Zebra mussel (*Dreissena polymorpha*)	[14]
2007	Krča et al.	CYP1A induction (EROD), GST induction; bioactivation potential (Ames test); OH-PAH bile metabolites	European chub (*Squalius cephalus*)	[15]
2007	Dragun et al.	Cytosolic concentrations of metals and proteins in the gills	European chub (*Squalius cephalus*)	[16]
2008	Kopjar et al.	Genotoxicity (comet assay)	Balkan loach (*Cobitis elongata*)	[20]
2009	Podrug et al.	Cytosolic total protein, metallothionein (MT), and metal concentrations	European chub (*Squalius cephalus*)	[17]

(continued)

Table 1 (continued)

Year	Authors	End point(s)	Species	Ref. no.
2009	Dragun et al.	Gill metallothionein (MT)	European chub (*Squalius cephalus*)	[18]
2011	Radić et al.	Peroxidase activity, lipid peroxidation, genotoxicity (comet assay)	Duckweed (*Lemna minor*)	[23]
2011	Pavlica et al.	Genotoxicity (comet assay; micronucleus test)	European chub (*Squalius cephalus*)	[21]
2012	Klobučar et al.	Genotoxicity (comet assay; micronucleus test)	Crayfish (*Astacus leptodactylus*)	[22]
2012	Marijić and Raspor	Trace metal concentrations, tissue metallothionein (MT)	European chub (*Squalius cephalus*)	[19]

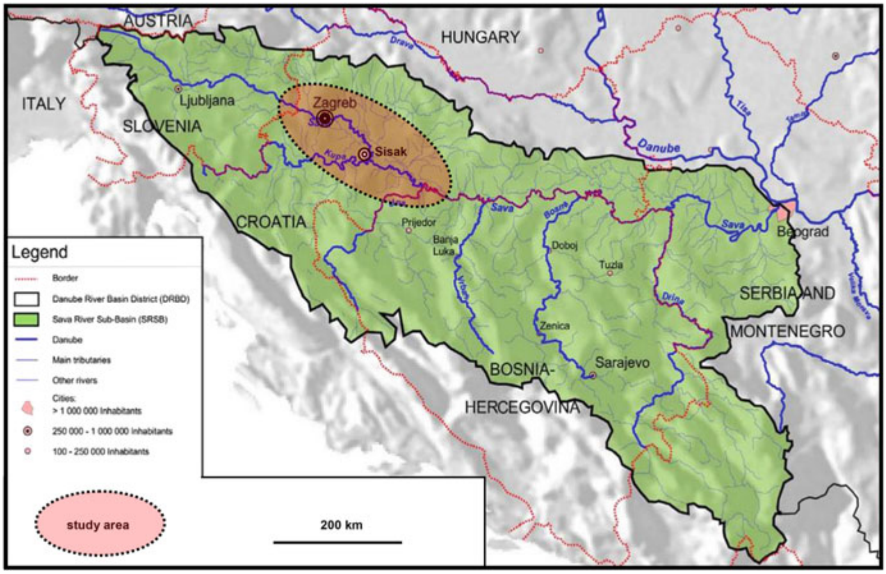

Fig. 1 Map of the Sava River basin. The most frequently studied section of the river, stretching from the Slovenian–Croatian border over the Zagreb City area to the confluence of the Una River, is encircled in red (study area)

section of the river starting at the Slovenian–Croatian border up to the confluence of the Una River has been by far the most studied part of the river (Fig. 1).

This section was often selected due to the well-defined gradient of pollution, ranging from low-to-moderately polluted sites before the city of Zagreb (1 mil. inhabitants, heavily industrialized) to the sites situated downstream from the

Zagreb and Sisak City areas, which are characterized by the enhanced pollution loads. The rest of the Sava River, however, has been much less studied.

Secondly, almost all of the studies utilized fish as indicator species (Table 1). Two studies were performed on a bivalve, one study on a native crayfish species, and only one study utilized a plant as indicator species.

Finally, less than 20 peer-reviewed articles were published on the subject in the course of over 30 years. All of these studies were not result of national monitoring programs but were rather carried out as integral parts of various national and international research projects. Therefore, although results of all these investigations represent a valuable and relatively solid data set, one has to be aware that there was no any systematic, long-term, scientifically sound biomonitoring program of the ecotoxicological status of the Sava River.

Fish are well known as species at the top of the food chain in aquatic ecosystems. They accumulate and bioconcentrate xenobiotics available in the water column or in the sediments. This line of reasoning was the base for the first large-scale biomarker studies in the Sava River watershed, performed in the early 1980s by the Kurelec group from the RBI in Zagreb. In their first attempt, focused on monitoring tumor frequencies in native fish populations as a proxy for detecting the effects of mutagenic/carcinogenic substances present in the heavily polluted Sava River, they did a massive scale work—almost 200,000 specimens belonging to 21 fish species were examined [3]. Data were collected by fish pathologists by direct observation of catches during official fishing competitions, and some competitions were even intentionally organized on certain heavily polluted stretches of the river contaminated by known quantity and type of contaminants. As a result, some 5.56 % specimens were necropsied, and most of the diseases observed were the consequence of either viral, bacterial, or helminth parasite infections. Surprisingly, however, there were no neoplasms detected. Five out of 21 fish species, caught at the most polluted locations downstream of the Zagreb City main wastewater outlet, were then chosen for determination of their liver B[*a*]PMO activities. The measured B[*a*]PMO activity of those fish species was invariably high. For example, B[*a*]PMO activity in wild carps from the Sava River was over ten times higher on average than in control carp specimens from local fish farms, clearly indicating a highly significant exposure to pollutants able to induce cytochrome P4501A (CYP1A)-dependent (phase I) liver detoxification enzymes. In the follow-up study [4], the same group introduced a few additional end points: (1) B[*a*]PMO activity was determined in the caged carps exposed from 5 to 140 days at polluted locations and compared with control specimens held in laboratory conditions, (2) B[*a*]PMO induction was measured in carps i.p. treated with hexane extracts of the Sava River collected at several locations (the so-called induct test), (3) the concentration of the B[*a*]PMO inhibitors in corresponding hexane extracts was evaluated in vitro, and finally (4) testing of the mutagenic potential of the Sava River extracts was performed by the Ames test. Overall, there were a good correlation between the level of pollution and B[*a*]PMO activity as determined both in the native and the caged specimens, but no correlation between the low water quality and frequency of neoplasia in native fish populations. Although the hexane extracts of a few liters of the Sava River surface water at some locations contained sufficient concentrations of mutagenic substances to yield significant increase in the number of revertants in the Ames test, the presence of these harmful substances neither affected the reproduction status of the fish nor increased the neoplasia frequency. Therefore, the authors concluded that monitoring of the fish tumor frequency for evaluation of the health hazard from waterborne mutagens/carcinogens does not appear to be a promising approach.

The potential of B[*a*]PMO determinations as an effective biomarker of exposure was then exploited in two studies that further evaluated putative correlation between the liver B[*a*]PMO activity and pollution load of the Sava River and some smaller, much less polluted rivers (Krka and Kupa) in the same area [5, 6]. Again, the RBI group examined B[*a*]PMO activity in the three chosen native species (European chub, barbel, and nase). Based on these initial data, 10- and 20-day cage exposure experiments with carps were performed at the three typical segments of the Sava River and one each in the Kupa and Krka Rivers. The obtained activity levels were compared with the domestic and industrial load of these rivers derived from data obtained from the Water Management Authorities in Slovenia and Croatia. The determined B[*a*]PMO activities in nonmigratory fish populations were highly correlated with the recent pollution history for the particular part of the river and were highly correlated with the pollution load as expressed in population equivalents. The very same set of data clearly revealed that the pollution of the Sava River, especially in the Zagreb City area, resulted in much higher biomarker (B[*a*]PMO) response in comparison to the responses measured in fish inhabiting less polluted Rivers Krka and Kupa. Therefore, the measurement of liver B[*a*]PMO activity in natural fish populations proved to be a useful tool both for detecting the presence and estimation of the quantity of xenobiotics in water. Furthermore, the use of caged experimental fish offered the same predictive validity as that of wild fish populations, with significant practical advantages that were frequently exploited in subsequent studies in the Sava River watershed.

In 1989 the ecotoxicology group from RBI published an interesting study that focused on the application of the measurement of specific DNA adduct concentration in target tissues of the Sava River fish as a key biological end point of exposure to environmental carcinogens [10]. Using a highly sensitive assay based on the ^{32}P-postlabeling technique, they found that natural populations of freshwater fish species (European chub, barbel, bream, and carp) from the Sava River revealed the presence of four to nine qualitatively similar adducts, irrespective of whether they were caught from unpolluted or polluted waters. No significant differences were observed between the adduct levels of fish from the unpolluted waters and those of fish from the polluted waters, and a dominant feature of the fish DNA adducts was species specificity. The finding that a vast majority of DNA modifications in fish were obviously caused by natural factors rather than by exposure to man-made contaminants offered a basis for a more realistic view in assessing the genotoxic risks in the Sava River basin.

Unfortunately, the warfare in the region started in the early 1990s, and the following postwar situation caused difficulties in the organization of any meaningful field studies, especially considering the fact that the Sava River basin became shared between four independent countries. As a result, almost no biomonitoring studies had been carried out in the 1990s. The exception was the study reported by Britvić and colleagues in 1993 [11]. This study was based on the data obtained by chemical determination of metabolites of compounds to which fish were exposed. As some of these compounds may cause profound biological effects in fish, the authors studied the correlation between the increase in bile fluorescence caused by petroleum hydrocarbon metabolites, the induction of liver B[*a*]PMO activity, and the increase in the liver potential for the bioactivation of promutagenic benzo[*a*]pyrene to *Salmonella typhimurium* TA100 mutagens. Seven fish species caught at polluted locations along the Sava River showed several-fold increase in the levels of all three parameters, as compared with their levels in fish living in the reference Korana River or with the responses determined in control carp specimens held in laboratory. These results offered qualitatively new support to the idea of using simple measurements of fluorescence of diluted bile as a rapid and cheap complementary investigative tool for monitoring and assessment studies.

The end of the 1990s denoted revitalization of biomarker studies in the Sava River basin, as well as the inclusion of new indicator species and new ecotoxicological biomarkers. Genotoxicity/mutagenicity determinations were updated with new methods, like micronucleus test as one of the most successful and reliable assays for detecting aneugenic and clastogenic genotoxicants or the detection of DNA damage at the level of the single cells using the comet assay. Kolak and colleagues [12] were the first to determine genotoxicity of the Sava River by the measurement of the micronuclei frequencies in European chub erythrocytes. The fish were caught at different seasons at three locations in Croatia and compared with data on chub caught from the unpolluted river Kupčina. Although there were no seasonal differences, the average frequency of micronuclei in erythrocytes from the Sava River specimens (0.89–0.93‰) was twice higher than in the controls (0.42‰). The fish in the laboratory were further i.p. injected with benzo[*a*]pyrene, and the results showed that the determination of micronuclei frequency in fish erythrocytes could serve as a useful and reliable part of genotoxic biomonitoring programs in the Sava River basin.

Then, the very first biomarker study done on non-fish species was the work published in 2003 by Klobučar and colleagues, who monitored genotoxicity of the Sava River using micronucleus test and comet assay on the mussel *Dreissena polymorpha* hemocytes [13]. Caged mussels were exposed for 30 days at four monitoring sites of different pollution intensity. The baseline level of micronuclei frequencies in the hemocytes of mussels from the reference site (River Drava) was 0.5‰. No increase in micronuclei frequency was found in mussels from the medium-polluted site while other, more polluted sites showed higher frequencies ranging from 2.7 to 5.2‰. Results from the comet assay showed concordance with micronucleus test, indicating higher intensity of DNA damage at polluted locations.

Again using the zebra mussel as indicator species, Smital and colleagues introduced in 2003 a new ecotoxicological end point, inducibility of the so-called multixenobiotic resistance (MXR) mechanism primarily mediated by the efflux activity of the P-glycoprotein as the phase III of cellular detoxification machinery [14]. The main goal of the study was to ascertain the rate-dynamic level as well as the possible usability of MXR in environmental biomonitoring. Since the primary result of MXR induction should be the decrease in intracellular accumulation of xenobiotics, the determination of MXR induction was performed using the measurement of P-glycoprotein transport activity. The authors measured the accumulation or the efflux rate of the model P-glycoprotein substrate rhodamine B in the gills of mussels previously exposed to polluted versus reference locations in the Sava River area. The results obtained showed that the P-glycoprotein transport activity was induced according to the level of pollution and that only a 4-day period was already long enough for the significant induction and deinduction of MXR activity. However, the inducibility of Pgp transport activity was significantly limited—the maximal level of induction obtained in this study resulted in 50–60 % lower rhodamine B accumulation in the gills of induced specimens when compared with control, non-induced animals, indicating that the use of the MXR as a relevant biomarker should be measured along with the determination of DNA, mRNA, and/or related protein expression.

The most ambitious biomarker study done recently in the Sava River basin was the extensive work accomplished within the EU FP6 project SARIB [8]. Considering biomarker determinations, the most significant contribution was published in 2007 by Krča et al. [15], reporting hepatic biomarker responses in European chub that was selected as indicator fish species within the SARIB project. In an attempt to first determine the species-specific physiological range of selected biomarkers (minimal and maximal responses) in European chub, juvenile specimens caught in the Sava River were laboratory-exposed to various (0.25–50 mg/kg) doses of either model polycyclic aromatic hydrocarbon (PAH) promutagen benzo[*a*]pyrene (BaP) or a well-known model CYP1A inducer β-naphthoflavone (β-NF) for 3–5 days. The responses of several hepatic biomarkers were determined in the exposed fish: 7-ethoxyresorufin-*O*-deethylase (EROD) activity, CYP1A content, glutathione S-transferase (GST) activity, liver bioactivation potential, and finally the amount of hydroxylated polycyclic aromatic hydrocarbon bile metabolites determined by the fixed wavelength fluorescence and the high-performance liquid chromatography (HPLC) technique. The relevance of determined biomarker responses has been analyzed further and cross-correlated with the same set of biomarkers, as well as with tissue concentrations of PAHs and polychlorinated biphenyls (PCBs), determined in European chub specimens collected simultaneously at five different polluted locations along the Sava River. The species-specific upper and lower limits in responses of studied biomarkers were determined and the obtained ranges successfully evaluated in field situation. With the exception of the GST activity, all other biomarkers determined in European chub proved to be valuable indicators of environmental pollution, clearly reflecting higher pollution in locations downstream of Zagreb City and at the sites downstream of the oil refinery of the town of Sisak. Furthermore, these data for the first time showed that even at the most polluted locations, the determined hepatic biomarker responses in feral chub specimens were well below maximal, species-specific physiological response. In the

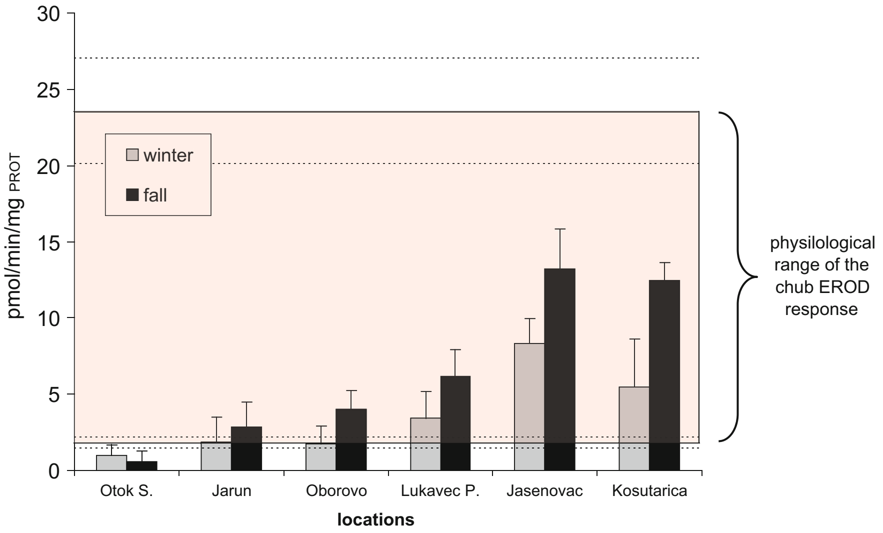

Fig. 2 Seasonal variability in the liver 7-ethoxyresorufin-*O*-deethylase (EROD) activity in chub specimens caught at denoted locations along the Sava River in the winter (February) and fall (September) of 2005. The upper and/or lower limits of the chub, species-specific EROD responses, as were determined in the β-naphthoflavone (25 mg/kg) laboratory exposure experiments, are given as additional information (*solid lines*, means; *dashed lines*, SDs)

follow-up investigations, the RBI group analyzed the possible influence of seasonal differences on selected biomarker responses. Most of the hepatic biomarkers determined in chub showed no significant variation in response, with the exception of the EROD (phase I) and GST (phase II) activities that were elevated in chubs caught in the fall (September) versus those analyzed in the winter months (February) (Figs. 2 and 3).

Additional important results of the SARIB project were investigations that were directed to the analysis of the concentration of metals, total cytosolic proteins, and metallothioneins—specific metal-binding proteins—in European chub gill tissue. In the first study published in 2007 by Dragun and colleagues [16], the authors analyzed the influence of the season and the biotic factors (age and gill mass) on metal and protein levels in the juvenile European chub gill tissue. Five metals were addressed (Zn, Fe, Cu, Mn, and Cd), and a clear, seasonally dependent influence of the gill mass on both the protein and the metal levels was observed. The proposed explanation for the different dependence of metal levels on the gill mass in autumn and spring was the seasonal difference in feeding intensity and metabolic rate, with presumably faster metabolism and water filtration through gills in spring. In the next study, the same group focused on the assessment of metal accumulation in the liver as a target organ [17]. The metallothionein concentrations did not differ between the study sites, and the authors suggested the main reason for this observation was relatively the low dissolved and labile concentrations of metals known as metallothionein inducers (Zn, Cu, and especially Cd) in the Sava River water

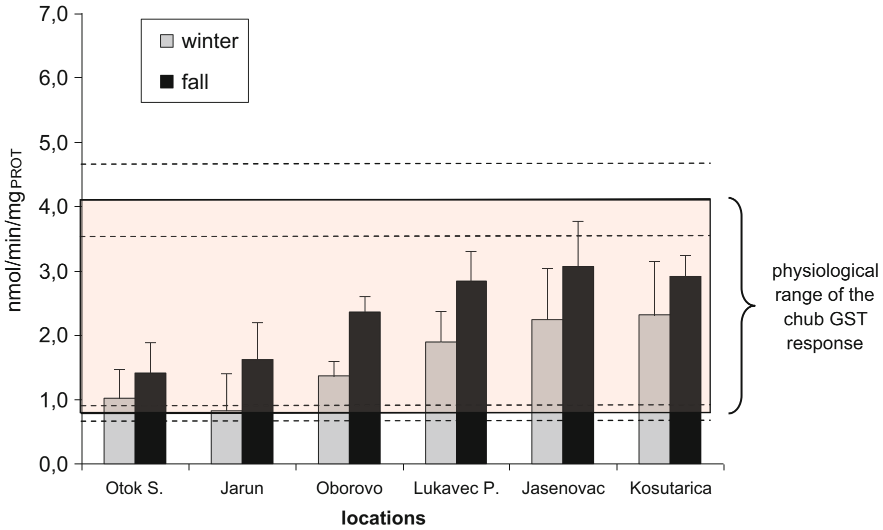

Fig. 3 Seasonal variability in the liver glutathione *S*-transferase (GST) activity (EROD) in chub specimens caught at denoted locations along the Sava River in the winter (February) and fall (September) of 2005. The upper and/or lower limits of the chub, species-specific GST responses, as were determined in the β-naphthoflavone (25 mg/kg) laboratory exposure experiments, are given as additional information (*solid lines*, means; *dashed lines*, SDs)

column. However, hepatic cytosol concentrations of Cd showed statistically significant increase from the less polluted sites upstream of Zagreb City towards more affected locations downstream of Zagreb City and the town of Sisak, respectively. Therefore, it has been suggested that Cd concentrations in hepatic cytosol of European chub can be recommended as an early-warning marker of fish chronic exposure to Cd from combined sources, both the water and ingested food.

Nevertheless, as the determined concentration of metallothioneins was highly variable among sampling campaigns and seasons, the possible causes of this variability were studied in more detail and resulting data published in 2009 by Dragun et al. [18]. Apart from the putative influence of metabolic activity on metallothionein levels, the correlation analysis indicated a significant association between metallothioneins and the fish size. Differences between males and females, as well as between mature and non-mature fish, were not observed in juvenile specimens, even in the spring reproductive season. Based on the analysis of the site-specific metallothionein variability, the authors concluded that, under the conditions of low dissolved metal concentrations in the river water (as was reported for the Sava River), the metal-binding proteins seem to be more affected by different biotic factors than by metal exposure. Therefore, the measured concentrations of metallothioneins were rather considered as the constitutive levels that differ between the season of lower metabolic rate (autumn) and the season of higher metabolic activity (spring). This assumption was further confirmed in a recent comprehensive field survey on the site-specific variability of trace metal concentrations in the gut content, gastrointestinal tissue, and two gastrointestinal subcellular fractions (defined as metal-sensitive fraction and metal-detoxified fraction, respectively) [19]. At five sampling sites along the Sava River, 1- to 5-year-old European chub specimens were caught in the post-spawning period (September) in order to estimate if metal concentrations in fish intestines are related to their levels in the gut content or fish age. Clear difference in metal abundance between the gut content and gastrointestinal tissue was observed, implying a selective metal absorption in fish intestines. Relationship among metal concentrations in the gastrointestinal tissue and two subcellular fractions was significant for all analyzed metals. Site-specific differences indicated the age-related increase of gastrointestinal Cu, Mn, and Cd concentrations towards more polluted sites, while significant correlation between metal concentrations in the gut content and fish age exists only for Mn. In the subcellular gastrointestinal fractions, site-specific differences were not recorded on total water-soluble protein and metallothionein concentrations, which was ascribed to the constitutional, basal metallothionein concentrations, as hypothesized in the previous study from the same group [17].

Several additional studies have been recently published on the assessment of the genotoxic effects in plant and animal species inhabiting the section of the Sava River in or close to the Zagreb City area. One new indicator fish species, the Balkan loach (*Cobitis elongata*) was introduced in the study reported in 2008 by Kopjar and colleagues [20]. The amount of DNA damage in the erythrocytes was estimated using the alkaline comet assay in loach specimens from the Sava River and the reference Kupa River. The obtained data revealed modest genotoxic damage in fish from the Sava River and demonstrated significantly lower levels of DNA damage in fish from the Kupa River. However, although a good DNA damage determination pattern was obtained for Balkan loach, due to its global and regional conservation status, only restricted use of a small number of specimens per sampling site was suggested. Another follow-up study of the SARIB project was published in 2011 by Pavlica et al. [21], again in native European chub specimens caught in different seasons at several locations that followed the pollution gradient of the Sava River. The extent of genotoxic damage was addressed by the comet assay and micronucleus test carried out on fish erythrocytes. The results of the comet assay showed the lowest genotoxic influence at the least polluted site, while higher DNA damage was observed at the polluted sites. Although the basal levels of DNA damage were also elevated, a clear gradation of DNA damage was found due to pollution intensity in all sampling periods. Likewise, the lowest cytogenetic damage as revealed by the micronucleus test was observed at the least polluted site. High variations in micronuclei frequency were observed between sampling periods, although the number of micronucleated erythrocytes was consistently the highest one at the most polluted site. The comet assay as a biomarker of genotoxic effect exhibited higher sensitivity in discriminating the genotoxic capacity of studied polluted sites while the micronucleus assay appeared to be less sensitive. However, the study demonstrated that in optimal biomonitoring programs, both tests should be used together as they can reveal different aspects of DNA damage.

As can be seen from this overview, most of the biomarker studies on the Sava River were traditionally performed on fish. However, apart from rather scarce studies that utilized mussels, two recent studies addressed genotoxic potential of the Sava River using new taxa. In 2012 Klobučar and colleagues assessed the genotoxicity by measuring DNA damage in hemocytes of the caged freshwater crayfish *Astacus leptodactylus* by means of comet assay and micronucleus test, integrated with the measurements of physiological (total protein concentration) and immunological (total hemocyte count) hemolymph parameters as additional stress biomarkers [22]. Crayfish were collected at the reference site (River Mreznica) and exposed in cages for 1 week at three polluted sites along the Sava River. The long-term pollution status of these locations was confirmed by chemical analyses of sediments. Statistically significant increase in DNA damage measured by the comet assay was observed at all three polluted sites comparing to the crayfish from the reference site. In addition, native crayfish from the mildly polluted site (Krapje) cage-exposed on another polluted site (Zagreb) showed lower DNA damage than crayfish from the reference site exposed at the same location, indicating adaptation and acclimatization of crayfish to lower levels of pollution. Micronuclei induction showed similar gradient of DNA damage as the comet assay. The observed increase in total hemocyte count and total protein content in crayfish from polluted sites also confirmed stress caused by exposure to pollution. The results of this study have proved the applicability of caging exposure of freshwater crayfish in environmental genotoxicity monitoring in the Sava River basin.

3 Evaluation of the Ecotoxicological Status of the Sava River Using Small-Scale or In Vitro Bioassays

Complementing biomonitoring programs traditionally based on the determinations of biomarker responses, our ability to monitor water quality has been additionally improved in recent decades through the use of ecotoxicological test methods based on the so-called small-scale or in vitro bioassays. Contrary to biomarker responses typically measured in biota collected from or exposed in situ to various environmental pressures in real environmental conditions, the bioassays are in aquatic toxicology mostly based on determinations of biological responses of various cellular components, cells, organs, or small animals that are laboratory-exposed to row environmental samples or more often to various chemical extracts of complex environmental samples [24, 25]. The use of these methods has the advantage of being highly sensitive, rapid, and reproducible. Furthermore, they require minute amounts of sample material and are thus well suited for screening large amounts of samples. These screening methods also have the advantage of being able to integrate the toxicological activity of multiple contaminants that act through a common toxic mechanism and making it possible to assess the total potential for a biological effect in complex samples. There are also disadvantages, however, as cell/tissue/organism-specific factors may result in data that are not applicable to other species or effects observed at the doses tested might not be environmentally relevant, making the ecological significance of bioassay data lower in comparison to biomarker responses.

Bioassays have rarely been used in the monitoring of the Sava River before the late 1990s, with the exception of the Ames test as a method of choice for detecting mutagenic/genotoxic potential. A more intensive application of bioassays actually started a decade ago, again mostly fostered by recent EU or other international research projects focused on chemical and ecological characterization of the Sava River basin. The first bioassay study performed after the war activities in the early 1990s was the study published in 1997 on the determination of MXR inhibitory potential of river water in the Sava River basin [26]. In this chapter we showed that the effect of MXR inhibitors present in water can be directly demonstrated in differently affected natural waters using the measurements of the rhodamine B accumulation in the gills of mussels exposed to either natural water samples or XAD-7 extracts of corresponding river waters. The sensitivity of direct measurement of MXR inhibitors in natural waters enabled the identification of the most significant point sources of contaminants within the stretch of the Sava River along the Zagreb City area. Water from the Sava River collected downstream of the inlet of municipal wastewaters had a higher MXR-inhibiting potential than water from the Sava River collected upstream of the inlet, even after a fivefold dilution. Furthermore, concentration of MXR inhibitors in the most polluted part of the Sava River appeared to be 3.6- and 5-fold higher than in the less polluted rivers Dobra and Korana, respectively.

A large-scale bioassay study focused on the evaluation of the chronic toxicity of the Sava River was more recently conducted within the EU FP6 SARIB project [8]. In the study published in 2008 by Källqvist and colleagues [27], the authors presented results on the analysis of the surface water and sediment samples that were in 2006 collected throughout the whole course of the Sava River, with 26 sampling positions selected in the riparian countries Slovenia, Croatia, Bosnia and Herzegovina, and Serbia. The sampling positions were chosen so as to encompass the Sava River basin and to consider the impact on the pollution of the Sava River by its major tributaries (Savinja River, Krka River, Kupa River, Una River, Vrbas River, Bosna River, and Drina River). The final samples were collected at Belgrade, just before the Sava River merges with the Danube. The algal growth inhibition test with the freshwater algae *Pseudokirchneriella subcapitata* was selected as a recommended method of choice for the determination of chronic toxicity of complex mixtures and wastewaters [28]. Although most of the samples were toxic to the algae, large differences in toxic potential were observed. The most toxic samples were up to 18,500 times (sediment extracts) and 32 times (pore water), respectively, more toxic than the least toxic sample. However, organic compounds in the water-soluble and particulate fraction of surface waters from the Sava River were less toxic to the algae. Only four (water-dissolved fraction) and nine (particulate fraction) of the total 21 surface water samples caused chronic toxicity to the algae. The results from this study clearly identified and confirmed several compartment-specific hot spots in the Sava River, and the performed toxicity screening revealed that sediments and river water from the some locations at the Sava River were sufficiently toxic to algae to cause growth inhibition when assessed by established classification and risk-assessment procedures.

As pointed out before, the majority of the studies described confirmed the Zagreb City wastewater treatment plant (WWTP) as the predominant input of pollution into the Sava River. Since Zagreb City has a mixed sewer system, including significant contributions from both domestic and industrial sources, the composition of contaminants in an untreated wastewater is rather complex [2, 25]. Considering this fact, it is especially relevant to discuss available bioassay data obtained after mid-2007, when the full-scale WWTP of Zagreb City becomes operational. According to our knowledge, there are two bioassay studies accomplished following this point, both performed by our group within the NATO Science for Peace and Security Programme directed to the assessment of hazardous chemical contamination in the Sava River basin using the so-called effects-directed analysis (EDA) approach [9]. The EDA protocols are, in principle, laboratory-based studies in which an environmental sample is treated using a variety of analytical chemical procedures and treated and untreated samples are tested for toxicity using various bioassays. EDA approach is today generally accepted as the most efficient way to accurately address problems associated with toxicity in water and sediments, offering a rational tool for risk characterization, toxicity reduction, and the identification of harmful substances in real-world matrices having impacts on aquatic ecosystems [29]. Our first study [30] was focused on the characterization of the Zagreb City wastewater as the major pollution input in the Sava River, using the EDA approach, i.e., a combination of bioassays and chemical analytical methods based on advanced sample preparation and analytical protocols, which allowed the identification of a wide variety of nontarget contaminants. The sampling strategy included analyses of raw wastewater and biologically treated effluents, and special attention was paid to the assessment of the relative importance of contaminants having different polarities. An integral part of the study was evaluation of the efficiency of removal of the observed toxic potential following the advanced WWTP recently established in Zagreb City. Over 100 individual contaminants or closely related contaminant groups were identified by high-resolution GC/MS and liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-QToF-MS). The identified compounds covered a wide range of chemical structures and physicochemical properties, in particular with respect to the chemical compound hydrophobicity and/or polarity. Furthermore, the comparison of their semiquantitatively determined concentrations indicated a large variability of their respective concentration ranges, spanning over five orders of magnitude. Considering the bioassay data, ecotoxicity profiling of the investigated primary and secondary effluent samples, including cytotoxicity, chronic toxicity and EROD activity, inhibition of the multixenobiotic resistance (MXR), and genotoxicity, and estrogenic potential, revealed the most significant contribution of toxic compounds to be present in polar fractions.

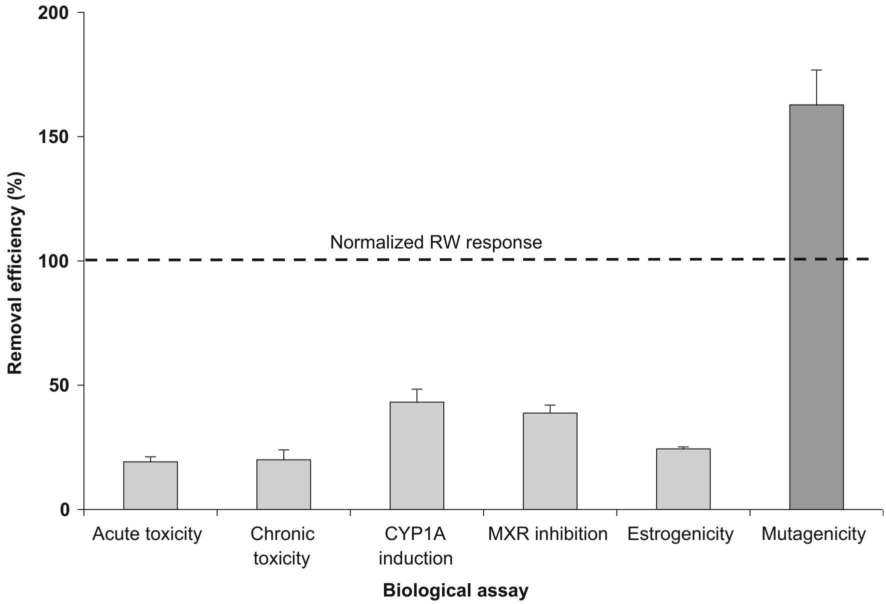

Fig. 4 Comparative presentation of biologically relevant efficiencies of removal of toxic substances from the wastewater samples collected from the Zagreb City WWTP, as determined using a series of bioassays in the study published in 2011 by Smital and colleagues [30]. Removal efficiency, determined as toxic response in the secondary effluent (SE) sample, is expressed in comparison to the toxic response of the corresponding raw wastewater (RW) sample set at 100 %. Acute toxicity (cytotoxicity) was determined using the MTT assay, chronic toxicity by the algae growth inhibition test, CYP1A induction potential by the EROD assay, MXR inhibition by the calcein-AM assay, estrogenicity by the YES test, and mutagenicity by the Ames test, as described in detail in [30]. Mean ± SDs are shown

Finally, the advanced wastewater treatment using conventional activated sludge process reduced the initial toxicity of raw wastewater to various extents. Although chemical analysis showed that the most efficient toxicity removal was observed for the polar compounds, various bioassay end points used in the study clearly confirmed significant, biologically relevant removal efficiency. Yet, the efficiency varied considerably, ranging from 80 % for acute (cytotoxicity) and chronic (algal) toxicity to 57.2 % decrease in toxicity response for the CYP1A induction (Fig. 4). Mutagenicity determination by the Ames test appeared to be the only exception, as our data indicated possible activation of promutagenic substances that could have been present in the raw wastewater sample. Overall, this study clearly emphasizes the importance of polar organic contaminants in the Sava River. Since the polar fraction, due to analytical limitations, represents the least studied fraction in environmental matrices, further efforts need to be directed towards more detailed analysis of polar environmental contaminants in order to identify novel candidates contributing to different ecotoxicological end points.

Subsequently, using the knowledge obtained during the previous study, we recently performed the first regional specific prioritization of organic contaminants in the Sava River, using the described EDA approach. In the recently published study [31], we analyzed ecotoxic potential of surface water and sediment samples collected at four locations covering the already emphasized and well-studied 150-km long river section from the Slovenian–Croatian border to the confluence of the Una River, characterized by well-defined pollution gradients. Total extracts of water and sediment samples were subjected to toxicity screening using a series of small-scale or in vitro bioassays designed to characterize the biological response of hazardous contaminants with different modes of action, as has been done in our previous study. The cytotoxicity of the Sava River water extracts was very low at all locations studied and no significant differences between the individual sampling stations were observed. In contrast, a significant cytotoxicity was detected in all sediment samples, in particular those collected downstream of the Sisak City area, in agreement with the data from bioassay-assisted monitoring of the Sava River using the freshwater algae *Pseudokirchneriella subcapitata* [27], indicating that the effects may be related to industrial effluents from the Sisak City area, in particular those originating from the oil refinery activities. The distribution of EROD induction potential was generally in agreement with the distribution of cytotoxicity. As expected, a significantly enhanced EROD activity was determined in the secondary effluent sample from the Zagreb City WWTP. However, all examined river water samples were characterized by rather low EROD induction potential, with moderately increased activity at the Oborovo location, downstream of the Zagreb City main wastewater outlet. In contrast, high EROD induction potential was determined in the sediment samples, in particular at the locations downstream from the Sisak City, which again probably reflected an additional input of CYP1A inducers such as multi-ring PAHs from the oil refinery. The distribution of MXR inhibitors was significantly different, indicating location-specific differences in compounds causing the bioassay responses that inhibit MXR. The results revealed that these contaminants were primarily associated with the aqueous phase, while their concentrations in analyzed sediments were rather low. The estrogenic potential of both surface water and sediment samples suggested rather modest presence of (xeno) estrogens in the Sava River, most probably reflecting an efficient removal of those substances in the Zagreb City WWTP. Finally, the mutagenic/genotoxic potential of the Sava River samples was generally very low.

Nevertheless, most of the compounds detected in the analyzed water and sediment samples from the Sava River cannot be clearly associated with the specific end points tested. Therefore, we believe it is reasonable to assume that nonspecific biotests, e.g., acute or chronic toxicity determinations, are related to the most abundant compound classes found in the samples, including PAHs, phthalates, sterols, and surfactants. Except for PAHs, the other groups of prominent chemicals identified in the Sava River are not highly toxic. However, although surfactants are only moderately toxic to aquatic life, they should not be neglected when assessing the overall toxic potential since their concentrations in the river water are often 1,000 times higher than the concentrations of the classical hydrophobic contaminants. The observed ratios of measured environmental concentrations (MECs) and predicted no-effect concentrations (PNECs) for moderately toxic chemicals can often be higher than the corresponding ratios of the classical pollutants. That means that even less toxic contaminants may well be responsible for the observed adverse effects, and our preliminary risk-assessment data indicate that this scenario might be correct for the Sava River as well. The risk quotients (RQs) calculated for selected organic contaminants identified in this study revealed that besides PAHs, linear alkylbenzene sulfonates (LAS), cationic surfactants, and alkylphenol polyethoxylates (APEO) may represent the greatest risks for aquatic organisms in the Sava River. It is important to emphasize that surfactants were also the most abundant contaminants in the Sava River sediments. Obviously, their hydrophobic moieties allow an efficient adsorption onto river sediments, warranting the careful monitoring of surfactant contaminants in order to assess the overall indices of water quality. Apart from surfactants, comparatively high RQs were obtained for the personal care products benzophenone and galaxolide, indicating that municipal wastewater is a major source for discharge of pollutants to the Sava River. In addition, a high RQ was obtained for the environmentally ubiquitous plasticizer diethylhexyl phthalate, which even exceeded the EU WFD recommended maximum allowable concentration in the present Sava River water samples.

In addition, a study that for the first time used a plant species (duckweed) for ecotoxicity monitoring of the Sava River has been recently published [23]. In this investigation growth parameters and several additional end points (pigment content, peroxidase activity, lipid peroxidation, and genotoxicity measured by the alkaline comet assay) were used to detect the toxic and genotoxic effects of surface water samples on duckweed plants. The surface waters of different origin and pollutant burdens were collected monthly over a 3-month period at three sampling sites along the Sava River and its confluents. Surface water samples collected from all three stations caused reduction of duckweed growth rates, chlorophylls and carotenoid contents, and peroxidase activity. In contrast, damage to membrane lipids (estimated by malondialdehyde content) and especially to DNA (estimated by tail extent moment) markedly increased in duckweed exposed to industrial wastewater samples. The results from this study demonstrated the potential of the use of a widely available plant species as a sensitive indicator of water quality, further increasing the portfolio of indicator species that may be used in biomonitoring of the Sava River basin.

In conclusion, although it would be premature to use these data for the fully quantitative risk evaluation, the assessment of contaminants in the Sava River watershed clearly emphasizes the possible importance of certain emerging classes of organic contaminants, which are not included in the European and national monitoring strategies. This is particularly true for the most polar fraction. Despite the fact that polar contaminants remain the least studied class in environmental matrices, their bioavailability potential in the aquatic environment is rather high compared to the classical hydrophobic pollutants [32]. Consequently, typical representatives of this class, such as surfactants and pharmaceuticals, should be included in the future region-specific monitoring activities.

4 Evaluation of the Current Ecotoxicological Status of the Sava River

We close this chapter in an attempt to do a preliminary evaluation of the current ecotoxicological status of the Sava River. We do it by comparison of relevant analytical chemical determinations and biomarker or bioassay responses determined in monitoring studies performed in the 1980s versus the most recent studies accomplished in the late 2000s.

As mentioned before, the early and mid-1980s were the years with the highest pollution pressure on the Sava River. The industrial and agricultural activities in the former Yugoslavia experienced historical peaks and the use of pharmaceuticals and personal care products in municipalities was relatively high, all of it combined with dominance of environmentally unfriendly technologies and lack of the advanced wastewater treatment practices. After this period, however, the three important factors actually contributed to significant improvement in the chemical and ecological quality of the Sava River: (1) the breakup of Yugoslavia and related decrease in industrial activities during the warfare in the early 1990s, (2) the collapse of many industrial complexes in the postwar period combined with gradual implementation of more advanced production technologies and wastewater treatment practices in Slovenia and Croatia, and (3) the activation of the full-scale wastewater treatment plant of Zagreb City as the most significant point source of pollution along the Sava River. A comprehensive inventory of the current knowledge on hazardous chemical contaminants in the basin, with a special emphasis on wastewaters as their primary source, can be found in several recent studies [30–34].

Therefore, the important question here is whether the available biomarker and bioassay data sets allow any reliable comparison or even evaluation of the past and present ecotoxicological status of the Sava River? And do the biomarker/bioassay data point to any significant improvement? As may be expected, the answers are neither easy nor unambiguous, as both the chemical analytical and ecotoxicological techniques and tools significantly changed over the past decades. The facts that only a relatively short section of the river has been thoroughly studied, that various species have been used in biomarker studies, that bioassay approach has been used only recently, and that a full-scale, systematic monitoring program of the chemical and ecological status of the Sava River has never been established further make a reliable interpretation of data a challenging task. Nevertheless, there are some biological indicators that in part allow a reasonable comparison of past and more recent ecotoxicological status of the river.

The first potentially useful comparative biomarker relates to the exposure of fish species to CYP1A inducers. The most commonly used biomarkers are involved in

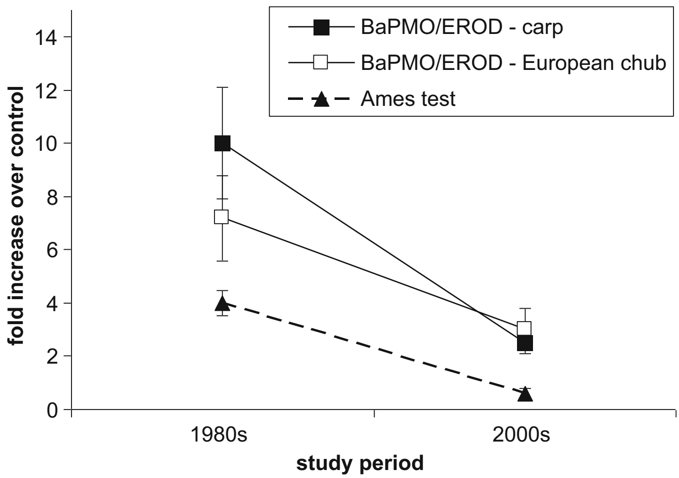

Fig. 5 Comparison of selected biomarker responses determined in the 1980s versus more recent determinations performed in the 2000s. The B[*a*]PMO, EROD, or Ames test data determined in corresponding study periods are expressed as fold increase in biomarker responses over related controls. These were in the 1980s studies performed in carps from the local fish farm and the European chub specimens caught before Zagreb City [3–6] or in the 2000s studies performed in chub specimens held in laboratory for 3 weeks and carps i.p. treated with XAD-7 extract of the surface water collected before Zagreb City ([15] and our unpublished data, respectively). Controls for the Ames test were the mutagenic potentials of the Sava River surface water samples collected in corresponding periods at locations upstream of Zagreb City [4, 31]

the detoxification of xenobiotics and their metabolites (biotransformation enzymes like CYP1A), and alterations in these enzymes are being used as biomarkers of induction or inhibition. The induction/inhibition of fish CYP1A had been in the 1980s measured as an increase in B[*a*]PMO activity. However, the CYP1A determination had been in the late 1990s improved by the use of another, this time non-promutagenic substrate 7-ethoxyresorufin, and the related liver 7-ethoxyresorufin-*O*-deethylase (EROD) activity is now being used as a gold standard in the determination of the environmental exposure to CYP1A inducers or inhibitors [35]. Therefore, comparison of the B[*a*]PMO activities determined in the 1980s in fish from the Sava River can be used in relation to the EROD activities measured more recently, providing that appropriate controls are available and the comparison is based on the same fish species. Having those prerequisites set, relatively correct comparison of the results is possible. As can be seen in Fig. 5, data from the 1980s clearly showed that native carp and European chub specimens from the Sava River, caught at the most polluted locations within or downstream of the Zagreb City area, had from seven to over ten times higher B[*a*]PMO activities in comparison with the carps from the local fish farms or European chub specimens caught before Zagreb City, respectively. However, data on EROD activities determined in the course of the most extensive biomarker study performed in 2007 in European chub specimens from the Sava River [15] showed only threefold induction of the liver EROD activity in specimens from the most polluted locations, in comparison to the basal EROD level measured in specimens held in laboratory for 3 weeks. Likewise, i.p. treatment of carps with XAD-7 extract of the Sava River surface water collected in 2008 at the location downstream of the Zagreb City wastewater outlet showed only 2.5 induction in comparison to the response determined in carps i.p. treated with XAD-7 extract of the Sava River surface water collected before Zagreb City (Fig. 5, our unpublished data). Therefore, the levels of the liver B[*a*]PMO and EROD activities, respectively, determined in the 1980s and the late 2000s indicate a highly significant decrease in exposure of the Sava River native fish populations to CYP1A inducers.

The second biological parameter of potential comparative value is the measurement of the mutagenic potential of the Sava River surface water samples, as has been in both periods determined by the use of Ames test. In the 1980s, the mutagenic potential of the Sava River water collected downstream of the Zagreb City wastewater outlet resulted in approximately fourfold increase in the number of bacterial revertants (higher mutagenic potential) in comparison to the mutagenic potential of less polluted locations upstream of Zagreb City (Fig. 5). In contrast, no significant differences in mutagenic potential were determined between the same locations in surface water samples collected in the summer of 2008 [31], again indicating marked improvement in comparison to the mutagenic profiles determined in previous decades. This observation is further supported by data on tissue concentration of PAHs and PCBs in chub specimens determined in 2007 in the SARIB project study and reported in related article published by Krča et al. [15]. As the authors reported, the concentrations of the seven PCB congeners and PAHs determined in the muscle and liver tissue of chub specimens sampled in September 2005 at several locations on the Sava River revealed relatively modest increase in tissue concentration of PCBs and PAHs along the pollution gradient from the location upstream of Zagreb City towards locations downstream of Zagreb City and Sisak City areas, respectively.

The observed decrease in intensity of biomarker and/or bioassay responses indicates that fish either acquired a highly effective adaptation of their cellular detoxification machinery to pollution pressure or, more likely, that the recent level of pollution of the Sava River decreased in comparison with the levels experienced in the 1980s. In support of the later scenario, chemical analytical determinations of organic contaminants in the same section of the Sava River reveal the same pattern of decrease in the overall pollution load. Two caveats, however, make the interpretation of chemical analytical data less reliable. Firstly, chemical analytical determinations in the 1980s mostly relied on the GC/MS techniques [2, 36] which did not allow reliable determinations of more polar contaminants that were monitored in recently published studies using the LC/MS methodology [30–34], along with the GC/MS determinations. Secondly, most of the available data from both periods are semiquantitative estimates. Nevertheless, a comparison of estimated concentration ranges of several classes of organic contaminants amenable by the GC/MS approach and determined in the Sava River in the 1980s versus the late 2000s clearly shows 10- to 100-fold decrease in concentrations of contaminants typically used in industrial processes or household activities. An overview of the existing water quality of the Sava River was prepared in 2009 under the framework of the International Sava River Basin Commission and is publicly available [1].

In summary, despite the described historical drawbacks and inadequacies in the biological monitoring of the Sava River basin, we believe it is reasonable to conclude that ecotoxicological status of the Sava River greatly improved in the last two decades. Unfortunately, any comprehensive biomonitoring study has not been performed after 2007, the year when a full-scale mechanical and biological treatment of the Zagreb City wastewater treatment plant actually started. As Zagreb City remains the most important source of pollution of the Sava River, however, it would be interesting to see if, and to which extent, the advanced treatment of wastewaters further improved ecological status of the river. Therefore, considering all of the points discussed in this chapter, a well-defined biomarker and bioassay study coupled with advanced chemical determinations, both in selected indicator species and in wastewater, surface water, and sediment samples, would be highly recommended. In this regard, data from previous studies can and should be used as a highly valuable input critical for a scientifically sound design of future biomonitoring studies in the Sava River basin.

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Microbial Characterisation of the Sava River

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Abstract Data on the microbiological quality of the freshwater systems under the anthropogenic influence, such as the Sava River, are of the major importance for the water resource management. Furthermore, analyses of the microbial quality of fish meat provide information of the fish as a valuable food resource from the investigated river basin. The health status of the fish, including dynamics of infection and biodiversity of endoparasites, is important bioindicator of changes in the ecosystem structure and function. For the ecosystem-based approach to the Sava River management, investigations of microbiological quality of the Sava River water and the meat of the European chub as the bioindicator organism, as well as dynamics of infection/biodiversity of intestinal parasites Acanthocephala, were performed. The survey comprised the data collected in periods 2005, 2006 and 2012. Microbiological investigation of water was performed in 2006 and 2012, while microbiological analyses of fish meat and ichthyo-parasitological investigation took place during 2005–2006. A high number of heterotrophic bacteria were recorded during 2006

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survey, confirmed by the distinctly higher values of the three faecal indicators (total coliform, *E. coli* and enterococci), and indicated poor water quality downstream of the cities Zagreb and Velika Gorica, as a result of the municipal sewage outlets. The results from 2012 survey indicated the existence of moderate to critical faecal and organic pollution in all samples. Accumulation of the bacteria in the European chub meat was mainly uniform along the watercourse within standards and limitations for the human consumption. Sampling sites downstream cities of Zagreb and Velika Gorica were characterised with the lower prevalence and abundance of two common species of the chub intestinal acanthocephalan parasites, *Pomphorhynchus laevis* and *Acanthocephalus anguillae*. Poor microbiological quality of the water and lower distribution of chub intestinal parasites were related to the anthropogenic influence, downstream of the urban areas.

Keywords Microbiological indicators • Water quality • Fish tissue • Microbial quality • Fish parasites infections

1 Introduction

The Sava River (945 km) is an important European watercourse and the largest tributary of the Danube River. The 95,551 km^2 catchment area extends over Slovenia, Croatia, Bosnia and Herzegovina and Serbia. It has a source in the Alpine region of Slovenia and inflows in the Danube River in Serbia. The Sava River belongs to the Black Sea basin and is the longest river in Croatia presenting the main water source for this country [1]. This river is a classic example of the ecosystem under high anthropogenic impact and the main wastewater recipient [2, 3]. In the upper course of the river, numerous artificial dams of the hydropower plants significantly affect water flow. The large river damming has impact on the concentrations of nutrients and increased sedimentation [4], which results in the increased numbers and diversity of prokaryotes [5]. In the middle and lower course, the Sava River flows through the regions of intense agriculture: Slavonija in Croatia, Bosanska Posavina and Semberija in Bosnia and Herzegovina and Srem in Srbija, which together cover area larger than 100,000 km^2 [6]. In the whole section, there are numerous pig, cattle and poultry farms. The impact of agricultural run-offs and animal farm wastewaters could lead to serious debasement of the water quality [7]. Along its flow, there are several point sources of high pollution, starting with the urban areas in Slovenia and continuing with the cities of Zagreb (the largest industrial zone and communication junction); Velika Gorica and Sisak in the middle course; and Šabac, Sremska Mitrovica and Belgrade city (2,000,000 inhabitants) in the lower course. The impact of untreated and improperly treated wastewaters is evident in high nutrient content, Biochemical oxygen demand (BOD) values and inorganic pollutant loads [8, 9].

The maximum river flows on the Sava River are usually in the October, November and December. Minimum water temperature is usually in January and

February, whereas the maximum is in July and August, and both are generally in accordance with the air temperature.

Human activities have been a major cause of eutrophication of freshwater systems [10] either by direct discharge of contaminating nutrients into the aquatic system or indirectly [11]. Direct contamination of water sources involves three main types of pollutant domestic discharges (particularly sewage), industrial effluent and agricultural waste. There are two main sources of nutrient entry into the freshwater system: (a) point source, where inflow into the lake or stream is localised (sewage, industrial effluent, agricultural pollution), and (b) diffuse source, where entry of organic pollutants occurs over a wide area (agricultural seepage, run-off from road systems, aerial pollution) [11].

In water bodies, microorganisms contribute to the biodegradation and transformation of organic matter, both of autochthonic and allochthonic origin, constituting an important link in the microbial loop [12].

The health status of fish, as well as microbiological quality of fish meat, is directly related to its habitat and environmental factors. With fishing opportunities, the Sava River is an important body of water, and half of all Croatian fishing catch comes from it.

There are more than 55 fish species, among which European chub (*Squalius cephalus*) is dominating in biomass, whereas in abundance is subdominant. In the Croatian rivers, the genus *Squalius* is represented by seven species and the European chub is the most abundant. There is a lack of information on the microbiological condition of the European chub, as well as about microbiological quality of fish from the Sava River.

1.1 Microbiological Indicators of Water Quality

Water bodies are natural environment for various groups of organisms, including microorganisms which carry out specific biochemical processes, forming groups with specific physiological properties [12]. Land use management associated with urbanisation can be responsible for changes of hydrology, geomorphology, stream chemistry and overall aquatic health. These changes can be reflected in the water quality [13], including microbial water quality.

In most freshwater environments, bacteria form the largest population of all free-living biota and are only exceeded by viruses in terms of total organisms present. The population ecology of freshwater bacteria is thus characterised by the high cell counts and the capacity for rapid rates of reproduction. Bacterial populations tend to show marked fluctuation in response to environmental factors that promote or deplete the increase in biomass [11].

In aquatic ecosystems, the microbial community constitutes a fundamental part, while heterotrophic bacteria play an important role in the biodegradation and transformation of organic matter and self-purification process in waters

[14–18]. Enumeration of the total heterotrophic bacteria is commonly used as the indicator of overall microbiological quality [19].

Counts of the viable heterotrophic bacteria (HPC) can be carried out by plating water samples onto nutrient agar plates and counting the number of colonies that develop. Although this approach can potentially give information of the total number of metabolically active heterotrophic bacteria present in the sample, with exceptions that all plating media are highly selective and many viable organisms with complex nutrient requirements will be excluded [11].

The number of heterotrophic bacteria in a water sample can be estimated from the colony-forming units (CFU mL^{-1}) on a recognised medium based on the specific incubation temperature and time. This parameter is closely related to the degree of eutrophication. Different techniques are used for the HPC determination, including the membrane filtration, the spread plating and the pour plating [20]. The conditions of the spread plate method are physiologically less stressful for bacterial growth because there is no heat stress and the colonies develop on the surface exposed to aerobic conditions. Moreover, the spread plate method almost always yields higher bacterial counts than the pour plate method, while it is less expensive than membrane filtration [21]. For example, the HPC method using a spread plate technique on a nutrient-poor medium within 7-day incubation has generally proven to be much more sensitive than the pour plate method using nutrient-rich agar [22]. An important parameter for bacterial growth is the temperature of incubation, which has a significant effect on the HPC results of any plate counting method [21]. Recommended incubation time ranges from 2 to 7 days, whereas acceptable incubation temperature varies from 20 to 35 °C [23].

To be safe for consumption, water must be free of pathogenic bacteria among which enteric pathogens are the ones most frequently encountered. Instead of detection and isolation of enteric pathogens, which is expensive and time consuming, coliforms and faecal streptococci are most commonly used as indicators of the presence of enteric pathogens [24]. However, because not all types of coliforms require the gut of a warm-blooded animal and some can grow in unpolluted water, *Escherichia coli* is a better indicator of the faecal contamination [10]. In addition, it is recognised that there is a strong correlation between the *E. coli* levels and both pathogenic organisms and gastrointestinal illnesses [10].

The enterococci group is a subgroup of faecal streptococci that includes *Streptococcus faecalis*, *S. faecium*, *S. gallinarum* and *S. avium*. Enterococci are a valuable indicator for determining the extent of faecal contamination in recreational surface waters.

2 Microbial Characterisation of the Sava River: 2005–2006 Survey

2.1 Microbiological Water Quality of the Upper Flow of the Sava River

2.1.1 Sampling Sites

Nine sampling sites were selected along investigated stretch of the Sava River: Otok Samoborski (OS); Zagreb (SZ); Oborovo, upstream from the Velika Gorica sewage outlet (US VGSO); Oborovo, downstream from Velika Gorica sewage outlet (DS VGSO); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); Jasenovac (JAS); and Košutarica (KOŠ) (Fig. 1). The first site was located near the Slovenian–Croatian state border and the last one on the point where the Una River inflows into the Sava River. The samplings on the Sava River were performed in 2006, from April 21st to June 21st.

2.1.2 Methods

The water samples were taken approximately 20 cm below the surface directly into sterile bottles, placed in the portable refrigerator and transported to the laboratory. For determination of the viable heterotrophic bacteria, the water samples were

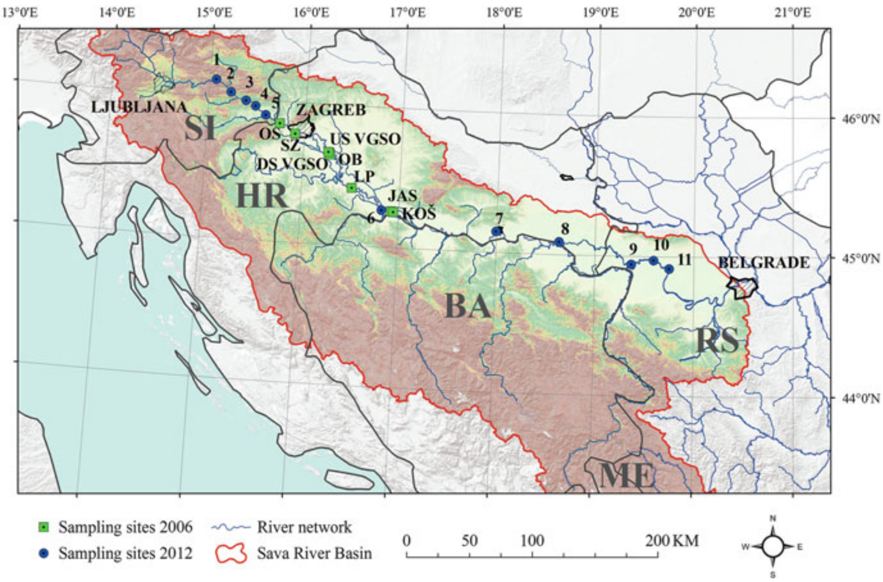

Fig. 1 The sampling sites along the Sava River

diluted (serial decimal) with sterile Ringer solution (Pliva, Zagreb, Croatia) pH 6.0 and inoculated by the spread plate method on R2A (Merck, Darmstadt, Germany) and yeast extract medium (YEA). YEA is the medium recommended by the European norm [25], while the use of R2A is applied for improved isolation of microorganisms from low-nutrient conditions [26, 27] and is routinely applied for enumeration of total heterotrophic populations in surface water [23, 28]. Bacterial colonies were enumerated after incubation at 35 °C during 24–48 h and at 22 °C during 3–5 days. Results were expressed as the colony-forming units (CFU) per mL.

Samples were also analysed for the total coliforms and *E. coli* using the Colilert® and the Quanti-Tray/2000. The Colilert® simultaneously detects total coliforms and *E. coli* density using the nutrient indicators *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-glucuronide (MUG), which are metabolised by total coliforms and *E. coli*, respectively. A product of total coliform metabolism of ONPG is yellow in colour, whereas positive yellow wells with *E. coli* and a by-product of metabolism of MUG fluoresce under UV light. Samples were diluted to approximately 1:100 and 1:1,000 before processing and incubated at 35 ± 0.5 °C for 24 h, and the results were estimated using the standard most probable number (MPN) method as MPN 100 mL^{-1}.

The Enterolert® defined substrate test was used for detection of the enterococci in the water. The Enterolert® use 4-methylumbelliferyl-β-D-glucoside as the defined substrate nutrient indicator. This substrate, when hydrolysed by enterococcus β-glucosidase, releases 4-methylumbelliferone which exhibits fluorescence under a UV light. The estimation of numbers of enterococci is obtained on the basis of positive fluorescent wells using an MPN method as MPN 100 mL^{-1} after incubation at 41 ± 0.5 °C for 24 h. Samples were diluted to approximately 1:10 and 1:100 before processing. The specificity and sensitivity of the Colilert® and the Enterolert® tests were good and obtained the results equal to standard methods for enumeration of the total coliforms, *E. coli* and the enterococci.

2.1.3 Results and Discussion

The main descriptive statistical results of the bacterial concentrations determined at eight sampling locations of the Sava River are summarised in Table 1.

The counts of the viable heterotrophic bacteria (HPC) cultivated on different media followed the same pattern, and excellent agreement between two media was confirmed by high positive statistically significant correlations (Table 2).

The HPC obtained at two incubation temperatures (22 and 35 °C) using YEA and R2A media was always higher on the same sampling sites. According to the obtained HPC at 22 °C using YEA medium, sampling sites Velika Gorica sewage outlet (DS VGSO) and Jasenovac (JAS) were identified as sites with significantly highest levels of HPC ($p < 0.05$) in relation to other sites (Otok Samoborski, OS; Zagreb, SZ; Lukavec Posavski, LP; and Košutarica, KOŠ), whereas at the same temperature using R2A medium only sampling site Velika Gorica sewage outlet

Table 1 Microbial characteristics (average; S.D., standard deviation; Min, minimum; Max, maximum) of the Sava River at sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, upstream from the Velika Gorica sewage outlet (US VGSO); Oborovo, downstream from Velika Gorica sewage outlet (DS VGSO); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP), Jasenovac (JAS); and Košutarica (KOŠ)

	OS				SZ				US VGSO				DS VGSO			
	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max
YEA 22 °C	1,186.7	110.6	1,070.0	1,290	943.3	47.3	890.0	980.0	52,466.7	52,171.2	10,000.0	160,000.0	84,000.0	47,512.4	18,000.0	160,000.0
R2A 22 °C	3,466.7	568.6	3,000.0	4,100	3,700.0	721.1	3,100.0	4,500.0	87,333.3	54,013.9	45,000.0	178,000.0	214,333.3	127,100.4	59,000.0	390,000.0
YEA 35 °C	1,840.4	2,094.4	390.0	134,000	4,398.0	5,517.3	330.0	134,000.0	17,650.0	12,559.7	3,300.0	134,000.0	4,4841.7	41,433.3	4,400.0	134,000.0
R2A 35 °C	1,068.0	1,005.0	450.0	2,800	5,120.0	4,899.7	1,000.0	11,000.0	23,144.4	11,950.7	9,200.0	38,000.0	61,544.4	60,618.2	8,500.0	160,000.0
T. C.	8,309.1	11,101.5	1,596.0	30,600	11,157.1	9,239.4	2,382.2	24,195.7	523,400.5	652,086.6	24,196.0	1,986,290.0	594,688.9	790,816.4	24,196.0	2,309,800.0
E.C.	2,114.1	3,610.7	202.0	10,000	1,645.4	1,607.2	512.1	4,105.8	61,390.1	78,528.7	11,000.0	261,250.0	103,414.2	115,491.8	10,000.0	368,400.0
ENT.	100.0	0	100.0	100.0	109.0	17.1	96.9	121.1	4,987.8	5,924.9	1,000.0	18,690.0	20,458.4	13,081.3	9,900.0	41,300.0
	OB				LP				JAS				KOŠ			
	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max	Average	±S.D.	Min	Max
YEA 22 °C	45,619.1	38,352.9	16,000.0	160,000.0	11,344.4	11,667.9	4,000.0	42,000.0	169,833.3	141,499.7	39,000.0	320,000.0	1,466.7	450.9	1,000.0	1,900.0
R2A 22 °C	87,666.7	42,938.9	51,000.0	159,000.0	9,055.6	3,631.8	4,800.0	15,200.0	17,7833.3	141,320.1	48,000.0	320,000.0	1,033.3	57.7	1,000.0	1,100.0
YEA 35 °C	15,867.5	13,123.1	2,100.0	41,000.0	3,698.1	1,692.1	1,160.0	6,200.0	121,712.5	144,775.3	1,000.0	300,000.0	466.7	57.7	400.0	500.0
R2A 35 °C	26,927.3	20,051.9	3,100.0	56,000.0	4,163.6	1,747.7	1,300.0	6,600.0	127,800.0	151,414.3	1,700.0	320,000.0	866.7	321.5	500.0	1,100.0
T. C.	518,994.3	597,431.3	24,196.0	1,986,290.0	27,657.5	8,714.7	17,310.0	44,120.0	45,873.3	61,740.9	2,010.0	137,610.0	2,020.0	0	2,020.0	2,020.0
E.C.	69,251.3	87,689.3	7,450.0	294,100.0	3,470.0	1,381.0	2,020.0	5,210.0	990.0	0	990.0	990.0	1,000.0	0	1,000.0	1,000.0
ENT.	5,130.0	5,454.8	1,810.0	21,570.0	357.3	270.0	2.4	708.3	12.5	5.1	9.9	20.2	10.0	0.1	9.9	10.0

Table 2 Pearson correlation coefficients (*r*) between the log HPC obtained on YEA and R2A media

Incubation temperature	*r*	*p*
22 °C	0.951	<0.0001
35 °C	0.989	<0.0001

(DS VGSO) had significantly highest levels of the HPC ($p < 0.05$) in relation to the other sites. At the incubation temperature 35 °C using YEA and R2A media, sampling site Velika Gorica sewage outlet (DS VGSO) had significantly highest levels of the HPC ($p < 0.05$) in relation to Otok Samoborski (OS) and Košutarica (KOŠ) sites, respectively. Most of the water quality differences between sampling sites were attributed to *E. coli* and enterococci. Regardless of which faecal indicator was selected, the most frequent water quality exceedances occurred at sites downstream Zagreb. Sampling sites Oborovo, upstream from the Velika Gorica sewage outlet (US VGSO); Oborovo, downstream from the Velika Gorica sewage outlet (DS VGSO); and Oborovo, downstream from the ferry (OB), showed statistically significant higher concentration of *E. coli* ($p < 0.05$) in relation to the concentrations at sampling sites Otok Samoborski (OS) and Jasenovac (JAS). In relation to the concentration of enterococci, the situation is more simple, because statistically significant concentration was at the sampling site Oborovo, downstream from the Velika Gorica sewage outlet (DS VGSO), in relation to the concentration at sites Zagreb (SZ) and Košutarica (KOŠ) ($p < 0.05$).

High bacterial load in the Sava River almost certainly is a result of anthropogenic input, e.g. from municipal sewage outlets of several major urban areas, especially Zagreb and Velika Gorica, respectively. The poor water quality of the Sava River, which was based on the high HPC values, was confirmed further by markedly higher values of the three faecal indicators in water samples of the Sava River (total coliforms, *E. coli*, enterococci). A high standard deviation determined at most sites indicated a pronounced temporal variability of bacterial density in river water.

We could conclude that the number of bacteria in the Sava River was considerably higher at the locations downstream from Zagreb and Velika Gorica (three sites in the Oborovo area: US VGSO, DS VGSO, OB) due to the influx of wastewater into the river water. Therefore, it is obvious that human activities were a major cause of direct discharge of contaminating nutrients into the aquatic system, namely, by point source of nutrient entry localised at few hot spots on the river stream.

2.2 *Microbiological Properties of European Chub Meat (*Squalius cephalus*) from the River Sava*

Staphylococcus aureus is a foodborne pathogen that causes staphyloenterotoxaemia. In the environment, the pathogen is most commonly isolated from raw food, soil, fug and water.

In foods, it has been found in the meat, milk, cheese, poultry, eggs, fish and sausages. The outbreaks of staphyloenterotoxaemia were associated with eating such diverse foods. Food poisoning is usually rapid and in many cases acute, depending on the individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested and the general health of the infected individuals. In infected individuals, staphyloenterotoxaemia is characterised by nausea, vomiting, retching, abdominal cramping and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. Cases of food poisoning and prevalence of *S. aureus* in different kinds of food like fish, meat products and dairy products have been reported mainly from the United Kingdom and France [29, 30]. In these countries, poisoning from fish makes 7 and 11 % of all food poisoning with *S. aureus*.

The objectives of this study were to investigate the hygienic quality and food safety of the European chub with regard to microbial spoilage and foodborne pathogens. The specific aim was to study the occurrence of *S. aureus* in the European chub.

2.2.1 Methods

A total of 90 fish, during two seasons (spring and autumn), were caught at five locations of the Sava River—Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS) (Fig. 1). Fish meat samples were cut aseptically into the slices. Ten grammes of skinless fish meat was homogenised for 5 min in the sterile bags with 90 ml of phosphate-buffered saline solution (Merck) using hand homogeniser. From the resulting dilution, appropriate decimal dilutions were prepared and plated in duplicate to enumerate the following microorganisms:

(a) Total viable bacteria were enumerated by the spread plate method using yeast extract agar [25]. Plates were incubated at 35 °C for 1 day and at 22 °C for 3 days.
(b) *S. aureus* was enumerated using the spread plate method on Baird-Parker agar (BD-BBL). Plates were incubated at 35 °C for 1–2 days.

Average results of duplicate measurements are presented as log colony-forming units per gramme (CFU g^{-1}) and for *S. aureus* confirmed using API Staph (bioMérieux, France).

2.2.2 Results and Discussion

The total viable bacteria (TVB) from the European chub skin and gills showed the same values during two sampling seasons, spring and autumn, except for sampling site Zagreb (SZ) (Figs. 2 and 3). In relation to previously determined values for the fish skin and gills, it is obvious that the TVB determined at both incubation temperatures with maximum values at skin and gills (<2.5 log CFU cm$^{2-1}$) were in normal ranges for both organs and below determined values for human consumption.

The TVB and *S. aureus* counts in the meat of the European chub from the each sampling site are presented in Table 3. The TVB counts determined at the incubation temperature of 22 °C were higher than those at 35 °C, but not statistically significant (Fig. 4). The maximum and minimum values of the TVB during the spring sampling were determined at the same sampling sites for both incubation temperatures. Maximum was at Lukavec Posavski site, and minimum was at Oborovo site. This similarity was not found during autumn sampling, where maximum TVB at 22 °C was at Lukavec Posavski site, whereas TVB at 35 °C was at Oborovo site. The TVB counts at 35 °C obtained during autumn sampling were higher at all sampling sites than in spring sampling, except at Lukavec Posavski site, but there were no statistically significant differences in the TVB (22 and 35 °C) between two sampling periods at each sampling site.

Sampling site with significantly higher TVB during spring sampling, at both incubation temperatures (22 and 35 °C), was Lukavec Posavski site (in relation to Otok Samoborski and Oborovo site; $p < 0.05$). During autumn sampling period, significantly higher TVB at 22 °C was at Oborovo and Lukavec Posavski sites (in relation to Otok Samoborski, i.e. Otok Samoborski and Jasenovac sites, respectively; $p < 0.05$), whereas TVB at 35 °C was at Oborovo site (in relation to Jasenovac site; $p < 0.05$).

All samples of the European chub contain *S. aureus* (Table 3). The content of *S. aureus* demonstrated variations in the number at different sampling sites (Fig. 5). Variations are possible result of the water quality impact. The highest prevalence of

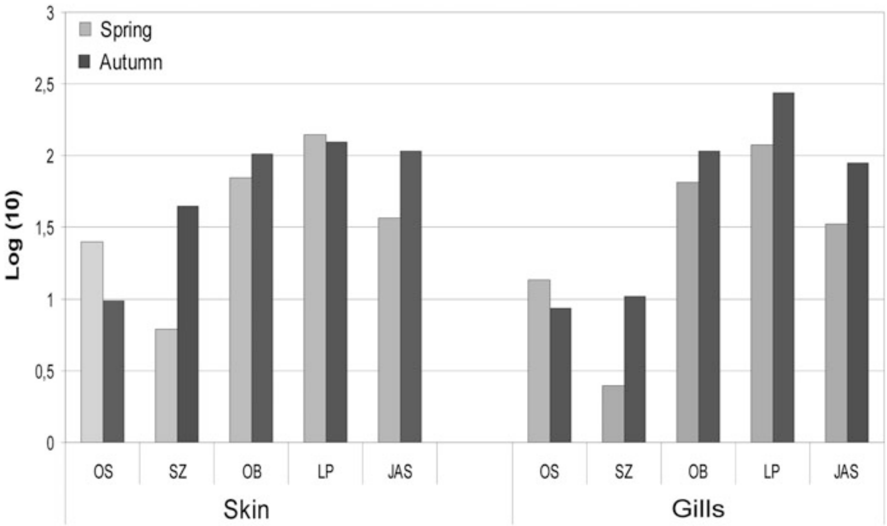

Fig. 2 Total viable bacteria (22 °C) from the skin and gills of the European chub during spring and autumn samplings at the sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS)

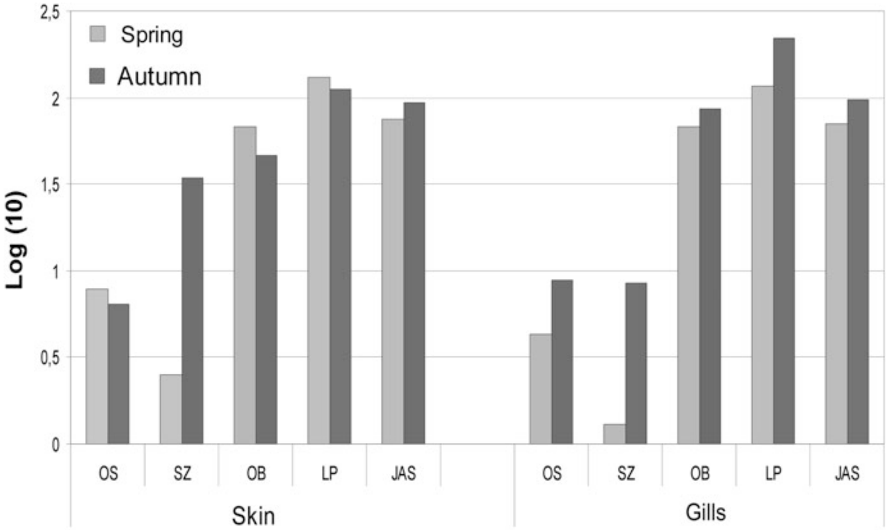

Fig. 3 Total viable bacteria (35 °C) from the skin and gills of the European chub during spring and autumn samplings at the sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS)

S. aureus was obtained during autumn sampling and was determined at sampling sites: Lukavec Posavski (300.0 ± 60.0 CFU g^{-1}) and Oborovo (155.0 ± 28.9 CFU g^{-1}), respectively. These maximum *S. aureus* counts were at sites downstream Zagreb, where the microbial water quality was poor.

In recent years, there has been considerable interest in the establishment of the microbial quality for the freshwater fish. It is crucial to determine initial microbial quality of the fresh European chub, because this fish is consumed without any prior sanitary or veterinary analysis. Their habitat seemed to affect the microbiological condition of the European chub meat more than the time of year. The microbial accumulation of the European chub varied, depending on the sampling site. In the European chub meat, determined values of the TVB (<4.5 log CFU g^{-1} at both incubation temperatures 22 and 35 °C) were higher than those determined on the skin and gills (<2.5 log CFU $cm^{2\text{-}1}$). Although these maximum values of the TVB (32.1×10^3 CFU g^{-1}) in the meat of the European chub were in accordance with the TVB in the fresh fish, where the microbiological limit for human consumption proposed by ICMSF [31] is 10^7 CFU g^{-1}, while other authors recommend 3×10^6 CFU g^{-1} [32]. The same pattern is in relation to *S. aureus* accumulation. *S. aureus* counts, as well as its consequences to the microbial quality of the European chub meat, were not observed. When judged by the ICMSF [31] standards (*S. aureus* less than 10^3 CFU g^{-1}), these findings with maximum obtained *S. aureus* level (3×10^2 CFU g^{-1}) are within limits for human consumption.

Table 3 Microbial characteristics (average, standard deviation—S.D.) of the European chub meat at sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS)

	Spring			Autumn		
	TVB 22 °C ($CFU \times 10^3\ g^{-1}$)	TVB 35 °C ($CFU \times 10^3\ g^{-1}$)	*S. aureus* ($CFU\ g^{-1}$)	TVB 22 °C ($CFU \times 10^3\ g^{-1}$)	TVB 35 °C ($CFU \times 10^3\ g^{-1}$)	*S. aureus* ($CFU\ g^{-1}$)
	Average ± S.D.	Average ± S.D.	Average ± S.D.	Average ± S.D.	Average ± S.D.	Average ± S.D.
OS	7.8 ± 1.2	3.2 ± 0.6	100.0 ± 0	2.5 ± 1.2	5.0 ± 3.9	3.3 ± 5.8
SZ	15.0 ± 4.6	3.8 ± 1.1	10.0 ± 0	9.7 ± 6.6	12.9 ± 7.7	3.3 ± 5.8
OB	1.6 ± 0.4	1.0 ± 0.1	5.0 ± 7.1	19.2 ± 0.4	15.2 ± 6.2	155.0 ± 28.9
LP	23.1 ± 2.3	29.8 ± 3.1	15.0 ± 7.1	21.7 ± 2.3	6.1 ± 2.1	300.0 ± 60.0
JAS	NA	NA	NA	4.1 ± 3.0	3.9 ± 2.8	95.0 ± 5.8

NA not analysed

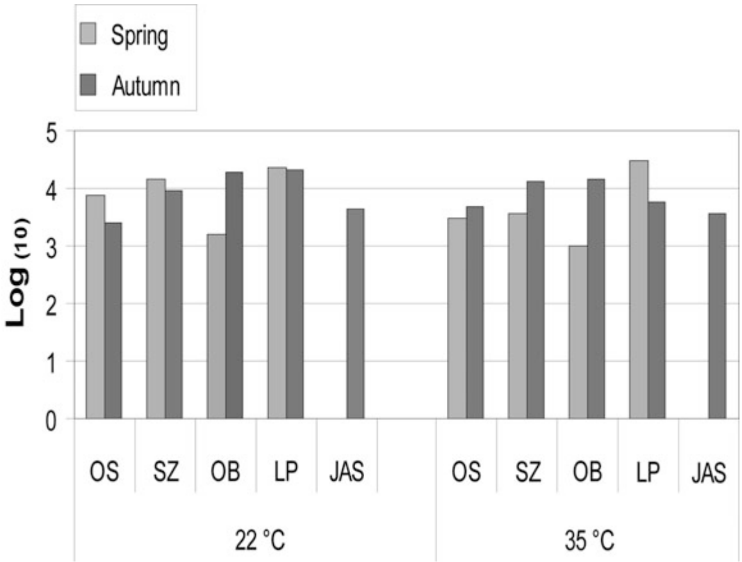

Fig. 4 Total viable bacteria (22 and 35 °C) in the muscle of the European chub during spring and autumn samplings at the sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS)

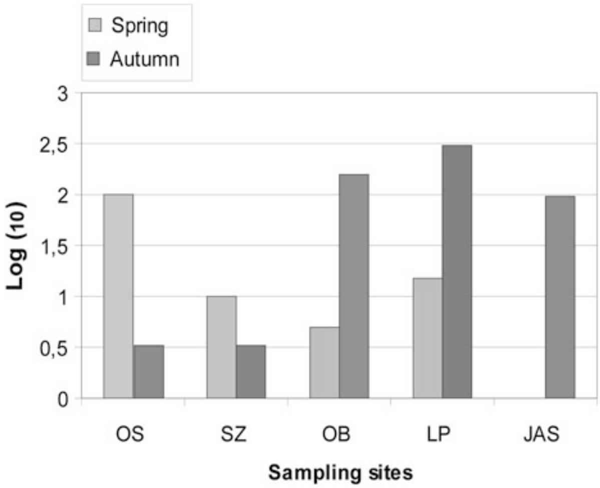

Fig. 5 *Staphylococcus aureus* in the muscle of the European chub during spring and autumn samplings at the sampling sites: Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS)

2.3 Dynamics of Infection and Biodiversity of Acanthocephala, Intestinal Parasites of European Chub from the Sava River

Fish endoparasites are extremely useful as management and conservation tool in the aquatic resources due to their unique site within food webs, their impacts on host biology and biodiversity as well as their reflection of changes in the ecosystem structure and function [33]. Members of the phylum Acanthocephala are fish intestinal parasites with complex life cycle involving invertebrate intermediate host (Crustacea) [34]. Acanthocephala can cause extensive damages to the definitive host digestive tract [35] and modification of intermediate host behaviour [36] and also are applicable as bioindicators for metal pollution in the aquatic environment [37]. Therefore, the aim of our study was to investigate dynamics of infection and biodiversity of acanthocephalans at five localities of the Sava River and to consider their ecological significance.

2.3.1 Methods

Parasitological examination of acanthocephalan specimens was performed on 267 European chubs (*Squalius cephalus*) sampled with the electrofishing device at the Sava River (Table 4) according to the Croatian standard: HRN EN 14011 [38].

Table 4 Sampling data for the European chubs caught in the Sava River from spring and autumn in 2005 and 2006, examined for prevalence and abundance of acanthocephalan infection

	Parasite prevalence (%) Mean abundance (min–max number of parasites) [number of sampled fish]			
	2005		2006	
Sampling site	Spring	Autumn	Spring	Autumn
1. *Otok Samoborski* N 45°50,543′ E 15°43,497′	86.7 [15]	72.3 [22]	80 3.3 (0–13) [15]	66.7 2.9 (0–10) [15]
2. *Sava Zagreb* N 45°46,572′ E 15°56,524′	100 [9]	80 [15]	69.2 4.5 (0–27) [13]	60 1.4 (0–3) [10]
3. *Oborovo* N 45°41,286′ E 16°14,875′	30 [10]	37.5 [8]	66.7 1.8 (0–7) [15]	30 0.6 (0–3) [10]
4. *Lukavec Posavski* N 45°24,081′ E 16°32,337′	56.3 [16]	60 [15]	72.7 4.8 (0–18) [22]	71.4 1.8 (0–5) [14]
5. *Jasenovac* N 45°15,825′ E 16°53,658′	–	70 [10]	53.3 2.9 (0–10) [15]	50 0.7 (0–2) [10]

Sampling was performed during spring and autumn in 2005 and 2006 at five sampling sites—Otok Samoborski (OS); Zagreb (SZ); Oborovo, downstream from ferry (OB); Lukavec Posavski (LP); and Jasenovac (JAS) (Fig. 1). Standard length, weight and sex of fish were determined and Fulton's condition index (FCI) was calculated [39]. Parasite prevalence for all seasons and abundance for spring and autumn in 2006 were calculated according to Bush et al. [40].

Fish were sterile dissected, and several acanthocephalan specimens were used for morphological identification by light microscopy [41, 42], while others were processed for molecular characterisation.

For genetic variability determination, 18S rRNA, cytochrome c oxidase subunit I (COI) and ITS (internal transcribed spacer) regions were analysed by PCR amplification and DNA sequencing [43].

Statistical analyses were performed using SigmaPlot for Windows, Version 11.0.

2.3.2 Results and Discussion

Acanthocephalans were the dominant intestinal parasites of the European chubs from the Sava River, and they were found at all investigated sites of the river (Table 4). Out of 267 specimens of the European chub, 65.2 % were infected by acanthocephalans. The statistically significant difference in acanthocephalan abundance was obtained between spring and autumn sampling in 2006 ($p < 0.05$, Mann–Whitney rank sum test, Fig. 6a, b). The seasonal difference in the acanthocephalans occurrence could be associated both with changes in the abiotic factors (e.g. temperature) and biotic factors (e.g. host diet) [34]. As water temperature raise

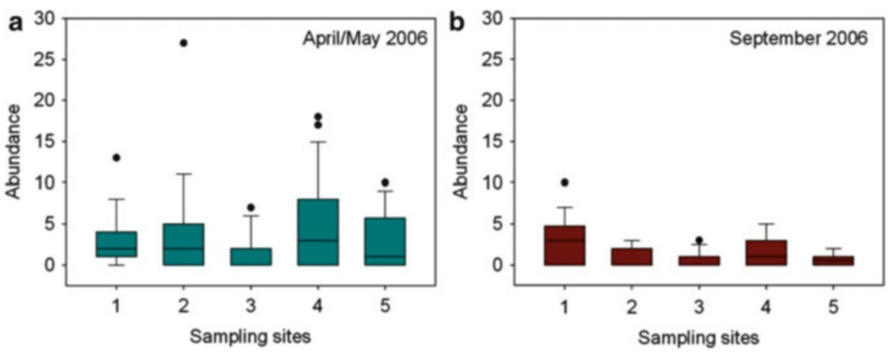

Fig. 6 The spatial distribution of the abundance of the acanthocephalan infections in the European chubs caught in the Sava River in Croatia, based on the data from spring (**a**) and autumn (**b**) sampling in 2006. The results are presented as *box plots*. The boundaries of *box plot* indicate the 25th and 75th percentiles; a *line* within the *box* marks the median value; *whiskers* above and below the *box* indicate the 10th and 90th percentiles, whereas *dots* indicate the 5th and 95th percentiles (Kruskal–Wallis one-way analysis of variance on ranks)

in the spring, chubs started to feed on amphipods more intensively, which may explain the increase of the infection level in the spring season.

Although the trend of lower parasite abundance was observed in both seasons at site 3 (Oborovo) and in the autumn at site 5 (Jasenovac), the differences between the investigated sites were not statistically significant ($p > 0.05$; Kruskal–Wallis one-way analysis of variance on ranks). This is in agreement with the spatial distribution of infection prevalence which was also the lowest at site 3 (Table 4). Site 3 (Oborovo) was generally characterised by the inferior river water quality, as seen from increased concentrations of dissolved and labile species of several metals in the river water [44, 45], increased organic and faecal water contamination (as described in Sect. 2.1) and increased water toxicity and moderate organic pollution [46, 47]. In addition, at site 5 (Jasenovac), higher accumulation of several metals (Cd, Cu) in various chub tissues (liver, gills, gastrointestinal tissue) was also observed in the autumn, indicating increased metal exposure compared to the remaining sampling sites [48–50]. Such characteristics of the ecosystem at the sampling sites 3 and 5 probably have affected intermediate host on the first level, which is known to be very sensitive to the pollution [51], and finally resulted in lower abundance and prevalence of infection.

Positive correlation between abundance and fish length was weak, statistically significant ($p < 0.05$) only for the spring sampling. With increasing fish size, the number of acanthocephalans can increase due to the fact that larger/older fish can accumulate more parasites and can feed on larger amphipods [52].

Morphological analysis of acanthocephalan specimens revealed the presence of two species: *Pomphorhynchus laevis* and *Acanthocephalus anguillae* (Fig. 7a, b). European chub is preferred definitive host for both species and their distribution within alimentary tract overlaps [34].

Although competition between those two species was demonstrated in laboratory infections of rainbow trout, *Oncorhynchus mykiss* [53], such competition could not be confirmed in other species of fish, including preferred chub [54].

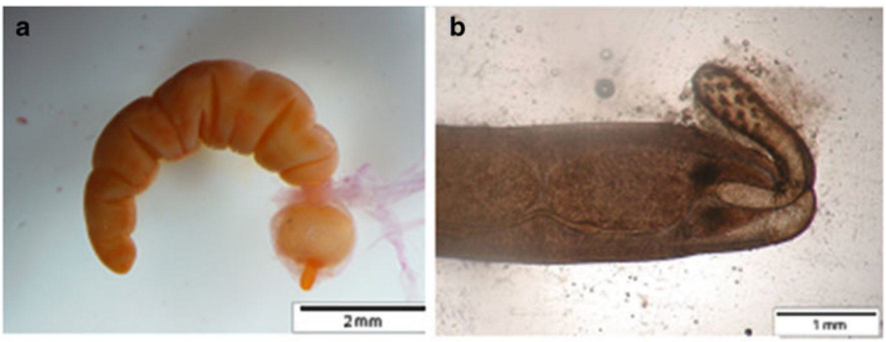

Fig. 7 Acanthocephala of the European chub from the Sava River: (**a**) *Pomphorhynchus laevis*. (**b**) *Acanthocephalus anguillae*

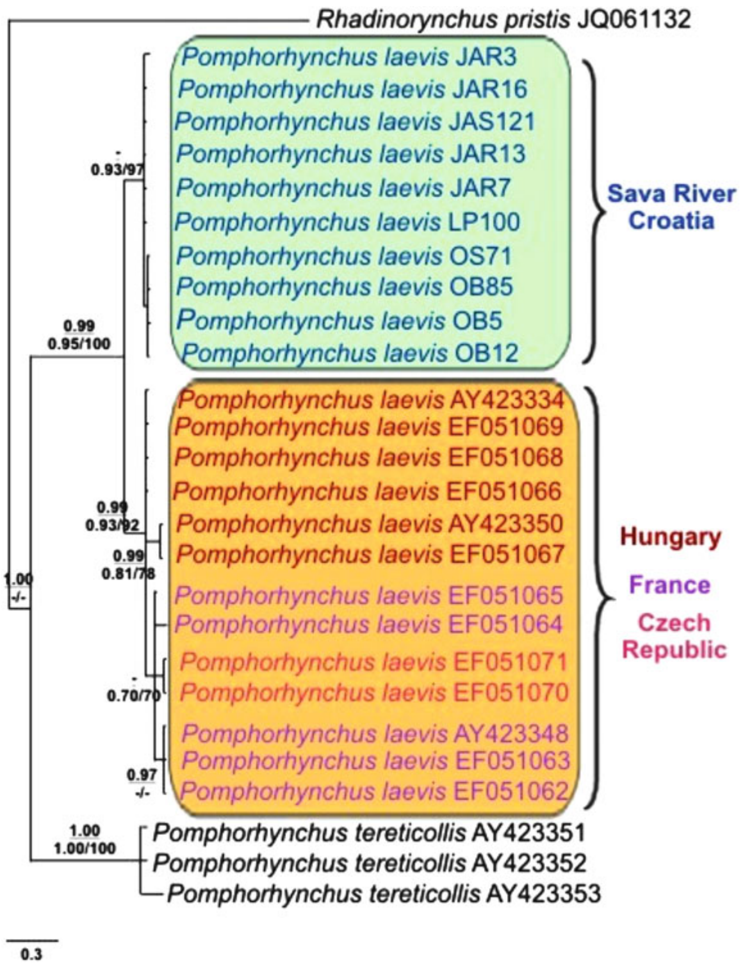

Fig. 8 Phylogenetic analyses inferred with COI data set. This tree shows systematic position of *P. laevis* from Croatia in relation to the other *P. laevis* and *P. tereticollis* strains from the continental Europe. The bootstrap values (1,000 replicates) of Bayesian PP support are given above and the ML (>70 %) and MP (>70 %) support below

Molecular analysis was performed on *P. laevis* specimens, as dominant acanthocephalan species of the European chub from the Sava River, to find out their genetic variability rate. COI proved to be the most effective marker (nucleotide similarity = 98.6–100 %, $n = 11$ sequences), while ITS regions (nucleotide similarity 99.8–100 %, $n = 13$ sequences) and 18S rRNA (nucleotide similarity = 100 %, $n = 10$ sequences) were more conserved. Based on partial COI sequence analysis, eight haplotypes ($H = 8$) were observed with no clear genetic clustering related to different sampling sites. Phylogenetic analysis confirmed subgrouping of *P. laevis* from the Sava River in Croatia separately from the other European specimens available from the GenBank (Fig. 8). These results suggest that widely specific species such as *P. laevis* shows local specificity and strain formation possibility in the continental Europe, based on COI DNA variability. In Britain and Ireland, two freshwater strains of *P. laevis* were confirmed [55], with difference probably more host than the geographically based. Although definitive host for the *P. laevis* specimens from the continental Europe used in our analysis was mainly the chub, differences in the intermediate hosts could be related to the strain formation. *P. laevis* uses the local species of *Gammarus* as its intermediate host [34]. It is possible that Croatian strain of *P. laevis* uses *Gammarus fossarum* as an intermediate host, whose distribution is described within the analysed part of the Sava River [56]. This species is different from the *Gammarus balcanicus*, *Echinogammarus stammeri* and *Gammarus roeseli*, which are described as intermediate hosts for other *P. laevis* strains in the continental Europe [57].

In summary, in the European chubs of good general health from the Sava River, two species of the intestinal acanthocephalan parasites were found, *P. laevis* and *A. anguillae*. The lower abundance of Acanthocephala at sampling site 3 (Oborovo) could be related to the increased pollution, caused by the main municipal sewage vent at this sampling site. Phylogenetic grouping of the *P. laevis* strain from the Sava River separately from the other known continental European strains indicates local specificity of this species which could be connected with the utilisation of different intermediate hosts.

3 Survey of the Microbiological Quality of Water of the Sava River: 2012 Survey

For the assessment of the present state of the microbiological quality of the Sava River, samples were collected during international survey in September 2012 from the 11 sampling sites in the upper, middle and lower course. The study included the investigation of the levels of sanitary pollution and organic contamination. A total of seven parameters were analysed. For the detection of sanitary pollution, total coliforms, faecal coliforms and faecal enterococci were analysed. For organic contamination assessment, heterotrophs, oligotrophs, aerobic heterotrophs and aerobic mesophilic bacteria were monitored.

3.1 Sampling Sites

For 2012 microbiological Sava survey, we have chosen five sites in Slovenia, three sites in Croatia and three sites in Serbia (Fig. 1). The coordinates of the sampling sites were measured by GPS ("Garmin eTrex") and charted by using ArcView software (map 1:300,000 system WGS_1984) (Fig 1).

Slovenia: The sites Hrastnik, Vrhovo, Blanca, Krško and Brežnica (1–5) are located upstream of the artificial dams of hydropower plants. The sites are only under the impact of wastewaters of minor settlements.

Croatia: The Drenov Bok site (6) is located in the area with intense agricultural activity, mainly represented by pig and poultry farms. The Slavonski Brod site (7) is located downstream of the town Slavonski Brod (60,000 inhabitants). The Štitar site (8) is mainly under the impact of agricultural run-offs. The site is situated about 30 km downstream the confluence of the Bosna River, significant right-hand tributary.

Serbia: The Bosut site (9) is located near the confluence of the small lowland Bosut River. However, about 15 km upstream of the Bosut site is the confluence of the Drina River, the largest tributary of the Sava River with significant hydrological input. The Sremska Mitrovica site (10) is under the impact of wastewaters from the town Sremska Mitrovica (40,000 inhabitants). The Jarak site (11) is located 15 km downstream of the Sremska Mitrovica. Except the wastewaters originating from the upstream located settlements, this site is also under the impact of agricultural run-offs.

3.2 Methods

Samples for analyses were collected in 0.5 L sterile glass bottles and transferred to the laboratory in dark cooling boxes. All samples were processed in the laboratory within 24 h from sampling.

Indicators of faecal pollution were isolated according to national legislation [58–60]. Total coliforms (cultivated on eosine-methylene blue agar at 37 °C for 24 h), faecal coliforms (cultivated on MacConkey agar at 44 °C for 24 h) and faecal enterococci (represented by *E. faecalis*, cultivated on dextrose tellurite agar at 37 °C for 24 h) were isolated by membrane filtration method. Isolation of total and faecal coliforms was performed with 0.45 μm cellulose nitrate filters, while for faecal enterococci 0.2 μm filters were used. Water quality was assessed in compliance with EU-Bathing Water Quality Directive 2006/7/EEC. The class limit values were as in the Joint Danube Survey [61]. Faecal coliforms-to-enterococci ratio was used to indicate origin of pollution. For the identification of some of the isolated total coliforms ($n = 50$), we have applied IMViC test (indole, methyl red, Voges–Proskauer and citrate) and additional identification by API 20E identification kit [62] and processed using bioMerieux online service.

For providing information about overloading of water with organic compounds, the presence of main groups of organotrophic bacteria was monitored. Among the organotrophs, special attention is paid to psychrophilic organotrophs (heterotrophs, oligotrophs and aerobic heterotrophs) as an autochthonous group which can be used for assessment of the ability of self-purification [63] and mesophilic organotrophs, as partially allochthonous group possibly containing pathogenic bacteria. Counts of aerobic heterotrophic bacteria were performed on nutrient agar (spread plate

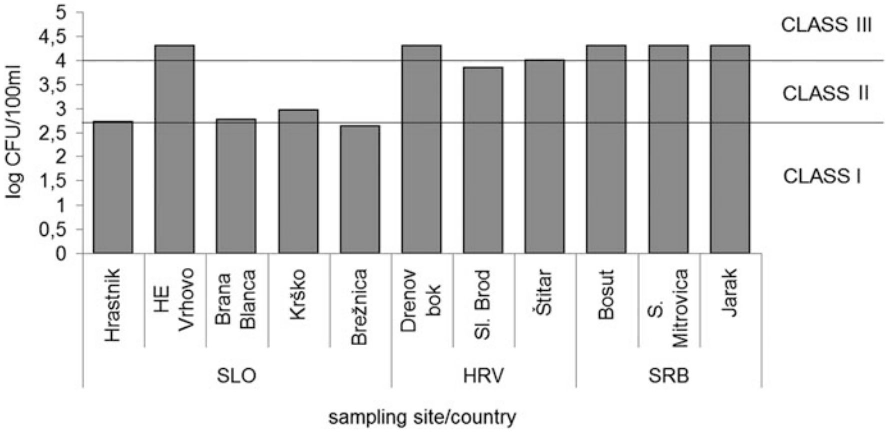

Fig. 9 Numbers of total coliforms in samples collected at the Sava River

technique, cultivation on 22 °C for 4 days). Water quality assessment based on this parameter was performed as purposed by Kohl [64]. The ratio of oligotrophic bacteria (pour plate technique with 1:10 diluted nutrient agar, incubation at 22 ºC for 4 days) to heterotrophic bacteria (pour plate technique with nutrient agar, incubation at 22 ºC for 4 days) (O/H ratio) was used for assessment of self-purification ability. The O/H ratio higher than 1 indicates satisfactory level of self-purification [63]. The mesophilic organotrophs were isolated by cultivation on nutrient agar for 24 h at 37 ºC.

3.3 Results and Discussion

3.3.1 Sanitary Pollution of Sava River

The results indicated the presence of the sanitary pollution at all sampling sites.

At the sites from the upper course of the Sava River, the number of total coliforms ranged from 430 CFU 100 mL^{-1} at the Brežnica site (class I) to >24,000 CFU 100 mL^{-1} at the Vrhovo site (class III) (Fig. 9). The numbers of faecal coliforms ranged from 380 to 1,090 CFU 100 mL^{-1} indicating moderate faecal pollution at all sampling sites with the exception of the Vrhovo site where critical faecal pollution was detected (Fig. 10).

The number of faecal enterococci was below the level of detection at the Brežnica site (<10 CFU 100 mL^{-1}), while the most polluted site was Hrastnik where moderate level of pollution was detected (Fig. 11). Considering that these sites are only under the impact of wastewaters originating from the minor settlements, increased numbers of bacterial can be attributed to the effect of damming.

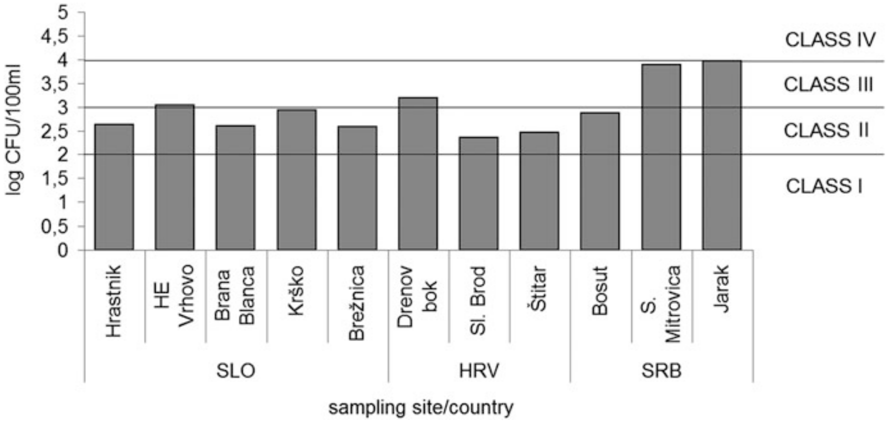

Fig. 10 Numbers of faecal coliforms in samples collected at the Sava River

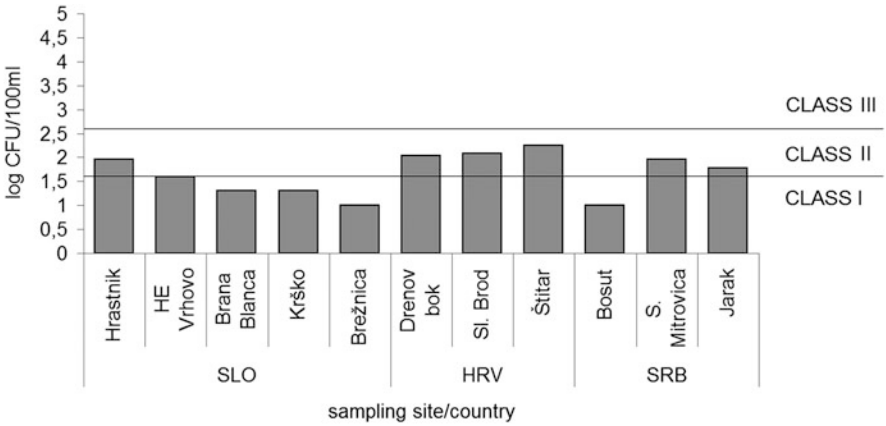

Fig. 11 Numbers of faecal enterococci in samples collected at the Sava River

At the sites in the middle course of the Sava River, the numbers of total coliforms ranged from 7,010 to >24,000 CFU 100 mL^{-1} (Fig. 9), while the numbers of faecal coliforms ranged from 230 to 1,540 CFU 100 mL^{-1} (Fig. 10). The counts indicated moderate pollution at the sites Slavonski Brod and Štitar and critical pollution at the Drenov Bok site. The numbers of faecal enterococci ranged from 120 to 180 CFU 100 mL^{-1} indicating moderate level of pollution (Fig. 11).

At the sites in the lower course of the Sava River, the highest numbers of total and faecal coliforms were detected. The numbers of total coliforms at all sites were >24,000 CFU 100 mL^{-1}, indicating critical pollution (Fig. 9). Faecal coliforms indicated moderate pollution at the site Bosut and critical pollution at the sites Sremska Mitrovica and Jarak (Fig. 10). The number of faecal enterococci was below the level of detection at the Bosut site (<10 CFU 100 mL^{-1}); moderate pollution was detected at the sites Sremska Mitrovica and Jarak (Fig. 11).

Among the isolated and identified coliforms ($n = 50$), 59 % belonged to *Citrobacter* sp., while 16 % were *E. coli*. *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Citrobacter braakii* which were also present in the samples.

The middle and the lower courses of the Sava River are under the impact of agricultural run-offs and wastewaters originating from settlements. The difference between the sites in the middle and lower course was observed in the numbers of the faecal coliforms and faecal enterococci. Although *E. faecalis* represents predominant species of enterococci in human faeces [65], it has been also isolated only in faeces of poultry [66]. Therefore, higher numbers of faecal enterococci in the middle course could be attributed to the wastewaters of the poultry farms in this area, while increased numbers of faecal coliforms in the lower course of the Sava River can be attributed to the wastewater from settlements. Moreover, faecal coliforms-to-enterococci ratio is much higher in the lower course of the river, indicating human origin of pollution [67]. The hydrological effect of the tributaries Drina and Bosut was evident only in reduced numbers of enterococci.

3.3.2 Organic Pollution of Sava River

The indicators of organic pollution showed moderate pollution and unsatisfactory level of self-purification at the majority of the sampling sites (Fig. 12). The counts of aerobic mesophilic bacteria ranged from 440 at the Slavonski Brod Site to 7,300 CFU mL^{-1} at the Hrastnik site.

Aerobic heterotrophs ranged from 580 to 17,300 CFU mL^{-1}. At the sites Slavonski Brod and Štitar, the lowest level of organic contamination was detected. The sites Hrastnik, Vrhovo, Brana Blanca, Krško, Brežnica, Drenov Bok and Jarak were moderately polluted (class II). The most polluted sites were Sremska Mitrovica and Bosut.

The highest numbers of oligotrophs and heterotrophs (14,160 and 14,140 CFU mL^{-1}, respectively) were detected at the site Sremska Mitrovica. The domination of heterotrophs over oligotrophs was detected at the majority of the sites, indicating unsatisfactory level of self-purification (Fig. 13).

The origin of organic pollution in an ecosystem can be attributed to organic manure, fertilisers, high stocking density, feed waste, faecal matter, algal bloom and human interference [68, 69]. The origin of organic pollution in the Sava River is probably the same as for the faecal pollution—high organic loads from urban wastewaters, animal farms and agricultural run-offs. Increased concentration of nutrients caused by damming can be linked to the increased numbers of organotrophs in the upper course of the river.

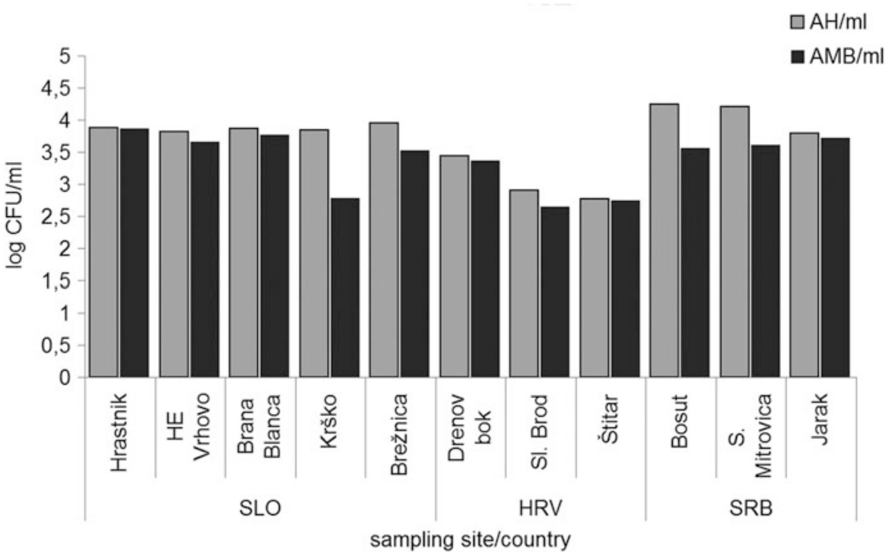

Fig. 12 Numbers of aerobic heterotrophs and aerobic mesophilic bacteria in the samples collected at the Sava River

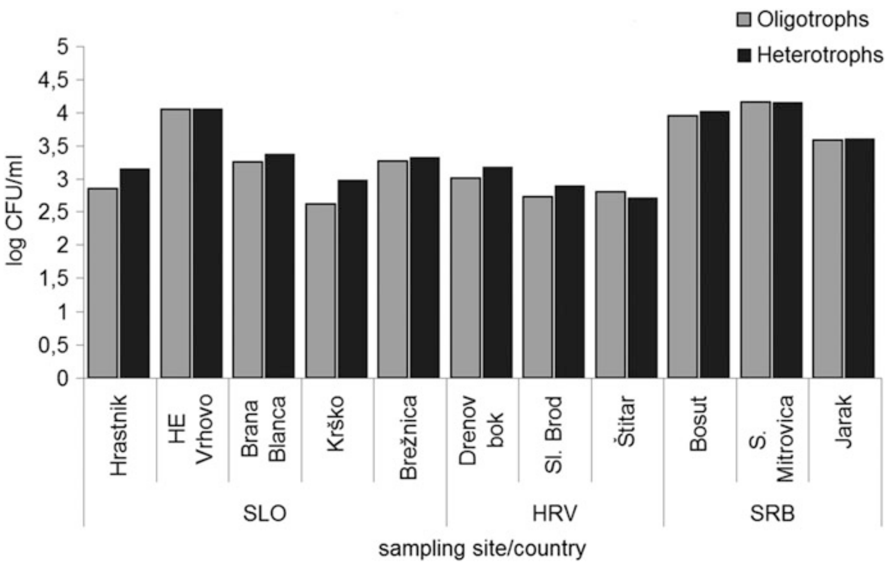

Fig. 13 Numbers of heterotrophs and oligotrophs in the samples collected at the Sava River

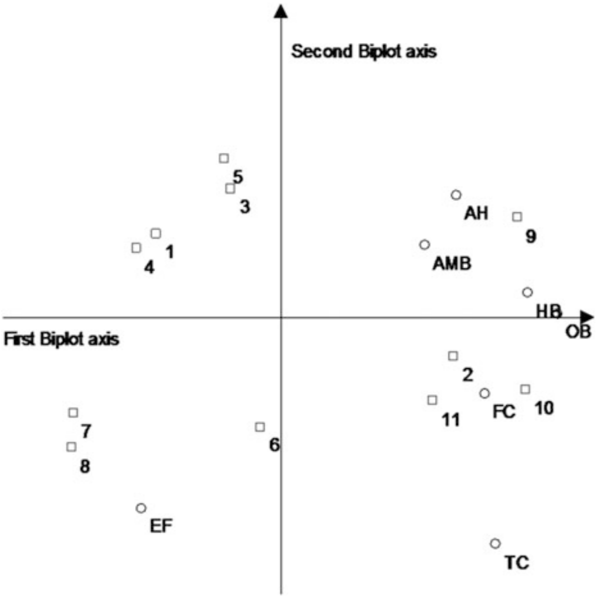

Fig. 14 Principal component analysis of samples collected at Hrastnik (1), Vrhovo (2), Blanca (3), Krško (4), Brežnica (5), Drenov Bok (6), Slavonski Brod (7), Štitar (8), Bosut (9), Sremska Mitrovica (10) and Jarak (11)

3.3.3 Principal Component Analysis of Obtained Data

To investigate the relationship between bacterial numbers and the sampling sites along the course of the Sava River, the results were analysed by principal component analysis (PCA, Flora software for floristic and vegetation analyses, [70]), commonly employed in environmental studies [71]. Although the existence of faecal and organic pollution was observed in all samples, the results of PCA analysis showed that samples from the upper and middle course formed one group and samples from the lower course the other group along the first biplot axis (Fig. 14). Along the second biplot axis, samples from the upper course formed one group, while samples from the middle course formed the other. Observed grouping can be attributed to the different types of the anthropogenic impacts in different parts of the river (damming, urban wastewaters and agricultural activity).

Our results confirm previous conclusions that large lowland rivers in Europe (Danube, Velika Morava, Tisza, etc.) [8, 61, 72–78] are under considerable microbiological contamination.

4 Conclusions

Our study shows that microbiological quality of the Sava River is unsatisfactory.

Based on 2006 survey, considerably higher microbiological contamination was recorded at the locations downstream from Zagreb and Velika Gorica (three sites in the Oborovo area: US VGSO, DS VGSO, OB) due to the influx of wastewater into the river water. The results from 2012 survey indicated the existence of moderate to critical faecal and organic pollution along the whole investigated stretch.

The pronounced anthropogenic pressure downstream Zagreb and Velika Gorica was confirmed by ichthyo-parasitological survey, since on those sites lower prevalence and abundance of two common species of the chub intestinal acanthocephalan parasites, *Pomphorhynchus laevis* and *Acanthocephalus anguillae*, were recorded.

Accumulation of the bacteria in the European chub meat was mainly uniform along the watercourse within standards and limitations for the human consumption.

It is obvious that human activities cause microbiological contamination of the Sava River along the whole investigated stretch. Thus, it is necessary to apply the measures for reduction of microbial pollution in considerable stretch of this mighty river. The effective reduction of pollution, including microbiological, could be done only within coordinated action of all riparian countries.

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Algal Communities Along the Sava River

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Abstract Field analysis of phytoplankton and phytobenthos communities of the river Sava has been performed, from Slovenia to Serbia, in August 2011 and September 2012 at 20 localities. A total number of 256 taxa have been determined, from eight divisions: Cyanobacteria (20), Rhodophyta (1), Dinophyta (6), Cryptophyta (1), Chrysophyta (1), Bacillariophyta (152), Chlorophyta (67) and Euglenophyta (8). In the phytoplankton samples, 188 taxa have been identified and in the phytobenthos samples 153 taxa. The most diverse divisions of phytoplankton of the river Sava were Bacillariophyta (46.28 % of total taxa number) and Chlorophyta (34.57 % of total taxa number). Biomass of phytoplankton was low, and the abundance of phytoplankton communities varied between 65,000 and 412,000 Ind L^{-1}. The biomass of phytoplankton of the river Sava was in the range of 41 to 564 μg fr. wt. L^{-1}. The phytobenthos dominated by the division of Bacillariophyta, making 81.7 % of the community. Visible macroaggregations were composed of *Cladophora glomerata* (Chlorophyta) and *Thorea hispida* (Rhodophyta).

Keywords Phytoplankton • Phytobenthos • Large lowland rivers • Diversity • Community

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1 Introduction

Apart from their importance as primary producers, algae are important as biological quality elements for river ecosystems. Both phytobenthos and phytoplankton communities are used in the determination of water quality in rivers according to the European Water Framework Directive [1].

Previous researches on algal communities of the river Sava were mainly related to phytoplankton, especially in the Serbian part of the stream. Numerous and important, on the phytoplankton communities both qualitative and quantitative were performed between 1939 and 2008 [2–14].

Some of the investigations also assessed water quality either by the saprobity degree based on the indicator algae species [2, 5–7, 9–17] or the trophicity degree based on the phytoplankton biomass and chlorophyll a concentration [14].

Researches on benthic algae of the river Sava were mainly concerned with the Bacillariophyta. A section of the river Sava's flow was partially investigated during the First and the Second Joint Danube Survey (JDS1 and JDS2) conducted in 2002 and 2007. The aim of investigations was to monitor the diversity of algal communities, both phytoplankton and phytobenthos, and the abundance of the taxa found and to determine the water quality according to the indicator species of algae and the saprobiological methods [18, 19]. Phytobenthos in the part of the river Sava flowing through Serbia has not been thoroughly investigated. Veljić and Cvijan [20] provide data on the qualitative composition of the benthic algal community in the river Kolubara, a right-side tributary, as well as in the smaller rivers Obnica and Jablanica in its basin. Data on the presence of red algae (Rhodophyta) in the Serbian stretch of the river Sava and its tributaries were reported by Čađo et al. [13], Simić et al. [21], Veljić and Cvijan [20] and Simić and Pantović [22].

The aim of this study is to present the results of qualitative and quantitative investigations on the composition of phytoplankton and phytobenthos assemblages in the river Sava, particularly from the Serbia stretch, and to review data on the water quality of this river.

2 Material and Methods

Hydrobiological survey of the Sava River was carried out in August of 2011 and September of 2012. During two cycles of research, phytobenthos samples were gathered at 20 localities, while phytoplankton samples were conducted at 14 localities during the second year of investigation (Table 1). Physical and chemical parameters were measured at the time of sampling as well.

Quantitative phytoplankton samples, 500 ml each, were taken at a depth of 0.1 m in the main flow of the river, put in plastic bottles and preserved in a 4 % solution of formaldehyde. Qualitative phytoplankton samples were collected by sweeping a plankton net of 25 cm diameter and ca. 22 μm mesh size. The collected material was

Table 1 Sampling sites of phytobenthos (Phb) and phytoplankton (Php) in Slovenia (SI), Croatia (HR) and Serbia (RS) in the river Sava

Sample number	Sampling site	Country	Year	Phb	Php
1	Hrastnik	SI	2012	+	+
2	Below HPP Vrhovo dam	SI	2012	+	+
3	Below HPP Blanca dam	SI	2012	+	+
4	Krško	SI	2012	+	+
5	Brežice	SI	2012	+	+
6	Rugvica	HR	2012	+	+
7	Lijeva Martinska Ves	HR	2011	+	–
8	Lukavec Posavski	HR	2012	+	+
9	Krapje	HR	2011	+	–
10	Mlaka	HR	2012	+	+
11	Orubica	HR	2011	+	–
12	Slavonski Brod	HR	2012	+	+
13	Slavonski Šamac	HR	2011	+	–
14	Štitar	HR	2012	+	+
15	Bosut confluence	RS	2012	+	+
16	Sremska Mitrovica	RS	2012	+	+
17	Jarak	RS	2012	+	+
18	Šabac	RS	2011	+	–
19	Ostružnica	RS	2011	+	–
20	Makiš	RS	2012	+	+

transferred to sample storage bottles and fixed with 4 % formalin. Qualitative and quantitative analysis of the collected material was performed in the laboratory at the Institute of Public Health of Serbia, Belgrade, Serbia.

Qualitative analysis was carried out to the species level or to the genus level, where it was impossible to identify the species. Quantitative analysis of phytoplankton was made using the Utermöhl method [23] with an Olympus inverted microscope, expressing data as number of cells per litre. The number of cells was converted to phytoplankton biomass by geometric approximations, using a standard formula [24, 25], and data are expressed in μg fr.wt. L^{-1}. Average cell dimensions were obtained by measuring at least 25 representatives of each taxon present.

Phytobenthos sampling was done according to the following standards: EN 13946 (Water quality. Sampling and processing of diatoms in rivers) [26] and CEN/TC 230 N 0540 (Water quality. Standard for monitoring, sampling and laboratory analysis of phytobenthos in shallow watercourses) [27].

Samples were taken from hard structures of substrate (gravel, stones and rocks) wherever it was possible on the left and the right bank of the main watercourse, in the illuminated zone at the depth of 1 m. Algal material was scraped off from approximately 10 cm^2 of surface from each of five stones using a small amount of water and transferred into a sampling bottle. Thread-like taluses were removed with a knife or tweezers and put in a bottle. The material was fixed with formaldehyde solution to final concentration of 1–4 %.

Algological samples are kept at the Department for Biology and Ecology of the Faculty of Science, Kragujevac, Serbia. Algae were observed with a microscope C. Zeiss-Amplival, with magnifications of up to 1,600×. Algae of all groups (except Bacillariophyta) were microscopically observed directly from the sample or using selective coloration (e.g. Lugol's solution for green and blue green). A portion of each sample was treated using a standard procedure with high concentrated sulphuric acid, and the obtained material was used for preparation of permanent diatom slides [28].

3 Results

Through qualitative analysis of the phytoplankton and phytobenthos communities during 2011 and 2012 along the river Sava in 20 localities, the presence of 256 taxa was recorded: Cyanobacteria (20), Rhodophyta (1) Dinophyta (6), Cryptophyta (1), Chrysophyta (1), Bacillariophyta (152), Chlorophyta (67) and Euglenophyta (8). In the phytoplankton samples 188 and in the phytobenthos samples 153 taxa were determined. In the part of Sava's flow through Slovenia, the presence of 176 taxa was recorded, through Croatia 178 and through Serbia 160 taxa (Table 2).

3.1 Results for Phytoplankton

The qualitative analysis of the phytoplankton during 2012 indicates the presence of 188 taxa from seven divisions: Cyanobacteria, Dinophyta, Cryptophyta, Chrysophyta, Bacillariophyta, Chlorophyta and Euglenophyta (Table 2). In the part of the Sava's flow through Slovenia, there were 139 taxa, through Croatia 129 and through Serbia 112.

The analysis of the cenotic composition of the phytoplankton of the river Sava (Fig. 1) by the number of taxa was dominated by two divisions: Bacillariophyta (46.28 % of the total number of taxa) and Chlorophyta (34.57 %). In the part of the flow of the river Sava through Slovenia, diatoms contribute 53.24 % of the total number of the taxa determined, while the green ones make up 27.34 %. In the Croatian part of the flow, the percentage of diatoms and green algae in the total number of the taxa determined is almost equal (43.41 % and 41.09 %), while in the Serbian part of the flow, that ratio is 58.04 %—Bacillariophyta and 29.46 %—Chlorophyta (Fig. 2).

The greatest number of the taxa was recorded at sampling stations 4—Krško (82 taxa) and 14—Štitar (81 taxa), and the smallest in 12—Slavonski Brod (42 taxa) and 8—Lukavec Posavski (44 taxa) (Fig. 3).

Table 2 Algal taxa identified during 2011/2012 hydrobiological survey of the river Sava

Taxa	Country		
	SI	HR	RS
Bacillariophyta			
Achnanthes Bory sp.	+*	+	+
Achnanthidium macrocephalum (Hustedt) Round et Bukhtiyarova	–	–	*
Achnanthidium minutissimum (Kützing) Czarnecki	+	+	+
Achnanthidium pyrenaicum (Hustedt) Kobayasi	–	*	*
Achnanthidium subatomoides (Hustedt) Lange-Bertalot	–	*	*
Amphora Ehrenberg ex Kützing sp.	+	+*	–
Amphora copulata (Kützing) Schoeman et Archibald	*	*	*
Amphora lybica Ehrenberg	+	+*	+*
Amphora ovalis (Kützing) Kützing	+*	+*	+*
Amphora pediculus (Kützing) Grunow	+	+*	+*
Amphora veneta Kützing	–	–	*
Asterionella formosa Hassall	+	–	+
Aulacoseira granulata (Ehrenberg) Simonsen	+	+	+
Aulacoseira muzzanensis (Meister) Krammer	+	+	+
Cocconeis Ehrenberg sp.	*	–	–
Cocconeis neodiminuta Krammer	*	–	–
Cocconeis pediculus Ehrenberg	+*	+*	+*
Cocconeis placentula Ehrenberg	+*	+*	+*
Cocconeis placentula var. *klinoraphis* Geitler	–	*	*
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	*	*	*
Craticula ambigua (Ehrenberg) D. G. Mann	–	*	–
Craticula cuspidata (Kützing) D. G. Mann	+*	+*	+
Cyclotella (Kützing) Brébisson sp.	+*	*	+*
Cyclotella meneghiniana Kützing	+*	+*	+*
Cyclotella ocellata Pantocsek	+	–	+
Cymatopleura elliptica (Brébisson) W. Smith	+*	+	+
Cymatopleura solea (Brébisson) W. Smith	+*	+	+*
Cymatopleura solea var. *apiculata* (W. Smith) Ralfs	+	–	–
Cymbella C. Agardh sp.	*	–	–
Cymbella affinis Kützing	+*	+	+
Cymbella cymbiformis C. Agardh	–	–	*
Cymbella lanceolata (Ehrenberg) Van Heurck	+*	+	*
Cymbella tumida (Brébisson) Van Heurck	+*	+*	+*
Cymbella turgidula Grunow	–	–	*
Diadesmis confervacea Kützing	–	*	–
Diatoma ehrenbergii Kützing	+	+*	*
Diatoma mesodon (Ehrenberg) Kützing	*	*	–
Diatoma moniliformis Kützing	–	–	+*
Diatoma vulgaris Bory	+*	+*	+*
Didymosphenia geminata (Lyngbye) M. Schmidt	+*	–	–

(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Diploneis oblongella (Nägeli ex Kützing) Cleve-Euler	+	*	*
Encyonema lunatum (W. Smith) Van Heurck	–	–	*
Encyonema minutiforme Krammer	–	–	*
Encyonema minutum (Hilse) D. G. Mann	+	+	+
Encyonema prostratum (Berkeley) Kützing	–	+	–
Encyonema silesiacum (Bleisch) D. G. Mann	+*	+*	+*
Encyonema ventricosum (C. Agardh) Grunow	–	*	–
Epithemia Kützing sp.	*	–	–
Fragilaria capucina Desmazières	+	–	+
Fragilaria ulna (Nitzsch) Lange-Bertalot Sippen *ulna* sensu Lange-Bertalot	+*	+	+*
Fragilaria ulna Sippen *acus* sensu Lange-Bertalot	+	+*	+
Fragilaria ulna Sippen *angustissima* sensu Lange-Bertalot	+	+	+
Fragilaria vaucheriae (Kützing) Petersen	*	*	–
Gomphonema Ehrenberg sp.	*	+	+
Gomphonema angustum C. Agardh	+	*	–
Gomphonema micropus Kützing	–	*	*
Gomphonema minutum (C. Agardh) C. Agardh	+	+	+
Gomphonema olivaceum (Horn) Brébisson	+*	*	+*
Gomphonema pala Reichardt	*	*	*
Gomphonema parvulum Kützing	+*	*	+*
Gomphonema subclavatum (Grunow) Grunow	*	–	–
Gomphonema tergestinum (Grunow) Fricke	*	–	–
Grunowia tabellaria (Grunow) Rabenhorst	*	–	–
Gyrosigma acuminatum (Kützing) Rabenhorst	+*	+*	+*
Gyrosigma scalproides (Rabenhorst) Cleve	+*	+*	+*
Gyrosigma sciotense (Sullivan et Wormley) Cleve	–	*	*
Halamphora montana (Krasske) Levkov	–	*	*
Hantzschia amphioxys (Ehrenberg) Grunow	*	–	–
Hantzschia spectabilis (Ehrenberg) Hustedt	–	–	*
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	+*	–	–
Lemnicola hungarica (Grunow) Round et Basson	+*	+	–
Luticola dismutica (Hustedt) D. G. Mann	–	–	*
Luticola goeppertiana (Bleisch) D. G. Mann	–	–	*
Luticola muticopsis (Van Heurck) D. G. Mann	*	–	–
Melosira lineata (Dillwyn) C. Agardh	–	–	+
Melosira varians C. Agardh	+*	+*	+*
Meridion circulare (Greville) C. Agardh	+*	*	–
Navicula Bory sp.	+*	+	+
Navicula antonii Lange-Bertalot et Rumrich	–	*	*
Navicula capitatoradiata Germain	+*	+*	+*
Navicula cincta (Ehrenberg) Ralfs	+	*	–

(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Navicula cryptocephala Kützing	+	+*	+*
Navicula cryptotenella Lange-Bertalot	–	*	–
Navicula gregaria Donkin	*	–	+*
Navicula lanceolata (C. Agardh) Ehrenberg	+	+*	+*
Navicula menisculus var. *menisculus* Schumann	+	+	+
Navicula phyllepta Kützing	–	–	+
Navicula radiosa Kützing	+*	*	*
Navicula recens (Lange-Bertalot) Lange-Bertalot	+*	–	+
Navicula tripunctata (O. F. Müller) Bory	+*	+*	+*
Navicula trivialis Lange-Bertalot	+	+*	+*
Navicula upsaliensis (Grunow) Peragallo	*	–	*
Navicula veneta Kützing	*	*	*
Navicula viridula (Kützing) Ehrenberg	+*	+*	+*
Navicula viridula var. *rostellata* (Kützing) Cleve	+	+*	+*
Navicula vulpina Kützing	*	*	*
Neidium ampliatum (Ehrenberg) Krammer	–	*	–
Neidium dubium (Ehrenberg) Cleve	+	+*	+*
Nitzschia Hassall sp.	+	–	+
Nitzschia gracilis Hantzsch	–	+	–
Nitzschia acicularis (Kützing) W. Smith	+	+	+
Nitzschia amphibia Grunow	*	*	*
Nitzschia capitellata Hustedt	–	–	*
Nitzschia communis Grunow	–	*	–
Nitzschia dissipata (Kützing) Grunow	+*	*	*
Nitzschia dissipata var. *media* (Hantzsch) Grunow	*	–	*
Nitzschia fonticola Grunow	+*	+	+
Nitzschia frustulum (Kützing) Grunow	–	–	*
Nitzschia fruticosa Hustedt	+	+	+
Nitzschia haufleriana Grunow	*	–	–
Nitzschia incognita Krasske	–	*	–
Nitzschia inconspicua Grunow	*	*	*
Nitzschia intermedia Hantzsch ex Cleve et Grunow	*	–	*
Nitzschia linearis (C. Agardh) W. Smith	+*	+*	+*
Nitzschia palea (Kützing) W. Smith	+*	+*	+*
Nitzschia paleacea (Grunow) Grunow	–	–	+
Nitzschia pseudofonticola Hustedt	*	–	–
Nitzschia recta Hantzsch	–	*	*
Nitzschia sigma (Kützing) W. Smith	+	–	+
Nitzschia sigmoidea (Nitzsch) W. Smith	+*	+*	+*
Nitzschia sublinearis Hustedt	–	*	–
Nitzschia tubicola Grunow	–	*	–

(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Nitzschia vermicularis (Kützing) Hantzsch	+	–	–
Pinnularia Ehrenberg sp.	*	–	–
Pinnularia viridis (Nitzsch) Ehrenberg	+	–	–
Placoneis placentula (Ehrenberg) Heinzerling	–	*	*
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	+*	+*	–
Planothidium septentrionalis (Østrup) Round et Bukhtiyarova	–	–	*
Reimeria sinuata (Greg.) Kociolek et Stoermer	*	*	*
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	+*	+*	+*
Rhopalodia gibba (Ehrenberg) O. Müller	–	+	–
Sellaphora bacillum (Ehrenberg) D. G. Mann	*	*	–
Sellaphora blackfordensis D. G. Mann et S. Droop	–	–	*
Sellaphora capitata D. G. Mann et S. M. McDonald	–	*	–
Sellaphora laevissima (Kützing) D. G. Mann	+	–	–
Sellaphora pupula (Kützing) Mereschkovsky	*	*	*
Skletonema potamos (Weber) Hasle	+	–	+
Stauroneis anceps Ehrenberg	–	–	*
Stauroneis phoenicenteron (Nitzsch) Ehrenberg	–	–	+
Staurosira pinnata Ehrenberg	*	–	*
Stephanodiscus Ehrenberg sp.	+*	+*	+*
Stephanodiscus hantzschii Ehrenberg	+*	+*	+*
Surirella Turpin sp.	–	+	–
Surirella angusta Kützing	+*	*	*
Surirella brebissonii Krammer et Lange-Bertalot	*	–	*
Surirella minuta Brébisson	+*	*	+
Surirella splendida (Ehrenberg) Kützing	–	–	+*
Surirella tenera Gregory	+	+*	+*
Tryblionella angustata W. Smith	*	*	*
Tryblionella calida (Grunow) D. G. Mann	*	–	–
Tryblionella gracilis W. Smith	–	*	–
Tryblionella levidensis W. Smith	–	+*	+*
Chlorophyta			
Actinastrum hantzschii Lagerheim	+*	+	+
Ankistrodesmus bibraianus (Reinsch) Korshikov	–	+	+
Ankistrodesmus gracilis (Reinsch) Korshikov	–	–	+
Cladophora glomerata (Linnaeus) Kützing	*	*	*
Closterium Nitzsch sp.	+	+	–
Closterium acerosum Ehrenberg	+	*	+
Closterium aciculare T. West	+	+	–
Closterium acutum var. *variabile* (Lemmermann) Krieger	+	–	–
Closterium moniliferum (Bory) Ehrenberg	+	+*	+*
Coelastrum astroideum De Not.	–	+	–

(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Coelastrum microporum Nägeli	+	+*	+
Coelastrum reticulatum (Dangeard) Senn	+	–	–
Cosmarium Corda sp.	+*	+	+
Dictyosphaerium pulchellum Wood	+	+	+
Eudorina elegans Ehrenberg	–	–	+
Eutetramorus fottii (Hindák) Komárek	+	+	+
Golenkinia radiata Chodat	+	–	+
Gonium pectorale O. F. Müller	–	–	+
Hydrodictyon reticulatum (Linnaeus) Lagerheim	–	+	–
Kirchneriella irregularis. var. *irregularis* (G. M. Smith) Korshikov	–	+	+
Micractinium pusillum Fresenius	+	–	+
Monoraphidium contortum (Thuret) Komárková-Legnerová	+	+	+
Monoraphidium griffithii (Berkeley) Komárková-Legnerová	–	+	+
Monoraphidium indicum Hindak	+	+	+
Monoraphidium komarkovae Nygaard	+	+	+
Mougeotia C. Agardh sp.	+	+*	+
Oedogonium Link ex Hirn sp.	*	*	*
Oocystis A Braun sp.	–	+	+
Pandorina morum (O. F. Müller) Bory	+	+	+
Pediastrum boryanum (Turpin) Meneghini var. *boryanum*	+	+*	–
Pediastrum duplex var. *duplex* Meyen	+	+	+
Pediastrum duplex var. *gracillimum* West et G. S. West	+	+	–
Pediastrum integrum Nägeli	–	+	–
Pediastrum simplex var. *simplex* Meyen	+	+	+
Pediastrum simplex var. *echinulatum* Wittz	–	+	+
Pediastrum simplex var. *sturmii* (Reinsch) Wolle	–	+	–
Pediastrum tetras (Ehrenberg) Ralfs	+	+	–
Scenedesmus acuminatus var. *acuminatus* (Lagerheim) Chodat	+*	+	–
Scenedesmus acuminatus var. *minor* G. M. Smith	+*	+	–
Scenedesmus acutus Meyen	+*	+*	+
Scenedesmus disciformis (Chodat) Fott et Komárek	+	+	+
Scenedesmus dispar Brébisson	+	+	–
Scenedesmus ecornis (Ehrenberg) Chodat	+*	+*	+*
Scenedesmus ellipticus Corda	–	+	–
Scenedesmus intermedius var. *intermedius* Chodat	–	+	–
Scenedesmus linearis Komárek	–	+	–
Scenedesmus magnus Meyen	+	+	+*
Scenedesmus obliquus (Turpin) Kützing	–	+	–
Scenedesmus obtusus Meyen	–	+	–
Scenedesmus opoliensis var. *mononensis* Chodat	+	+	+
Scenedesmus pecsensis Uherkovich	–	+	–

(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Scenedesmus quadricauda (Turpin) Brébisson	+*	+*	+*
Scenedesmus quadrispina Chodat	+	+	–
Scenedesmus semicristatus Uherkovich	+	–	–
Scenedesmus semipulcher Hortobágyi	–	+	–
Scenedesmus sempervirens Chodat	+	+	–
Scenedesmus smithii Chodat	–	+	–
Schroederia setigera (Schröder) Lemmermann	+	–	–
Sphaerocystis planctonica (Korshikov) Bourrelly	–	+	–
Spirogyra Link sp.	*	+*	+*
Staurastrum chaetoceras (Schröder) G. M. Smith	+	–	–
Staurastrum furcigerum Brébisson	–	+	–
Staurastrum Meyen ex Ralfs sp.	–	+	–
Stigeoclonium Kützing sp.	+*	–	*
Tetraedron minimum (Braun) Hansgirg	–	+	+
Tetrastrum glabrum (Roll) Ahlstrom et Tiffany	–	+	+
Treubaria C. Bernard sp.	–	+	–
Chrysophyta			
Dinobryon divergens Imhof	+	–	–
Cryptophyta			
Cryptomonas Ehrenberg sp.	–	+	–
Cyanobacteria			
Anabaena Bory sp.	+	+*	+
Aphanizomenon flosaquae Ralfs ex Bornet et Flahault	+	–	–
Chroococcus Nägeli sp.	+*	*	+
Chroococcus limneticus Lemmermann	+*	*	–
Geitlerinema amphibium (C. Agardh ex Gomont) Anagnostidis	+	–	–
Komvophoron minutum (Skuja) Anagnostidis et Komárek	+	–	–
Leptolyngbya Anagnostidis et Komárek sp.	+	–	+
Leptolyngbya foveolarum (Rabenhorst ex Gomont) Anagnostidis et Komárek	+	–	+
Leptolyngbya valderiana (Gomont) Anagnostidis et Komárek	+	–	–
Merismopedia elegans A. Braun	–	+*	–
Oscillatoria Vaucher sp.	–	+	–
Oscillatoria amoena (Kützing) Gomont	–	+	–
Oscillatoria limosa C. Agardh ex Gomont	*	+*	+*
Oscillatoria tenuis C. Agardh ex Gomont	+	+*	+*
Phormidium Kützing ex Gomont sp.	+	+	+*
Phormidium chlorinum (Kützing ex Gomont) Umezaki et Watanabe	+*	+	+
Phormidium tergestinum (Kützing) Anagnostidis et Komárek	+	+	+
Pseudanabaena catenata Lauterborn	–	+	–
Pseudanabaena limnetica (Lemmermann) Komárek	+*	–	–
Spirulina major Kützing ex Gomont	+	+*	+*

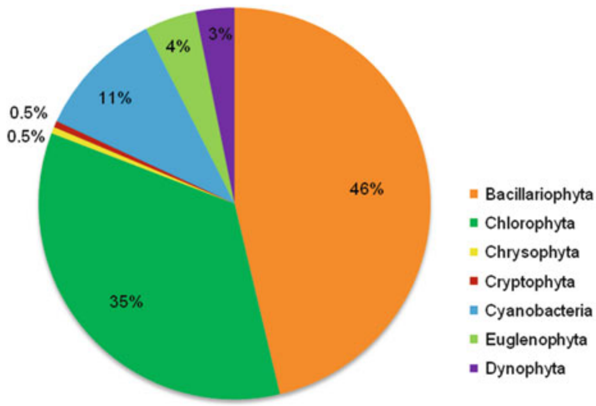
(continued)

Table 2 (continued)

Taxa	Country		
	SI	HR	RS
Euglenophyta			
Euglena Ehrenberg sp.	+	–	+
Euglena acus Ehrenberg	+	–	–
Euglena obtusa Van Goor	+	–	–
Lepocinclis Perty sp.	–	+	–
Strombomonas Deflandre sp.	+	–	–
Trachelomonas Ehrenberg sp.	–	+	+
Trachelomonas planctonica Svirenko	+	–	–
Trachelomonas volvocina Ehrenberg	+	+	–
Dinophyta			
Ceratium hirundinella (O. F. Müller) Bergh	–	+	–
Gymnodinium (Stein) Kofoid et Swe sp.	+	+	–
Peridiniopsis Lemmermann sp.	+	+	–
Peridinium Ehrenberg sp.	+	+	+
Peridinium cinctum (O. F. Müller) Ehrenberg	+	+	+
Peridinium umbonatum Stein	+	–	–
Rhodophyta			
Thorea hispida (Thore) Desvaux	–	–	*

+, taxon recorded in phytoplankton samples; *, taxon recorded in phytobenthos samples; –, no record of taxon

Fig. 1 Cenotic composition of the phytoplankton in the Sava River by the number of taxa



The results of the quantitative analysis of the phytoplankton of the river Sava are shown in Fig. 4. The greatest number of phytoplankton cells was recorded at station 6—Rugvica (412,000 Ind L^{-1}), and the smallest at station 16—Sremska Mitrovica (65,000 Ind L^{-1}). The biomass of the phytoplankton was in the range of 41 to 564 μg fr.wt. L^{-1}. In the part of the flow of the river Sava through Serbia, the

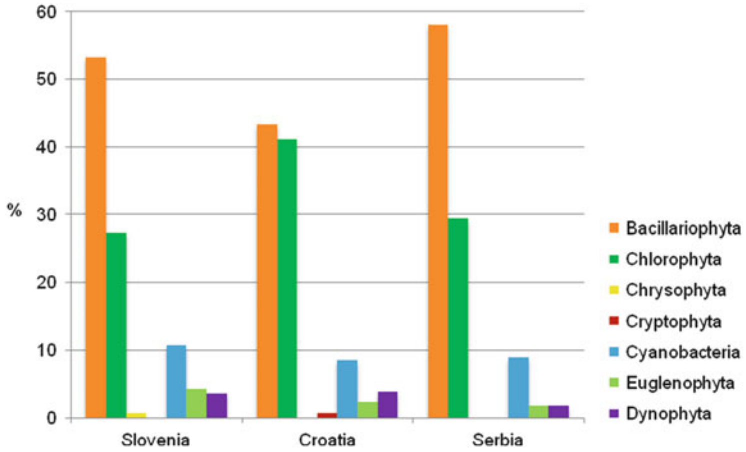


Fig. 2 Percentage representation of algal divisions in the phytoplankton community of the Sava River in Slovenia, Croatia and Serbia

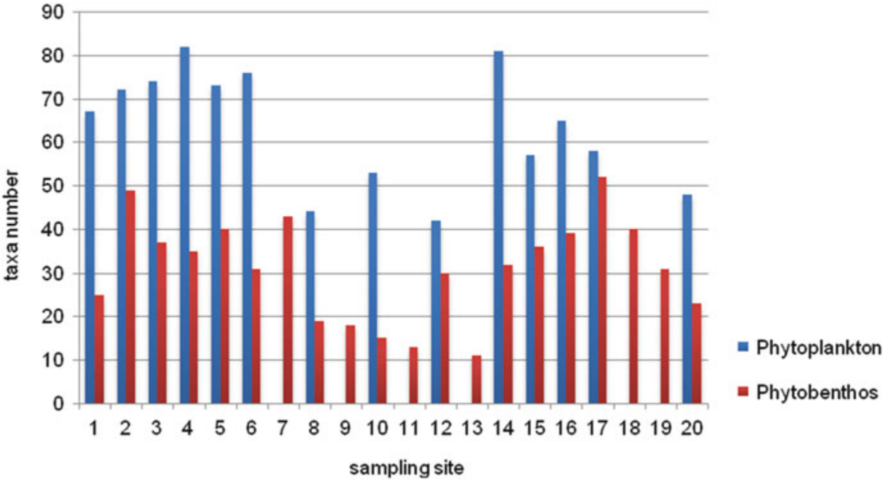


Fig. 3 Comparison of the number of taxa in the phytoplankton and phytobenthos in the localities along the Sava River. The real distances between sampling sites are not indicated

greatest number of phytoplankton was recorded in locality 17—Jarak (279,000 Ind L^{-1}).

The largest biomass was recorded at sampling station 3—below the HPP Blanca dam (564 μg fr.wt. L^{-1}), where the species from the genus *Navicula* and *Diatoma vulgaris* dominated, and the smallest was found at stations 15—Bosut confluence and 16—Sremska Mitrovica (41 μg fr.wt. L^{-1}) where the species from the genus *Stephanodiscus* dominated.

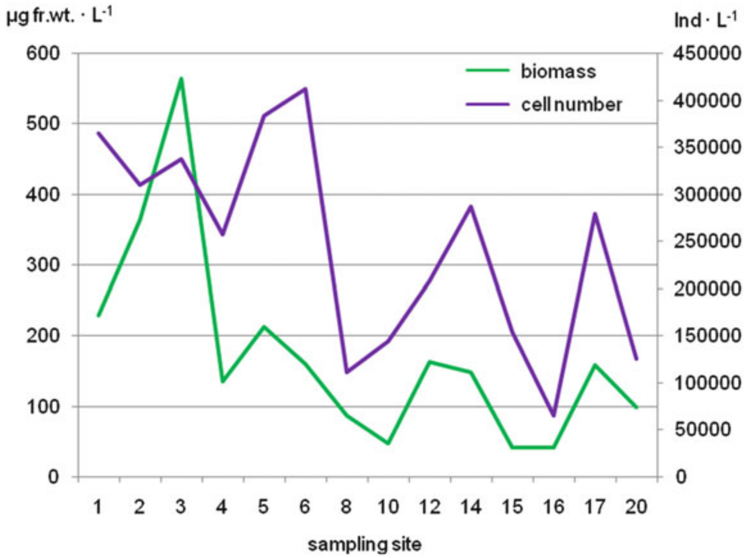


Fig. 4 The number (Ind L^{-1}) and biomass (μg fr.wt. L^{-1}) of the phytoplankton along the flow of the Sava River. The real distances between sampling sites are not indicated

Bacillariophyta have the largest percentage share in the total biomass of the phytoplankton of the river Sava (Fig. 5). The share was over 90 % at stations 2, 3, 16, 17 and 20. The largest share of the green algae (46 %) was recorded at station 6—Rugvica, where the species of the genera *Scenedesmus*, *Pediastrum* and *Closterium* dominated. The diatoms *Stephanodiscus hantzschii*, *Cyclotella meneghiniana* and *Aulacoseira granulata* were most often found. The upper flow of the river Sava is characterised by an increased frequency of the species of the genus *Navicula* (*N. tripunctata, N. lanceolata, N. capitatoradiata, N. cryptocephala*) and *Diatoma vulgaris*.

3.2 Results for Phytobenthos

The qualitative analysis of the phytobenthos sampled in August 2011 and September 2012 indicates the presence of 153 taxa from five divisions: Cyanobacteria, Rhodophyta, Chrysophyta, Bacillariophyta and Chlorophyta. In the part of the flow of the river Sava through Slovenia, the presence of 90 taxa was determined, through Croatia 95 and through Serbia 93 (Table 2).

The cenotic composition of the phytobenthos of the river Sava was dominated by the division of Bacillariophyta which was 81.7 % of the total number of taxa (Fig. 6). A smaller share was composed of Chlorophyta (11.11 %) and Cyanobacteria (6.54 %). In the part of the flow of the river Sava through Slovenia,

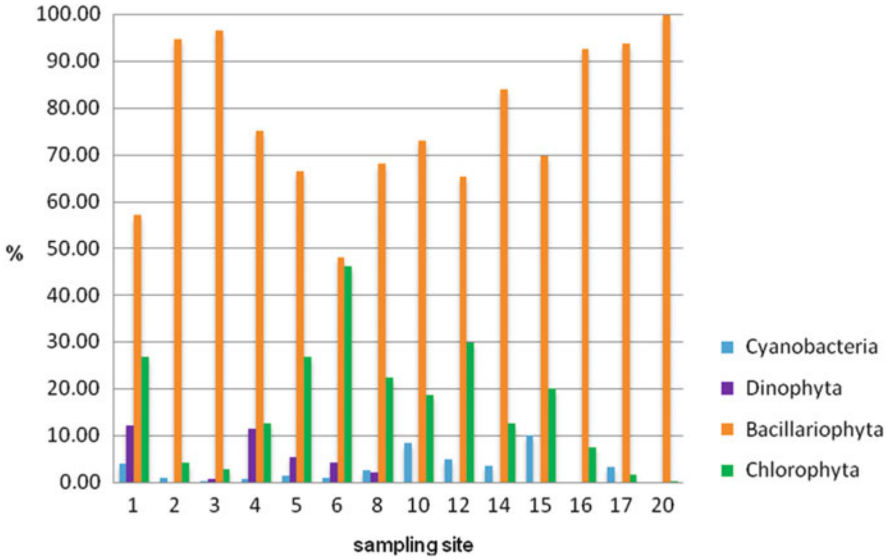
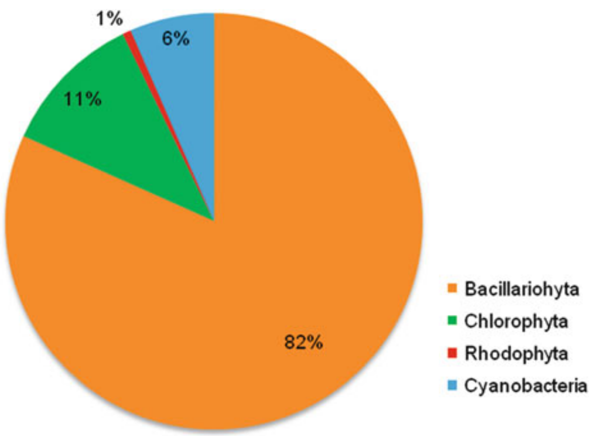


Fig. 5 Percentage share of the algal divisions in the total biomass of the phytoplankton of the River Sava. The real distances between sampling sites are not indicated

Fig. 6 Cenotic composition of benthos algae of the Sava River by the number of taxa



diatoms make up 82.22 % of the total number of the taxa determined, while the green algae share was 12.22 %. In the Croatian part of the flow, the percentage share of the total number of the taxa of the diatoms and green algae is similar to the previously mentioned one (Bacillariophyta 81.05 %, Chlorophyta 11.58 %), while in the part of the flow through Serbia, that ratio is 86.02 %—Bacillariophyta and 8.6 %—Chlorophyta (Fig. 7). Cyanobacteria were represented by 5.56 % (Slovenia), 7.37 % (Croatia) and 4.3 % (Serbia).

The greatest number of the determined taxa was recorded in the following localities: in Serbia at station 17—Jarak (52 taxa), in Slovenia at station 2—

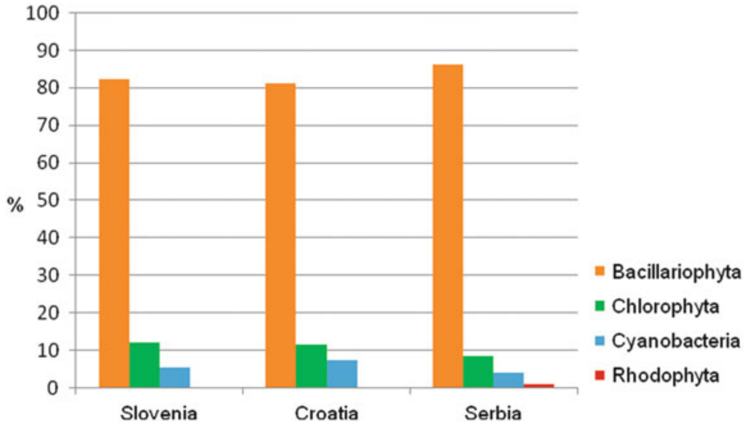


Fig. 7 Percentage representation of the algal divisions in the phytobenthos community in the Sava River in Slovenia, Croatia and Serbia

Fig. 8 Thallus of *Thorea hispida* (Thore) Desvaux (sampling site 16, Sremska Mitrovica, Serbia)

below HPP Vrhovo dam (49 taxa) and in Croatia at station 7—Lijeva Martinska Ves (43 taxa), and the smallest number was recorded in Croatia in the sampling station 13—Slavonski Šamac (11 taxa) (Fig. 3).

Green filaments of the macroalgae *Cladophora glomerata* were found in nine localities along the entire flow of the river Sava from Slovenia to Serbia, *Oedogonium* spp. and *Spirogyra* sp. in eight and *Stigeoclonium* sp. and *Mougeotia* sp. in two localities. Branched thalli, up to 50 cm long, of the red alga *Thorea hispida*, were found in locality 16, Sremska Mitrovica, in the Serbian part of the river Sava (Fig. 8).

4 Discussion

The qualitative analysis of the algal community investigated in August 2011 and September 2012 in the river Sava from Slovenia to the confluence with the Danube in Serbia indicates the presence of algae from the divisions of Cyanobacteria, Rhodophyta, Dinophyta, Chrysophyta, Bacillariophyta, Chlorophyta and Euglenophyta. While the phytoplankton contains almost identical number of diatoms (Bacillariophyta) and green algae (Chlorophyta), the phytobenthos is dominated by Bacillariophyta.

By comparing these results with the results of the previous, numerous phytoplankton and the rather rare phytobenthos observations, it is evident that the qualitative composition has not changed much over time.

The phytoplankton of the river Sava in Serbia was always characterised by the presence of algae from Cyanophyta, Pyrrophyta (primarily the class of Dinophyceae), Bacillariophyta, Chlorophyta and Euglenophyta. Algae from the divisions of Chrysophyta and Xanthophyta were sparsely present or absent.

The number of taxa in the Serbian part of the river Sava differed from previous investigations, since it depends on the number of sampling stations and the time period when the research was performed. The greatest number of taxa (227) was found in the period from 1982 to 1989 [29]. Slightly less taxa (185) occurred in the period from 2003 to 2004 [13]. Čađo et al. [14] confirmed the presence of 121 taxa in August 2006. A similar number (112 taxa) was determined in this study in September 2012.

In most recent investigations and those 75 years ago [2], comprising different localities and seasons, Bacillariophyta were the dominant group in terms of number of taxa, occasionally summing up to 90 %. The genera (represented by a small number of species) *Navicula* sp., *Diatoma* Bory de St.-Vincent, *Surirella* sp., *Aulacoseira* Thwaites sp. (including *Melosira* C. Agardh), *Cymbella* sp., *Cyclotella* sp., *Gomphonema* Ehrenberg sp., *Stephanodiscus* sp. and *Nitzschia* sp. were most frequent in the findings. The frequency and number of *Cyclotella meneghiniana*, *Fragilaria ulna* (Nitzsch) Lange-Bertalot, *Melosira varians*, *Aulacoseira granulata*, *Diatoma vulgaris*, *Cocconeis placentula* and *Encyonema minutum* are especially noticeable, since the first four of the species mentioned above are characteristic for many rivers (67 different rivers analysed in [30]). The species *Cyclotella meneghiniana* deserves special attention [8] considering that mass development was often observed in the last 20–30 years, while it has not been found at all or has been found rarely in the previous observations (see [3] and [6] and especially [2]). Protić [2] does not find this species at all and mentions *Stephanodiscus hantzschii*, *Fragilaria arcus* (Ehr.) Cleve, *F. crotonensis* Kitton and *Asterionella formosa* as numerous. By comparing his and more recent researches, changes in the qualitative composition as well as the quantitative representation of certain taxa are noticed, which can be explained by altered environmental conditions, and changes in the water quality of the river Sava.

In terms of number of taxa, Chlorophyta are a subdominant group of algae (phytoplanktons) in the river Sava in Serbia. The presence of the following genera, represented by a small number of species, especially stands out: *Scenedesmus* Meyen sp. (especially *S. quadricauda*), *Closterium* sp., *Ankistrodesmus* Corda sp., *Pediastrum* E. Hegewald sp., *Tetrastrum* Chodat sp., *Monoraphidium* Komárková-Legnerová sp. and to a lesser extent *Crucigenia* Morren sp. and some members of Volvocophyceae (e.g. *Chlamydomonas* Ehr. sp) [2, 3, 5–13]. Čađo et al. [14] recorded the dominance of Chlorophyta in the phytoplankton of the river Sava in August 2006. In 2011, Chlorophyta are also the subdominant group of algae in the phytoplankton, with similar dominance of species and genera as previously found.

Comparison of benthic algae composition with previous investigations cannot be made since such studies are lacking. The observation in 2011 and 2012 indicates that the number of species is smaller than in the phytoplankton and that an absolute dominance of Bacillariophyta is present at all localities. Filamentous green algae are particularly noticeable as a significant element of the phytobenthos community, especially *Cladophora glomerata* and *Oedogonium* sp. The finding of a rare red alga *Thorea hispida* in locality 16—Sremska Mitrovica—is also significant. The locality of Sremska Mitrovica is a new habitat of this species in Serbia, which has been defined as critically endangered species in Serbia (CR) by the number of findings, area of distribution, population density and endangerment degree [22]. The coverage of over 30 % in this locality indicates that it is the richest population of this alga in Serbia. So far, this alga was only found in the river Sava at Šabac [22]. The species of *T. brodensis* Klas [31] was found and described in the previous century in the part that flows through Croatia, through the town of Slavonski Brod, which is upstream of Sremska Mitrovica. Our investigations in 2011 and 2012 have not confirmed this finding of *Thorea* Bory de St.-Vincent sp. in this locality. Besides the red algal genus *Thorea*, the species *Audouinella chalybaea* (Roth) Bory de St.-Vincent has been found in the river Sava [13].

In earlier studies of quantitative structure of phytoplankton of the river Sava, the maximum development of this community was recorded in different seasons, mainly spring [2, 6, 8] or autumn [3, 4, 9]. The number of Bacillariophyta and Chlorophyta sometimes alternate in their seasonal appearance. Green algae become more important in summer, but diatoms of the autochthonous potamoplankton usually dominate [8]. Bacillariophyta are a taxonomic group best adapted to live in highly unstable environmental conditions, such as rivers [30].

The number of algae in the phytoplankton ranges in extremely wide limits on different localities—from 15,000 Ind L^{-1} [6] to 3,162,000 Ind L^{-1} [5]. The most commonly quoted values were between 73,000 Ind L^{-1} [4] and 256,000 Ind L^{-1} [7]. The last research of the river Sava in 2011 and 2012, in the part that flows through Serbia, also indicates that number is different in certain localities, from 65,000 Ind L^{-1} (locality16—Sremska Mitrovica) to over 279 000 Ind L^{-1} (locality 17—Jarak).

The biomass of the phytoplankton of the river Sava in September 2012 was in the range from 41 to 564 μg fr.wt. L^{-1} which indicates a low productivity of the river Sava in this period [30]. The part of the river Sava that flows through Serbia has until now mostly been classified as moderately [8] or low productive [13, 14]. Values of chlorophyll a concentration in 2006, in range from 1.15 to 1.5 μg L^{-1}, match class I of water quality according to ICPDR standard for river classification [32] and indicate an oligotrophic status [14].

In previous years, water quality of the river Sava was defined using plankton organisms as bioindicators. Lists of indicator organisms after СЭВ [33] and Gulyás [34] were used, as well as the saprobiological method according to Pantle and Buck [35]. Data on the water quality of the river Sava before World War 2 indicate that it was between classes I and II [5]. More recent data indicate different degrees of organic pollution in different years and seasons, as well as in different localities in Serbia. Water of the river Sava has been classified as class II [9], classes II and III [7, 12], III class [6] or classes III and IV [5]. Sometimes, the quality of the water of the river Sava is defined as in transition from class II to III or III to IV [15].

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Aquatic and Wetland Vegetation Along the Sava River

Branko Karadžić, Snežana Jarić, Pavle Pavlović, and Miroslava Mitrović

Abstract Diverse hydrological, climate, and soil conditions along the Sava River caused significant diversification of vegetation. Therefore, the objective of this chapter is to integrate and present all the available data on variability of the aquatic and riparian plant communities along the Sava River and its main tributaries as well as to identify the environmental factors, which are related to the distribution of different vegetation types. Special attention has been also paid on the detection of threats for rare and endangered plant species and fragile wetland ecosystems along the Sava River. Based on data review, syntaxonomic revision of aquatic and riparian vegetation based on common, pan-European databank is required. Ecological studies that involve inventory, monitoring, modeling, and prediction of changes in populations, ecological communities, and ecosystems require both georeferenced databases and computational tools for application of statistical methods.

Keywords Aquatic vegetation • The Sava River Basin • Community structure • Species richness • Riparian vegetation

1 Introduction

Ecotone is a transitional zone between two or more ecosystems that differ with respect to species composition [1]. Ecotonal communities are characterized by high biological diversity because they contain species from all neighboring communities. Such species mixtures are additionally enlarged by eurytopic species that are adapted to a wide spatiotemporal variability of environmental conditions in ecotones. The wetland ecosystems may be considered as large ecotones (transitional aquatic/helophytic, helophytic/terrestrial, forest/grassland zones), supporting high biodiversity. Global importance of wetlands is clearly elaborated in the Convention on Wetlands of International Importance, often referred to as the "Ramsar" Convention after the Iranian town of Ramsar where the treaty was adopted in 1971.

Due to the ecotone effects and broadly overlapping distribution of hygrophilous species, the classification of wetlands is an extremely problematical issue.

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According to Scott and Jones [2], the inland wetlands may be grouped into *riverine* (river/stream), *lacustrine* (lake/pond), and *palustrine* ecosystems, covering freshwater springs, peat bogs, fens, marshes, swamps, shrub-dominated swamps, and swamp forests. These ecosystems may be divided further into permanent and seasonal wetlands.

Investigation of aquatic and wetland communities in Europe has a long tradition [3–12]. These communities are extremely diverse due to wide (pan-European) distribution, heterogeneous environmental conditions, and periodic disturbance of ecosystems caused by seasonal floods.

Chemical and physical conditions (biochemical oxygen demand, pH, and concentrations of oxygen, nitrogen, and phosphorus, river depth, river width, substrate type, temperature, conductivity, turbidity, water velocity, etc.) in European rivers vary in a wide range, affecting diversification of aquatic and wetland vegetation. The trophic level (dis-, oligo-, meso-, and eutrophy) that indicates the concentrations of organic and inorganic compounds is an important parameter for differentiation of aquatic and wetland communities.

Due to seasonal floods and periodic disturbance of habitats, wetland plant communities have unstable species composition, and such situation additionally complicates classification of wetland vegetation. Communities of periodically flooded habitats are ephemeral. On the other side, numerous plant communities represent different successional stages with variable structure.

Despite numerous articles on aquatic and wetland vegetation [13–56] and syntaxonomic reviews [57–68], integrated analyses of the Sava River vegetation are missing. In this article we described variability of the aquatic and riparian plant communities along the Sava River and its main tributaries.

Diverse hydrological, climate, and soil conditions along the Sava River (running waters with different turbidity and different water depth, slow streams and standing waters; flat or slightly undulating relief with meanders, by-channels, old river courses, river islets, and ridges) caused significant diversification of vegetation.

Due to regular disturbance by stream power, the river communities rarely reach a climax condition but frequently occur as transient communities and are strongly influenced by prevailing weather conditions [5, 69]. Such situation, and the fact that aquatic and hygrophilous plant species have broadly overlapping distribution, creates objective problems in syntaxonomy of aquatic and wetland vegetation.

Syntaxonomic revision of aquatic and riparian vegetation requires creation of a common, pan-European databank. Ecological studies that involve inventory, monitoring, modeling, and predicting of changes in populations, ecological communities, and ecosystems require both georeferenced databases and computational tools for application of statistical methods. Theoretical basis of uni- and multivariate statistical methods that are used in ecological studies is described in numerous monographs [70–75].

However, implementation of these methods is impossible without powerful computational tools. Rapid development of information technologies resulted with proliferation of software packages [76–88]. These software packages differ significantly with respect to analytic abilities and flexibility in data manipulation (i.e., data editing and data exchange with other data banks). The FLORA package [88] integrates abilities of all existing packages, but also offers some general purpose routines that enable application of both uni- and multivariate analyses in ecological research. The newest version of the package is the culmination of a programming project running continuously since 1999 [74, 89–91]. Authors in Slovenia and Croatia generally use Turboveg database [92]. Investigators in Serbia prefer the FLORA package and BAES database (Biodiversity of Aquatic Ecosystems in Serbia—Ex situ protection http://baes.pmf.kg.ac.rs/), created by the Institute of Biology and Ecology Faculty of Science Kragujevac [93]. These systems are compatible, so they should be integrated in a common databank on wetland flora and vegetation in Southeastern Europe.

In this review we integrated all existing data in order:

1. To describe aquatic and wetland vegetation along the Sava River
2. To identify the environmental factors, which are related to the distribution of different vegetation types
3. To detect threats of rare and endangered plant species and fragile wetland ecosystems along the Sava River

2 Ecological Groups of Hygrophilous Plants

Plant species of aquatic communities (macrophytes) are adapted to specific ecological conditions. The term "macrophytes" is used to denote ecological group of aquatic, amphibian, and hygrophilous plants that dominate wetlands, shallow lakes, and running waters [94–97]. This group of taxonomically different species involves macroscopic algae, liverworts, mosses, ferns, and flowering plants.

Adaptations to similar conditions resulted with convergent evolution of aquatic plants. A process of convergent evolution results with similar physiological and anatomical adaptations of species belonging to different taxa [98, 99]. Flexible stems and leaves; firm attachment by adventitious roots, rhizomes, or stolons; the *aerenchyma* (tissue with large intercellular spaces); vegetative reproduction; and similar inconspicuous inflorescences for water pollination are common characteristics of most macrophytic species.

Threats of existence of aquatic plants are numerous (fast water flow, fluctuating water level, light, hypoxia, critical level of mineral compounds essential for metabolic processes).

Flow is a very powerful selective factor, to which stream macrophytes must be adapted. Reduction of stress-resistant tissues saves material and increases flexibility in running water; it also enables leafstalks to stretch according to changes in water level. Hydrophytes can withstand fast currents and turbulence. This explains why their *sclerenchyma* (protective tissue with thickened walls) is centrally placed rather than in the form of a ring, as it is in terrestrial plants, which bend and run the risk of breaking.

Fluctuating water flow is also a strong selective force in streams. Extreme high water (floods) can mechanically disturb the stream bottom and have disastrous effects on the populations there. The structure of the stream bottom is rapidly altered by gravel and stones rolling downstream, destroying the habitats of the organisms.

Light is one of the most limiting factors for aquatic plants. Proceeding towards the bottom, light varies in frequency and wavelength, because it is absorbed or dispersed by organic molecules, dissolved silt, and phytoplankton. Dense population of macrophytes may reduce the light penetration into the water.

Oxygen is a limiting factor in hypoxic conditions that occur during intense eutrophication process. Hydrophytes generally have well-developed aerial tissues. In rooting species, these tissues grow from the leaves to the roots and are used to carry and store gas, enabling oxygen to spread to the whole plant. The *aerenchyma* (tissue with large intercellular spaces) is also present in helophytes and, when the substrate is submerged, these species also suffer from lack of oxygen. The aerenchyma diminishes the weight of the plants, so that their floating leaves can emerge quickly after being occasionally submerged.

Submerged leaves of macrophytes are divided and elongated. Such morphology increases surface-to-volume ratio, favoring gaseous exchange. Moreover, narrow and elongated submerged leaves are evolved to withstand water currents. Stomata are absent in leaves of submerged plants. Many aquatic plants have stomata on the upper side of floating leaves.

Roots may be absent (as in *Utricularia* and hornwort) or very small. Rootless plants can both exchange gas and obtain the minerals they need through stems and leaves. Lack of mineral compounds essential for metabolic processes (inorganic phosphorus and nitrogen compounds) is a limiting factor for most of plant species. In dystrophy conditions, some plants evolved insectivory adaptations. Aquatic carnivorous plants involve the species *Aldrovanda vesiculosa* L. (*Droseraceae*) and about 50 species of the genus *Utricularia* L. (*Lentibulariaceae*) [100–102]. The majority of these plants usually grow in shallow dystrophic (humic) waters and most of them are considered rare and strongly or critically threatened [101, 103]. *Drosera* (*Drosera rotundifolia* L., *Drosera anglica* Huds., *Drosera intermedia* Hayne) species are also insectivorous plants that inhabit oligotrophic mires and bogs.

In order to pass the winter, hydrophytes produce winter shoots (turions) a few centimeters thick and which survive on the bottom.

Macrophytes play an important role for both invertebrates and fish as habitat and as refuge from predators. Moreover, they strongly affect the physical environment in the water. Water plants suppress water turbulence. By slowing the current, macrophytes can trap sediments and particulate organic matter. Within stands of aquatic vegetation, the light intensity quickly decreases with depth. Not only the light regime but also the temperature in plant stands differs from open water sites.

Metabolic activities of macrophytes control biogeochemical cycles in aquatic ecosystems. Macrophytes are primary producers of organic matter in aquatic ecosystems. Producing oxygen during photosynthetic process, they contribute to the oxygen concentration in the water. Many aquatic plants have aerenchyma, in which photosynthetically produced oxygen is transported by diffusion. In this way the plants transport oxygen to their roots. Subsequently the oxygen is often released in the sediment. On the other hand, aquatic macrophytes may also indirectly cause oxygen depletion. Decay of macrophytes will directly take oxygen from the water. During periods of active growth, macrophytes act as a sink for nutrients (phosphorus and nitrogen).

According to Raunkiaer [104], aquatic and wetland plants belong to *therophytes* (annual species which survive unfavorable conditions in the form of seed), *geophytes* (with underground organs), *hemicryptophytes* (with perennating buds at ground level), *helophytes* (tall marshy grasses), *hydrophytes* (with underwater perennating buds), *chamaephytes* (suffruticose, partially woody), and *phanerophytes* (shrubs and trees).

Macrophytes can be classified according to their growth form and their manner of attachment. Wetzel [105] distinguished two main groups of macrophytes (aquatic macrophytes rooting in sediment and freely floating macrophytes), with three subdivisions, on the basis of their emergence or submergence and the manner of attachment or rooting in the bottom sediment.

Rooting *macrophytes* involve emergent aquatic plants, the floating-leaved plants and submersed plants.

Emergent macrophytes are rooted in the sediment and may grow to relatively shallow water. During the growing season, all members of this group produce aerial leaves and flowers. Reed (*Phragmites communis Trin.*) and many other species (*Typha* spp., *Scirpus lacustris* L., *Acorus calamus* L., *Iris pseudacorus* L., *Butomus umbellatus* L., and *Sagittaria sagittifolia* L.) belong to this ecological group.

The *floating-leaved plants* may root in deep water and have floating leaves or aerial flowers (reproductive organs). Common representatives of this group of plants are *Nymphaea* spp., *Nuphar lutea* (L.) Sibth. & Sm., *Nymphoides peltata*, *Potamogeton natans L.*, *Polygonum hydropiper L.*, etc.

The *submersed macrophytes* complete their life cycle under the water surface. This group of plants includes the stoneworts (*Charophytes*) *Chara* and *Nitella*, a few moss species like *Fontinalis antipyretica* Hedw., and many flowering plants, e.g., *Myriophyllum spicatum* L., *Elodea nuttallii* (Planch.) H. St. John, *Potamogeton pectinatus* L., *Elodea canadensis* Rich, etc.

Freely floating (rootless) macrophytes live unattached to sediments. The life-forms within this group range from macrophytes with floating or aerial leaves and well-developed submersed roots (*Hydrocharis morsus-ranae L.*) to very small surface floating or submersed plants with few or no roots (*Lemna* sp. and the water ferns of the genus *Azolla* Lam.). Some plants in this group have aerial flowers (*Utricularia vulgaris* L.); others complete their life cycle under the water surface (*Ceratophyllum demersum* L.).

3 Syntaxonomy of Aquatic and Wetland Vegetation Along the Sava River

Ecological valorization of a region depends not only on taxonomic but also on ecosystem's diversity. Ecosystems can be grouped and classified in different ways [106]. In general, there are two approaches of ecosystem classifications. *Habitat-oriented approach* groups ecosystems which are similar with respect to environmental conditions (climate, hydrology, geology, soil) within their biotopes. On the other hand, *community-oriented approach* groups ecosystems which are similar with respect to physiognomy or floristic (faunistic) composition of biotic communities.

Habitat-oriented classifications of ecosystems are specified by the Birds Directive (EEC/79/409 directive) and the Habitats Directive (EEC/92/43 directive). More elaborated classification system involves CORINE Biotopes Classification [107, 108], Palaearctic Habitats Classifications [109], and the habitat classification based on European Union Nature Information System (EUNIS), developed by the European Environment Agency's European Topic Centre on Nature Protection and Biodiversity [110]. The PHYSIS database [111] covers Palearctic ecosystems, and it has been used to define NATURA 2000 and EMERALD networks of protected areas.

Biotic-oriented approach of ecosystem classification is based on similarity of biotic components of ecosystems. Vegetation is the most important structural (and functional) part of ecosystems [57, 60, 62, 112]. Therefore, the classification of vegetation corresponds to detailed classification of ecosystems. Rodwell et al. [113] and Lakušić [112] harmonized vegetation syntaxonomy with habitat-oriented classifications. In this article we classified vegetation along the Sava River using syntaxonomic approach.

Due to diverse environmental conditions, the aquatic and wetland vegetation along the Sava River are extremely complex. It may be divided into three distinct zones: (sub)alpine zone, mountainous zone, and lowland (peri-Pannonian floodplain) zone. Within each zone we analyzed riverine, lacustrine, and palustrine communities covering freshwater springs, peat bogs, fens, marshes, swamps, shrub-dominated swamps, freshwater swamp forests, and peat swamp forests.

Lacustrine communities along the Sava River are represented by vegetation of oligotrophic, mesotrophic, and eutrophic lakes and ponds. This vegetation belongs to alliances:

- *Charion fragilis* Krausch 1964 and *Charion vulgaris* (Krause *ex* Krause & Lang 1977) Krause 1981 (vegetation of submerged stonewort swords of oligotrophic and mesotrophic water bodies)
- *Eleocharition acicularis* Pietsch ex Dierssen 1975 (vegetation of amphibious plants in the littoral zone of fluctuating shallow oligotrophic and mesotrophic waters)

- *Potamion pectinati* (W. Koch 1926) Libbert 1931 (vegetation of rooted and floating macrophyte potamogetonid communities in mesotrophic and eutrophic water bodies)
- *Ceratophyllion demersi* Hartog & Segal *ex* H. Passarge 1996 (eutrophic vegetation of submerged macrophytes)
- *Lemnion minoris* O. Bolòs & Masclans 1955 and *Lemnion trisulcae* Hartog & Segal 1964 (free-floating duckweed communities of still, eutrophic waters)
- *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 (eutrophic vegetation of free-floating communities of macrophytes in fairly nutrient-rich waters)
- *Nymphaeion albae* Oberd. 1957 (eutrophic vegetation of floating-leaved rooting macrophytes)
- *Nanocyperion* W. Koch 1926 (pioneer dwarf-cyperaceous vegetation in the littoral zone of mesotrophic and eutrophic waters)
- *Phragmition communis* W. Koch 1926 (reed swamp vegetation of mesotrophic and eutrophic standing freshwater bodies or gently moving streams)
- *Magnocaricion elatae* W. Koch 1926 and *Caricion gracilis* Neuhäusl 1959 (vegetation of tall sedges on borders of eutrophic lakes and ponds).

Oligotrophic phosphate-poor, calcareous (sub)alpine lakes are colonized by populations of *Charophyceae* (*Charetea* Fukarek 1961 ex Krausch 1964). Representatives of genera *Chara* and *Nitella* form dense submerged algal carpets. These species-poor communities of oligotrophic alpine lakes belong to the alliance *Charion fragilis* Krausch 1964. Oligotrophic lakes are mineral poor. Low concentration of dissolved nitrogen and phosphorus in (sub)alpine lakes is a limiting factor for many species. Moreover, due to low content of nitrogen and phosphorus, the primary production of alpine lakes is low. Consequently, the oligotrophic lakes are clear, there is little accumulation of organic matter, and the substrate is often comprised of hard rocks. Lacustrine vegetation of subalpine lakes is also represented by oligomesotrophic submerged communities (alliance *Potamion polygonifolii* Hartog & Segal 1964 of the class *Potametea pectinati* Klika *in* Klika & Novák 1941). Dominant species of oligomesotrophic submerged communities of subalpine lakes are *Chara contraria* Mig., *Chara delicatula* Ag., *Chara aspera* Deth. Ex Wild., *Chara hispida* L., *Chara rudis* Leonh., *Chara baltica* Bruz., *Myriophyllum spicatum* L., *Potamogeton pectinatus* L., *Potamogeton alpinus* Balb., *Potamogeton lucens* L., *Potamogeton perfoliatus* L., *Potamogeton praelongus* Wulfen, *Potamogeton pusillus* L., *Ranunculus circinatus* Sibth., and *Ranunculus trichophyllus* Chaix.

The *littoral zone* of subalpine lakes is colonized by small-sized, hairgrass, amphibious plants in fluctuating shallow oligotrophic and mesotrophic waters (alliance *Eleocharition acicularis* Pietsch ex Dierssen 1975 of the class *Isoëto-Littorelletea* Br.-Bl. & Vlieger in Vlieger 1937). Dominating plants in these communities are small hairgrass amphibious helophytes that belong to genera *Juncus* (*Juncus bufonius* L., *Juncus bulbosus* L., *Juncus capitatus* Weigel., *Juncus effusus* L., *Juncus sphaerocarpus* Nees, *Juncus tenageia* Ehrh.), *Eleocharis*

(*Eleocharis acicularis* (L.) Roem. & Schult., *Eleocharis carniolica* Koch, *Eleocharis ovata* (Roth) Roem. & Schult.), *Cyperus* (*Cyperus flavescens* L., *Cyperus fuscus* L., *Cyperus michelianus* (L.) Link), and *Scirpus* (*Scirpus radicans* Schkuhr).These species form lawns along lacustrian banks.

In the mountainous region, the lacustrine vegetation is developed in numerous temporary, intermittent lakes within the "Karst of Notranjska" (karst in the Ljubljanica River Basin). This vegetation is represented by mesotrophic freshwater pond communities (*Potametea* Klika in Klika & Novák 1941) and the alliance *Charion vulgaris* (Krause *ex* Krause & Lang 1977) Krause 1981 of the class *Charetea* Fukarek 1961 ex Krausch 1964. Moreover, communities of the alliance *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 are distributed in temporary lakes of the "Karst of Notranjska." Mesotrophic water bodies are characterized by a moderate level of nutrients that can support a diverse macrophyte flora but with relatively clear water and limited growth of planktonic or filamentous algae. Mesotrophic waters support the highest diversity of submerged water plants. They also often support nationally threatened, scarce, or declining plant species.

Due to extreme water level fluctuations, the littoral zone of intermittent lakes is not well defined, but it represents a sort of ecotone, with extremely diverse floristic composition. Numerous ecotone communities have species of calcareous fens and swamps. Calcareous fens are represented by communities of the alliance *Caricetalia davallianae* Br.-Bl. 1949 of the class *Scheuchzerio-Caricetea fuscae* (Nordh. 1936) R. Tx. 1937. Communities of swamp helophytes belong to alliances *Phragmition communis* Koch 1926, *Magnocaricion elatae* Koch 1926, and *Caricion gracilis* Neuhäusl 1959 of the class *Phragmito-Magnocaricetea* Klika in Klika et Novak 1941.

Numerous ponds in lowland peri-Pannonian region of the Sava River are colonized by eutrophic communities of submerged, freely floating, and rooted leaf-floating macrophites.

Submerged vegetation belongs to the alliance *Ceratophylletea* Den Hartog & Segal 1964 of the class *Lemnetea* Tüxen ex O. Bolòs & Masclans 1955 and alliance *Potamion pectinati* (W. Koch 1926) Libbert 1931 of the class *Potametea* Klika *in* Klika & Novák 1941.

Freely floating vegetation is represented by alliances *Lemnion minoris* O. Bolòs & Masclans 1955, *Lemnion trisulcae* Hartog & Segal 1964, and *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 of the class *Lemnetea* Tüxen ex O. Bolòs & Masclans 1955. Dominating plants in these communities are *Lemna gibba* L., *Lemna minor* L., and *Lemna trisulca* L.

The eutrophic vegetation of floating-leaved rooting macrophytes belong to the alliance *Nymphaeion albae* Oberd. 1957 (class *Potametea* Klika *in* Klika & Novák 1941). Eutrophic water bodies are characterized by high concentrations of inorganic nitrogen and phosphorus. Such conditions promote algal blooms in some sites during summer. It is important to distinguish between water bodies that are naturally eutrophic and those that have been artificially enriched through agricultural runoff and sewage effluents.

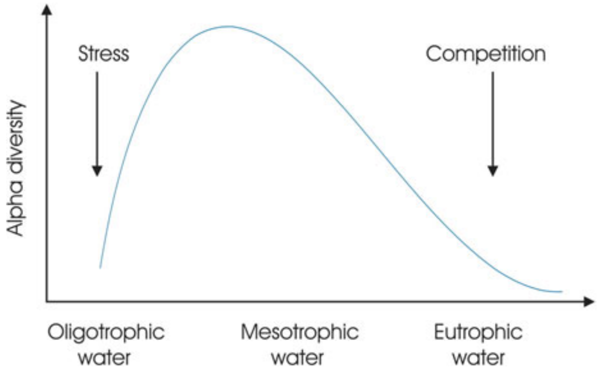

Fig. 1 The relationship between alpha diversity and trophic level of lacustrine communities along the Sava River

The littoral of peri-Pannonian ponds is very complex. Perennially submerged hydrophytes occupy central parts of ponds. Proceeding towards the banks, there is a zone of floating-leaved rooting macrophytes. Next zone is represented by a belt of tall marsh grasses that belong to the alliance *Phragmition communis* W. Koch 1926. This zone is almost exclusively colonized by *Phragmites communis* Trin, *Typha angustifolia* L., or *Typha latifolia* L. The borderline of ponds, where changes in water accumulate mud and silt, is colonized by pioneer ephemeral dwarf-cyperaceous vegetation on muddy, periodically flooded habitats (alliance *Nanocyperion* W. Koch 1926 of the class *Isoëto-Nanojuncetea* Br.-Bl. *et* Tx. 1943). Fragments of tall herbaceous ruderal vegetation (alliances *Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen and *Chenopodion rubri* (Tüxen ex Poli & J. Tüxen 1960) Kopecký) may be present within this zone.

Next belt is represented by vegetation of perennial caespitose or rhizomatous tall sedges on nutrient-rich clayey soils (alliances *Magnocaricion elatae* W. Koch 1926 and *Caricion gracilis* Neuhäusl 1959, of the order *Magnocaricetalia elatae* Pignatti 1954).

Although lacustrine vegetation in peri-Pannonian lowland area is heterogeneous, alpha diversity within particular community may be very low. This is a consequence of intense competitive interactions. Despite favorable condition for intense primary production of many species, the most successful competitors usually suppress other species, forming almost monodominant, low-diversity communities (e.g., *Phragmitetum communis sensu lato*, with dominance of *Phragmites communis* Trin., *Typhetum angustifoliae* (Allrge 1922) Soó 1927, with dominance of *Typha angustifolia* L. or *Nymphaetum albae* (Now. 1930) Tomaš. 1977 with dominance of *Nymphaea alba* L.).

A short syntaxonomic review clearly indicates that the species richness of lacustrine communities along the Sava River is related to the trophic level of lakes or ponds (Fig. 1).

Species richness is relatively low in oligotrophic lakes, since low concentrations of phosphorous and nitrogen (a trophic stress) eliminate all species except “stress tolerators” (mainly representatives of *Characeae*). The species richness increases in mesotrophic waters. On the other side, species richness is also low in eutrophic and dystrophic waters, where most species are excluded by intense competition, leaving only the "competitive dominants" (e.g., *Nymphaea alba* L. in open waters or *Phragmites communis* Trin. and *Typha latifolia* L. in the littoral zone of eutrophic ponds). Grime [114, 115] detected similar pattern of the relationship between species richness and habitat fertility. However, such trend does not indicate that biodiversity of oligotrophic and eutrophic lacustrine communities is low.

Biodiversity (biotic variability) may be classified using different approaches [116–118]. Since species is basic evolutionary and ecological unit, Karadžić and Marinković [74] distinguished intraspecies and interspecies diversity.

Intraspecies diversity is a result of genetic variability (genetic structure of populations), environmental heterogeneity, and genetic-environment interactions. Evolutionary factors such as mutations, migrations, assortative breeding, natural selection, and genetic drift may change genetic structure of populations.

According to Whittaker [119], the *interspecies diversity* (diversity of biotic communities) may be divided into *alpha diversity* (within-community diversity), *beta diversity* (between-communities diversity or diversity along environmental or spatial gradients), and *gamma diversity* (combined alpha and beta diversity within a region).

Alpha diversity depends on species richness (number of species within community) and dominance of species (proportion of individuals of particular species with respect to individuals of all species within community). Dominance of species is frequently referred to as the "species equitability."

Anderson et al. [120] and Vellend [121] distinguish two types of beta diversity: *directional turnover along a gradient* and *nondirectional variation among communities*. Directional turnover represents the change in community structure from one sampling unit to another along a spatial, temporal, or environmental gradient. Nondirectional beta diversity represents a variation among all possible pairs of sampling units, without reference to any particular gradient or direction.

Although alpha diversity of nutrient-poor and eutrophic communities is low, beta diversity in both cases may be very high. For example, in the littoral zone of dystrophic or eutrophic ponds and lakes, beta diversity is high because of presence of numerous communities (e.g., *Phragmitetum sensu lato*, *Typhetum s.l.*, *Glycerietum s.l.*, *Nasturtietum s.l.*, *Phalaradietum s.l.*, etc).

Riverine vegetation along the Sava River is represented by communities of alliances:

- *Ranunculion fluitantis* Neuhäusl 1959 (vegetation of rooted, floating or submerged, and temporary emerged macrophytes of stagnant mesotrophic freshwaters, capable to support periodic (usually autumn) low water table)
- *Ranunculion aquatilis* Passarge 1964 (=*Callitricho-Batrachion* Den Hartog & Segal 1964) (vegetation of crowfoot and milfoil rooted macrophyte communities of shallow-moving freshwaters of Europe)
- *Potamion pectinati* (W. Koch 1926) Libbert 1931 (aquatic vegetation of rooted and floating macrophyte potamogetonid communities in mesotrophic and eutrophic waters)

- *Phragmition communis* W. Koch 1926 (reed swamp vegetation of mesotrophic and eutrophic standing freshwater bodies or gently moving streams)
- *Phalaridion arundinaceae* Kopecký 1961 (reed vegetation along freshwater flowing and seasonally fluctuating streams)
- *Glycerio-Sparganion* Br.-Bl. & Sissingh in de Boer 1942 (helophyte vegetation of tall herbs and grasses along small freshwater streams and in shallow water bodies and ditch banks)
- *Oenanthion aquaticae* Heijný *ex* Neuhäusl 1959 (helophyte vegetation on unstabilized organic substrates of banks)
- *Nanocyperion* W. Koch 1926 (pioneer dwarf-cyperaceous vegetation of temporarily flooded muddy habitats)
- *Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen and *Chenopodion rubri* (Tüxen ex Poli & J. Tüxen 1960) Kopecký (summer-annual, nitrophytic ruderal vegetation of periodically flooded shores)
- *Alnion viridis* Aichinger 1933 (subalpine green alder scrub vegetation on gravel and fertile soils of the Alps and Balkans)
- *Epilobion fleischeri* G. Br.-Bl. & J. Br.-Bl. 1931 (tall herbaceous vegetation of montane-subalpine riverine gravel terraces on scree habitats and pebble alluvia)
- *Adenostylion alliariae* Br.-Bl. 1926 (tall-herb and scrub communities on fertile soils at high altitudes of temperate and mediterranean Europe)
- *Salicion incanae* Aich., 1933 (scrub vegetation of montane-subalpine riverine gravel terraces of the Alps)
- *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993 (willow scrub of stream banks of the montane to subalpine belts of the Alps and Carpathians)
- *Salicion albae* Soó 1930 (willow scrub and woodlands of lowland to submontane river alluvia of temperate Europe)
- *Populion albae* Br.-Bl. 1931 (poplar galleries on alluvia of large rivers)

Due to fast currents and fluctuating water, the *aquatic communities in alpine rivers* (Sava Bohinjka and Sava Dolinka) are sparsely developed. However, the littoral communities along these rivers are very diverse. The littoral zone of (sub) alpine rivers is characterized by an alternation of flooding and drying periods. Such ecological conditions are favorable for the development of pioneer vegetation of tall herbaceous species and shrubby alpine communities.

Pioneer vegetation, with a prevalence of tall herbaceous alpine species, colonizing pebbly and sandy shores, is represented by communities of alliances *Adenostylion alliariae* Br.-Bl. 1926 and *Alnion viridis* Aichinger 1933 (class *Mulgedio-Aconitetea* Hadač & Klika in Klika 1948), and alliance *Epilobion fleischeri* G. Br.-Bl. & J. Br.-Bl. 1931 (class *Thlaspietea rotundifolii* Br.-Bl. 1948). These communities are subject to abrupt, short-lived, heavy floods, and in summer, mainly towards the end of the period, they are subject to drought. Stones, gravel, and coarse-grained sand are redeposited from place to place during flooding. Vegetation overgrows the ridges of gravelly alluvia and protected zones of flooded areas. Pioneer shrubby vegetation of the (sub)alpine region belongs to alliances *Salicion incanae* Aich., 1933 and *Salicion eleagno-daphnoidis* (Moor 1958) Grass

1993 of the class *Salicetea purpureae* Moor 1958 [122, 123]. These communities are exposed to abrupt, short-lived, heavy floods, and summer droughts. Communities in this region have numerous species of chasmophyte (*Asplenietea trichomanis* (Braun-Blanq. *in* H. Meier & Braun-Blanq. 1934) Oberd. 1977) and scree (*Thlaspietea rotundifolii* Br.-Bl. 1948) vegetation.

Within the montane zone, the Sava River and its tributaries (Ljubljanica and Krka rivers) have relatively slow flow velocity. Such non-torrential situation is favorable for the development of mesotrophic communities of flowing waters (alliances *Ranunculion fluitantis* Neuhäusl 1959 and *Potamion polygonifolii* Hartog & Segal 1964, within the class *Potametea* Klika in Klika & Novák 1941). Dominating plants in these communities are *Ranunculus trichophyllus* Chaix, *Ranunculus fluitans* Lam. = *Batrachium fluitans* Wimm., *Ranunculus aquatilis* L. = *Batrachium aquatile* (L.) Dum., *Myriophyllum* spp., *Callitriche* spp., *Sium erectum* Huds., *Zannichellia palustris* L., *Potamogeton acutifolius* Link ex Roem. & Schult., *Potamogeton berchtoldii* Fieber, *Potamogeton crispus* L., *Potamogeton filiformis* Pers., *Potamogeton lucens* L., *Potamogeton nodosus* Poir., *Potamogeton obtusifolius* Mert. & Koch, *Potamogeton pectinatus* L., *Potamogeton perfoliatus* L., *Potamogeton praelongus* Wulfen., *Potamogeton pusillus* L., *Potamogeton trichoides* Cham. & Schltdl., *Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L., and *Najas marina* L.

The littoral zone of the mountainous sector of the Sava River, and its tributaries is represented by ephemeral vegetation of periodically inundated shores. Pioneer willow communities on boreo-alpine stream gravel habitats (alliance *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993 of the class *Salicetea purpureae* Moor 1958) are developed along the upper part of the Sava River. Communities of the alliance *Salicion albae* Soó 1930 are developed within the confluence area of the Krka River. Ephemeral, herbaceous vegetations on shores of rivers (oligotrophic communities of the class *Mulgedio-Aconitetea* Hadač & Klika and Klika & Hadač 1944 and mesotrophic communities of alliance *Phalaridion arundinaceae* Kopecký 1961 within the class *Phragmitetea* Tüxen & Preising 1942) are also developed on shores.

The aquatic vegetation of the Sava River and its tributaries within the peri-Pannonian lowland region is represented by meso-eutrophic submerged and freely floating vegetation. Submerged vegetation involves communities of the alliance *Ranunculion fluitantis* Neuhäusl 1959 within the class *Potametea* Klika in Klika & Novák 1941 and communities of the alliance *Potamion pectinati* (W. Koch 1926) Libbert 1931 (class *Potametea* Klika *in* Klika & Novák 1941). In slow-flowing channel communities, aquatic vegetation is represented by submerged vegetation (alliance *Ceratophylletea* Den Hartog & Segal 1964 of the class *Lemnetea* Tüxen ex O. Bolòs & Masclans 1955) and freely floating vegetation (alliances *Lemnion minoris* O. Bolòs & Masclans 1955, *Lemnion trisulcae* Hartog & Segal 1964 and *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 of the class *Lemnetea* Tüxen ex O. Bolòs & Masclans 1955).

The *littoral zone* of the Sava River within the peri-Pannonian region is represented by communities of dwarf-cyperaceous vegetation of temporarily flooded muddy habitats (*Isoëto-Nanojuncetea* Br.-Bl. *et* Tx. 1943 and alliances of ruderal communities on muddy shores *Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen and *Chenopodion rubri* (Tüxen ex Poli & J. Tüxen 1960) Kopecký of the class *Bidentetea tripartitae* Tüxen, Lohmeyer & Preising ex von Rochow).

Communities of these alliances colonize riverbanks with annual pioneer nitrophilous species such as *Bidens tripartita* L., *Bidens frondosa* L., *Bidens cernua* L., *Bidens connata* Mühlenb. *ex* Willd., *Chenopodium rubrum* L., *Chenopodium album* L., *Chenopodium ficifolium* Sm., *Chenopodium glaucum* L., *Chenopodium opulifolium* Schrad. ex Koch & Ziz, *Chenopodium polyspermum* L., *Atriplex patula* L., *Atriplex prostrata* Bouch. *ex* DC., *Barbarea vulgaris* R. Br., *Brassica nigra* (L.) Koch, *Calystegia sepium* (L.) R. Br., *Amaranthus blitoides* S. Watson, *Amaranthus retroflexus* L., *Agrostis stolonifera* L., *Echinochloa crus-galli* (L.) P. Beauv., *Epilobium hirsutum* L., *Epilobium roseum* Schreb., *Erigeron annuus* (L.) Pers., *Galium aparine* L., *Galium palustre* L., *Mentha aquatica* L., *Mentha longifolia* (L.) Huds., *Microrrhinum minus* (L.) Fourr., *Myosotis scorpioides* L., *Myosoton aquaticum* (L.) Moench, *Oenanthe aquatica* (L.) Poir., *Polygonum aviculare* L., *Polygonum hydropiper* L., *Polygonum lapathifolium* L., *Polygonum minus* Huds., *Polygonum mite* Schrank, *Polygonum persicaria* L., *Rorippa amphibia* (L.) Besser, *Rorippa palustris* (L.) Besser em. Jons., *Rorippa sylvestris* (L.) Besser, *Rumex crispus* L., *Rumex palustris* Sm., *Rumex stenophyllus* Ledeb., *Solanum dulcamara* L. *Leersia oryzoides* (L.) Sw., *Lycopus europaeus* L., *Lythrum salicaria* L., *Veronica anagallis-aquatica* L., *Veronica beccabunga* L., *Xanthium strumarium* L., etc. During the spring and at the beginning of the summer, sites look like muddy banks without any vegetation (develops later in the year). If the conditions are not favorable, this vegetation has a weak development or could be completely absent.

In slow-flowing channels, the littoral zone is represented by communities belonging to the alliance *Glycerio-Sparganion* Br.-Bl. & Sissingh in de Boer 1942 of the order *Nasturtio officinalis-Glycerietalia fluitantis* Pignatti 1953.

Besides herbaceous vegetation, the littoral zone of the peri-Pannonian lowland part of the Sava River is represented by riparian gallery forests (*Salicion albae* Soó 1930, *Populetalia albae* Braun-Blanq. *ex* Tchou 1948).

Sylvicultures of both allochtonous and autochtonous poplars and willows occupy large riverine zone along the Sava River [124–128].

Diverse ***palustrine vegetation*** along the Sava River is represented by communities around springs, calcareous fens, transitional mires, communities developed in peat depressions, peat-forming ombrotrophic raised bogs, swamps, swamp forests, swamp forest edges, and wet meadows. These communities belong to alliances:

- *Cratoneuron commutati* Koch 1928 (moss-rich vegetation of calcareous springs in supramontane and subalpine belts of Europe)
- *Caricion davallianae* Br.-Bl. 1949 (small-sedge fen vegetation on calcareous peaty soils and oligomesotrophic shallow water)
- *Caricion lasiocarpa* Vanden Berghen in Lebrun et al. 1949 (small-sedge mires developing on oligotrophic and oligomesotrophic peats)

- *Caricion canescenti-nigrae* Nordhagen 1937 (fen meadows with dominating sedges and forbs on noncalcareous peats or peaty mineral soils of temperate Europe and high altitudes of the Mediterranean)
- *Rhynchosporion albae* Koch 1926 (vegetation of stagnant, acid, dystrophic waters in pools of Sphagnum bogs on deep peats)
- *Sphagnion medii* Kästner & Flößner 1933 and *Sphagnion magellanici* Pawl 1928 emend Moore 1968 (bogs of subcontinental and montane regions from the mountain belt spanning the mediterranean and boreal regions of Eurasia)
- *Bolboschoenetalia maritimi* Hajny 1967 (graminoid and sedge vegetation of brackish waters and soils in the Pannonian region)
- *Alno-Quercion roboris* (Balkan and Apenine ash-alder forests on temporary flooded plains)
- *Alnion glutinosae* Malcuit 1929 (alder and willow woodlands of swamps, fens, and wet pastures)
- *Calthion palustris* Tüxen 1937 (permanently wet meadows of tall herbaceous plants on fertile mineral soils of temperate Europe)
- *Molinion caeruleae* W. Koch 1926 (hayed or grazed wet meadows at low altitudes on unfertilized, nutrient-poor soil, dominated by *Molinia caerulea* (L.) Moench, colonizing more or less moist, clayey/silty or peaty soils, both on siliceous and carbonatic substrata)
- *Juncion acutiflori* Braun-Blanq. *in* Braun-Blanq. & Tüxen 1952 (meadows and pastures of moist peaty mineral soils with flushing or impeded drainage)
- *Alopecurion pratensis* H. Passarge 1964 (perennial, mesophilous, regularly mowed and sometimes even grazed, non-intensively fertilized, species-rich meadows and pastures, dominated by graminoids, in floodplains of large rivers in central and eastern Europe)
- *Cnidion dubii* Bal.-Tul. 1966 (meadows in large lowland river floodplains that are characterized by an alternation of flooding and summer-drying periods)
- *Deschampsion caespitosae* Horvatić, 1958 (floodplain alluvial meadows of subcontinental regions of Europe)
- *Convolvulion sepium* Tüxen *in* Oberd. 1957 (seminatural tall-herb riparian vegetation on banks of rivers and other water bodies)
- *Filipendulion ulmariae* Segal 1966 (tall-herb riparian vegetation)
- *Petasites officinalis* Sill. 1933 (tall-herb vegetation of raw alluvium soils on montane streamsides)
- *Senecio fluviatilis* Tüxen 1967 (communities of nitrophiles tall herbs and ferns around eutrophic lakes and ditches)
- *Potentillion anserinae* Tx. 1947 of order *Potentillo anserinae-Polygonetalia avicularis* Tüxen 1947 (low herb communities of variable habitats with wet–dry or brackish–fresh habitat conditions)
- *Agropyro-Rumicion crispi* Nordh. 1940 (pioneer vegetation of coastal gravel, boulders, or rocky cliffs, enriched with organic detritus)

Communities of mountain and subalpine springs and wet rocks (*Montio-Cardaminetea* Br.-Bl. Et Tx. 1943) colonize fast-flowing cold brooks, rills, and springs. These communities are divided into orders *Montio-Cardaminetalia* Pawłowski et al. 1928 (vegetation of cold, oligotrophic water springs of the nemoral and boreal zones and of oro-mediterranean mountain belt of Europe) and *Cardamino-Chrysosplenietalia* Hinterlang 1992 (vegetation of soft-water springs in shady forest habitats in the submontane and montane belts of Central European mountains). Alliance *Cratoneuron commutatum* Koch 1928 of order *Montio-Cardaminetalia* Pawłowski et al. 1928 involves moss, tuffa-forming communities on calcareous substrate. These communities are developed in the Julian Alps.

Calcareous fens are represented by communities of the alliance *Caricetalia davallianae* Br.-Bl. 1949 of the class *Scheuchzerio-Caricetea fuscae* (Nordh. 1936) R. Tx. 1937. Alkaline fens are dominated by small-sized sedges and other *Cyperaceae*, growing on permanently flooded peat soils, with base-rich water. Small-sized sedges, other *Cyperaceae*, and brown moss species dominate in calcareous peaty soils and shallow fens. Dominant *Cyperaceae* of these communities are *Schoenus nigricans* L., *Schoenus ferrugineus* L., *Eriophorum latifolium* Hoppe, *Carex davalliana* Sm., *Carex flava* L., *Carex lepidocarpa* Tausch, *Carex hostiana* DC., C*arex panicea* L., *Scirpus cespitosus* L., etc. Prominent "brown moss" carpet is formed by *Campylium stellatum* (Hedw.) C. Jens.; *Drepanocladus intermedius* (Lindb.) Warnst.; *Drepanocladus revolvens* (Sw.) Warnst.; *Cratoneuron commutatum* (Hedw.) Roth.; *Acrocladium cuspidatum* (Hedw.) Lindb.; *Ctenidium molluscum* (Hedw.) Mitt.; *Fissidens adianthoides* Hedw.; *Bryum pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey., & Scherb.; and other species.

Transitional mires on mesotrophic and oligomesotrophic peats and peaty mineral soils are represented by communities of the alliance *Caricetalia fuscae* Koch 1926 em. Br.-Bl. 1949 (class *Scheuchzerio-Caricetea fuscae* (Nordh. 1936) R. Tx. 1937). These ombrotrophic/minerotrophic peats receive nutrients by both rain and groundwater. Acidophilous small-sized sedges (*Carex lasiocarpa* Ehrh., *Carex limosa* L., *Carex rostrata* Stokes) are dominant in these communities. Other species that frequently occur in these communities are *Drosera anglica* Huds., *Drosera rotundifolia* L., *Menyanthes trifoliata* L., *Oxycoccus palustris* Pers., *Potentilla palustris* (L.) Scop., *Pseudocalliergon trifarium* (Weber & D. Mohr) Loeske, *Scorpidium scorpioides* (Hedw.) Limpr., *Juncus articulatus L.*, *Trichophorum cespitosum* (L.) Hartm., *Pedicularis palustris* L., etc.

Vegetation developed in stagnant, dystrophic waters in pools of *Sphagnum bogs* on deep peats or sandy bare substrata with oligotrophic waters (alliance *Rhynchosporion albae* Koch 1926 of order *Scheuchzerietalia palustris* Nordhagen 1936 and class *Scheuchzerio-Caricetea fuscae* (Nordh. 1936) R. Tx. 1937) is sporadically developed. Dominant species in these species-poor communities are *Rhynchospora alba* (L.) Vahl, *Rhynchospora fusca* (L.) W. T. Aiton, *Drosera intermedia* Hayne, *Drosera rotundifolia* L., *Carex limosa* L, *Gymnocolea inflata*, *Lycopodiella inundata* (L.) Holub, *Menyanthes trifoliata* L., *Pseudocalliergon trifarium* (Web. & Mohr) Loeske, *Scheuchzeria palustris* L., *Scorpidium cossonii* (Schimp.) Hedenäs, *Sphagnum* spp., etc.

Peat-forming, ombrotrophic (mainly fed by rainwater), *raised bogs* are represented by the vegetation order *Sphagnetalia magellanici* (Pawlowski 1928)

Moore (1964) 1968 of the class *Oxycocco-Sphagnetea* Br.-Bl. & Tx. 1943. Dominating plants in these communities are representatives of genus *Sphagnum* (*Sphagnum capillifolium* (Ehrh.) Hedw., *Sphagnum cuspidatum* Ehrh. ex Hoffm., *Sphagnum fallax* Klingg., *Sphagnum flexuosum* Dozy & Molk., *Sphagnum centrale* C. Jen., *Sphagnum fuscum* (Schimp.) H. Klinggr, *Sphagnum girgensohnii* Russ., *Sphagnum magelanicum* Brid., *Sphagnum palustre* L., *Sphagnum nemoreum* Scop., *Sphagnum papillosum* Lindb., *Sphagnum rubellum* Wilson, *Sphagnum tenellum* Ehrh ex Hoffin., *Sphagnum angustifolium* (C. Jens. ex Russ.) C. Jens., *Sphagnum flexuosum* Dozy & Molk.), other mosses (*Polytrichum strictum* Hedw., *Mylia anomala* (Hooker) Gray, etc.) and vascular plants *Andromeda polifolia* L., *Carex limosa* L., *Carex pauciflora* Lightf., *Drosera rotundifolia* L., *Eriophorum vaginatum* L., *Lycopodiella inundata* (L.) Holub, *Oxycoccus microcarpus* Turcz. ex Rupr., *Oxycoccus palustris* Pers., *Rhynchospora alba* (L.) Vahl, *Scheuchzeria palustris* L., *Trichophorum cespitosum* (L.) Hartm., *Vaccinium uliginosum* L., *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., *Scheuchzeria palustris* L., *Trichophorum cespitosum* (L.) Hartm., *Carex nigra* (L.) Reichard, and *Carex panicea* L.

Swamp forests are developed mainly within the lowland region of the Sava River. Alder and willow woodlands of swamps, fens, and wet pastures belong to alliances *Alnion glutinosae* Malcuit 1929 and *Salix cinerea* Th. Müll. *et* Görs 1958 of the class *Alnetea glutinosae* Braun-Blanq. & Tüxen 1943.

Temporary flooded forests on nutrient-rich alluvial soil are included in alliance *Alno-Quercion roboris* of the class *Populetea albae* Br.-Bl. 1962.

Edges of hygrophylous forests are represented by communities of the alliances *Filipendulion ulmariae* Segal 1966, *Petasites officinalis* Sill. 1933, *Senecio fluviatilis* Tüxen 1967, and *Convolvulion sepium* Tüxen *in* Oberd. 1957. Light is the main limiting factor in humid, nutrient-rich, open habitats. Due to strong competition for light, the lianas such as *Echinocystis lobata* (Michx.) Torr. & A. Gray., *Clematis vitalba* L., *Solanum dulcamara* L., *Cuscuta europaea* L., *Humulus lupulus* L., *Vitis vinifera* L. subsp. *sylvestris* (C. C. Gmelin) Hegi, etc. are frequent in these communities. Other species that occur in these communities are *Filipendulion ulmariae* (L.) Max.; *Hypericum tetrapterum* Fries; *Mentha longifolia* (L.) Huds.; *Thalictrum flavum* L.; *Geranium palustre* L. (in communities of the *Filipendulion* alliance); *Barbarea stricta* Andrz.; *Eupatorium cannabinum* L.; *Fallopia dumetorum* (L.) Holub; *Senecio fluviatilis* Wallr. (in *Senecio fluviatilis* communities); *Petasites hybridus* (L.) P. Gaertn., C. A. Mey., and Scherb; and *Cirsium erysiphales* (Jacq.) Scop. (in *Petasites officinalis* communities).

Halophytic vegetation of herbaceous plants is represented by communities of alliance *Bolboschoenetalia maritimi* Hajny 1967. These communities are highly influenced by a Pannonic climate with extreme temperatures and aridity in summer. The enrichment of salt in the soil is due to high evaporation of groundwater during summer. These habitat types are partly of natural origin and partly under a distinct influence of cattle grazing.

Wet anthropogenic meadows and pastures are extremely heterogeneous. These pan-European communities belong to the class *Molinio-Arrhenatheretea* Tüxen 1937 and include secondary mesic and wet grasslands on nutrient-rich soils. They have developed due to regular mowing or grazing on sites of deciduous, mixed, or coniferous forests.

There is little consensus over the classification of lowland wet meadows. They occur across a broader geographic gradient from the suboceanic to subcontinental areas within Central and Western Europe. Wet meadows and pastures are assigned to several vegetation alliances, but the conceptual basis of these alliances varies between countries and authors [129, 130]. These communities represent unstable successional stages. Moreover, communities that belong to different syntaxa have a high proportion of common species. Overlapping species distribution prevents unambiguous delimination of different syntaxonomic units. Such situation requires a revision of syntaxonomic relations of wet meadows and pastures, at pan-European level.

These communities represent different successional stages that are primarily affected by frequency and intensity of mowing and/or grazing. Besides biotic factors, the wet meadows and pastures are affected by climate, water regime of habitats (frequency and intensity of flooding, groundwater level), and soil conditions (exchangeable basic cations contents, fertilization level, salinization level, sediment deposition, soil texture, etc.). Soil texture refers to the proportion of minerals of varying sizes that comprise the solid fraction of the soil. Gravel and coarse sand correspond to the parent material with particle size greater than 2 mm. The smaller particles are denoted as fine sand, silt, and clay. Physical properties of soil such as water retention capacity, aeration, and water permeability depend on the soil texture. Physical properties of soils in wet meadows and pastures are usually degraded by permanent trampling and treading.

Depending on duration of flooding period, groundwater level, and soil properties, these communities may be divided into alliances *Calthion palustris* Tüxen 1937, *Cnidion dubii* Bal.-Tul. 1966, *Molinion caeruleae* W. Koch 1926, *Alopecurion pratensis* H. Passarge 1964, *Potentillion anserinae* Tüxen 1947, and *Deschampsion caespitosae* Horvatić, 1958.

Mesotrophic *Calthion* meadows are dominated by tall broad-leaved herbs, while the percentage of grasses and sedges is low. These communities occupy the alluvia of small streams and near springs, where the soil is moist even during dry summer months and is usually well supplied with nutrients. They require regular management by mowing. In the abandoned stands, these communities are replaced by the communities of the *Filipendulion ulmariae* Segal 1966 alliance.

Oligotrophic moist *Molinion* meadows are usually dominated by *Molinia arundinacea* or *Molinia caerulea* and thrive on peaty, nutrient-poor, and acidic soils, often containing a high proportion of organic matter. They are located in shallow meadow depressions and at margins of river arms and may occur in mosaic with *Cnidion*, *Magnocaricion*, and *Phragmition* communities.

Cnidion meadows are regularly flooded for a few weeks every spring but in summer the water table can often drop to approximately 1 m below ground level

[131]. As a result of decreasing water table, these communities are dry in summer. Due to summer drought, these communities have a high number of xerophytic species with continental distribution. Periodic floods bring nutrients, sand, and mud, so these communities are developed on nutrient-rich, relatively light soil. *Cnidion* meadows are regularly flooded but, unlike the *Calthion* meadows, they dry out in summer due to the dry continental climate. They also differ from the *Molinion* meadows in that they develop on mineral soils with a good supply of nutrients.

The *Deschampsiom cespitosae* meadows are ecologically (and floristically) similar to *Cnidion* meadows. Therefore, some authors assume that the alliance *Deschampsia cespitosa* is a synonym for alliance *Cnidion dubii* [132]. Periodic alternation of spring floods and summer drought is common to both alliances. The *Deschampsia cespitosa* meadows are induded in spring due to excessive precipitation rather than riverine floods [133]. After the retreat of spring floods, water table usually decreases several meters below the ground surface. The alternation between two extremes—pronounced wetting and drying of the soil profile, as well as low permeability for water—are essential ecological factors influencing the thriving of *Deschampsia* communities [16, 134, 135]. Highly productive *Deschampsia* meadows can be usually mown more times a year.

Despite similar ecology, communities of alliances *Deschampsia cespitosa* and *Cnidion dubii* are floristically different. The group of Illyrian (karst) *Deschampsia* meadows is different from middle European meadows by the presence of some plant species with Illyric-Dinaric distribution which does not appear in Central Europe, e.g., *Gladiolus illyricus* Koch, *Peucedanum coriaceum* subsp. *Pospichalii* (Thell.) Horvatić, *Iris errirhiza* Pospich, *Scilla litardierei* Breistr., and *Succisella inflexa* (Kluk) G. Beck. *Cnidion* communities have a higher percent of continental and haloxeric species with Pannonian distribution.

Floristic differences are caused by the continentality gradient. Increased continentality (from maritime submediteranean region to Pannonian and east-European regions) has profound effect on ecosystems. For example, the periodic flood-drought alternation cycles are more extreme in continental than in submaritime climates. Extreme alternation of wet–dry seasons is favorable for salinization process (i.e., the process of salt enrichment in soil). Consequently, the soil salinity increases from submaritime to continental regions (from oligohaline *Deschampsia cespitosa* communities over more continental *Cnidion dubii* communities, mesohaline *Bolboschoenetalia maritimi* Hajny 1967 communities to euhaline *Salicornia* communities). As *Deschampsia cespitosa* is a eurytopic, widespread species, it is not a good synataxonomic indicator [133]. Further investigation of ecology and syntaxonomic relation between alliances *Deschampsia cespitosa* and *Cnidion dubii* should be performed at pan-European level.

Lowland hay meadows (*Alopecurion pratensis* H. Passarge 1964) are species-rich communities, occurring on lightly to moderately fertilized soils of the plain to submontane levels. These communities are periodically flooded. However, in contrast to *Cnidion* or *Deschampsia* meadows, the soils of *Alopecurion* meadows do not dry out for longer periods and most of continental species are missing.

The alliance *Potentillion anserinae* Tx. 1947 includes the nitrophilous ruderal vegetation of short to medium-tall plant communities. These species-poor communities form dense mats of treading (trampling)-resistant and grazing-tolerant hemicryptophytes (*Agrostis stolonifera* L., *Alopecurus geniculatus* L., *Potentilla anserina* L., or *Potentilla reptans* L.). Floods regularly affect stands located in alluvial rivers. These communities are traditionally grazed by poultry (mainly geese) and other domestic herbivores. The water and animal influences lead to soil compression and nitrification. The natural and anthropic stands of the association are distributed along rivers, on banks of water bodies, in depressions located in alluvial river, gravel pits, etc.

Natural ecosystems along the Sava River area are increasingly endangered due to changes in land use. Numerous synanthropic communities are developed on fertile alluvial area of the Sava River [126, 136–139].

3.1 Alpine Region

Sava Dolinka and Sava Bohinjka collect the water from alpine belts of the Julian Alps and the Karavanke Mountains. Vegetation of these two draining regions is diverse. Forest vegetation is represented by broad-leaved beech forests, mixed fir-beech forests, and coniferous forests [140–145]. The alpine shrub vegetation above the upper tree line is represented by dwarf pine (*Pinus mugo* Turra) and other highland vegetation types within the classes *Asplenietea trichomanes* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdörfer 1977, *Thlaspietea rotundifolii Br.-Bl.* 1948, *Elyno-Seslerietea* Br.-Bl. 1948, *Loiseleurio-Vaccinietea* Eggler 1952, *Mulgedio-Aconitetea* Hadač & Klika and Klika & Hadač 1944 [146–149].

The high-mountain lakes involves seven lakes (Lake Jezero pod Vršacem, Lake Rjava Mlaka, Lake Zelena Mlaka, Lake Jezero v Ledvicah, Lake upper Dvojno Jezero, Lake lower Dvojno Jezero, and Lake Črno Jezero). The highest lake, Rjavo Jezero, is located 2,002 m a.s.l. The lowest lake, Črno Jezero, is located at 1,319 m a.s.l. The Valley of Seven Triglav Lakes (Dolina Sedmerih Triglavskih Jezer) is characterized by a transverse (E–W) profile, a very steep eastern slope, a relatively flat valley, and a relatively gentle western slope.

Blaženčić et al. [150] described aquatic vegetation of the glacial lakes within the Triglav National Park. The dominant aquatic species in these lakes are *Chara delictuala* A. N. Desvaux., *Chara aspera* C. L. Willdenow., *Chara rudis* (A. Braun) H. von Leonhardi., *Chara contraria* A. Braun *ex* Kützin., *Cinclidotus fontinaloides* (Hedw.) P. Beauv., *Platyhypnidium riparioides* (Hedw.) Dix., *Batrachium trichophyllum* (Chaix) Bosc., *Myriophyllum spicatum* L., *Potamogeton alpinus* Balbis., *Potamogeton crispus* L., *Potamogeton lucens* L., *Potamogeton perfoliatus* L., *Potamogeton praelongus* Wulfen, and *Potamogeton pusillus* L.

The Savica River receives most of its water from the high mountain from the Valley of Seven Triglav Lakes. Water emerging from a water-filled gallery, in the form of a waterfall of the Savica River, sinks about 500 m from higher up on the plateau, where the Valley of Seven Triglav Lakes is located. Through vertical underground channels, water is drained into horizontal channels and finally appears in the form of a waterfall in the middle of a vertical cliff.

The Savica River is the main affluent of Lake Bohinjsko Jezero. The lake is situated in Triglav National Park, in the northwestern part of Slovenia, within the Julian Alps biosphere. Lake Bohinj is located at the end of a long valley, formed by a glacier. The catchment area of the lake includes the highland karst area. Mostnica, a short river that flows from Lake Bohinjsko Jezero and numerous springs from neighboring mountains, forms the Sava Dolinka River. Urbanc-Berčič [52, 53] described floristic change of aquatic vegetation in the Bohinj Lake. Central parts of the lake are covered by dense mats of *Charophytes* (*Chara delicatula* A .N. Desvaux., *Chara aspera* Deth. *ex* Willd., *Chara rudis* (A. Braun) H. von Leonhardi., *Chara contrana* A. Br.) and other submerged plants (*Myriophyllum spicatum* L., *Potamogeton alpinus* Balbis, *Potamogeton crispus* L., *Potamogeton lucens* L., *Potamogeton perfoliatus* L., *Potamogeton pusillus* L., *Batrachium circinatum* (Sibth.) Spach). In the littoral zone of the lake dominate helophytes *Phragmites australis (Cav.)* Trin. *ex* Steud., *Carex flava* L., *Lythrum salicaria* L., *Filipendulion ulmariae* (L.) Maxim., etc.

Lake Bled is smaller and located in the middle of an urban surrounding. Contrary to Bohinj lake, intensive eutrophication has been an outstanding process in this century, accompanied occasionally with algal bloom Urbanc-Berčič [52]. Among submerged vegetation dominate *Myriophyllum spicatum* L., *Batrachium trichophyllum* (Chaix) F. Schultz, *Potamogeton perfoliatus* L., *Scirpus lacustris f. fluitatis* (L.) Palla, and *Chara sp.* Vegetation of floating plants is represented by communities of *Nuphar luteum* (L.) Sibth. et Sm. and *Nymphaea alba* L. Diverse emergent helophytes (*Alisma plantago-Aquatica* L., *Acorus calamus* L., *Carex riparia* Curt., *Eleocharis* sp., *Iris pseudacorus* L., *Menyanthes trifoliata* L., *Mentha aquatica* L., *Lycopus europaeus* L., *Phragmites australis* (Cav.) Trin. *ex* Steud., *Solanum dulcamara* L., *Sparganium emersum* Rehmann) occupy the littoral zone.

The glacier of Bohinj carried the huge amounts of a material at first, which ground and deepened the valley of the *Sava Bohinjka River*.

The *Sava Dolinka* rises at the Zelenci Pools near Kranjska Gora, in a valley separating the Julian Alps from Karavanke range. The spring is located at 833 m above sea level, in area of drainage divide between Adriatic and Danube basins. The source of the Sava Dolinka River belongs to the wetland at the foot of alpine slopes, in the alluvial and glacial deposits, surrounded by wetland vegetation. The source area is located within the national reserve Zelenci. Draining region of Sava Dolinka is extremely diverse.

Alpine rivers Sava Bohinjka and Sava Dolinka are characterized by an almost total lack of macrophytes. Sparse distribution (or complete absence) of macrophytes in alpine rivers may be explained by strong erosive power of fast mountain stream that prevents establishment and persistence of aquatic plants. Rock debris carried down the rivers after snow melting and storm flows obstruct formation of macrophyte communities.

Moreover, extreme seasonal fluctuations in water level, due to periodic autumn drying and high flows in spring and summer, are unfavorable for development of macrophyte vegetation.

Despite lack of macrophytes, littoral vegetation of these two rivers is diverse and represented by rich mixture of forest/meadow flora. Forest vegetation is represented by coniferous and deciduous forest communities [144]. Coniferous forests involve acidophilous spruce-fir communities (*Piceetalia excelsae* Pawłowski in Pawłowski et al. 1928), pine forests of nutrient-poor and hydromorphic, sandy soils (*Pinetalia sylvestris* Oberd. 1957), and montane calcareous relict pine forests of the Balkans, Apennines, Alps, and Carpathians (*Erico-Pinetalia* Horvat 1959). Deciduous forests involve acidophilous beech forests on nutrient-poor soils (*Luzulo-Fagetum* Scamoni & Passarge 1959) and beach forests of nutrient-rich soils (*Fagetalia sylvaticae* Walas 1933). Forest elements are mixed with other vegetation types including wet meadows (*Calthion palustris* Tüxen 1937), calcareous fens (*Caricion davallianae* Br.-Bl. 1949), mires (*Caricion lasiocarpa* Vanden Berghen in Lebrun et al. 1949), chasmophytic vegetation (*Asplenietea trichomanis* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberd. 1977), and scree vegetation (*Thlaspietea rotundifoliae* Br.-Bl. 1948). Syntaxonomic status of such mixtures is not so clear. Moreover, littoral communities along these alpine rivers are subjected to permanent change due to strong erosive power of fast mountain streams. Littoral with undeveloped soil on deposed boulders, gravel, and pebbles colonize either ephemeral herbaceous communities (alliances *Epilobium fleischeri* G. Br.-Bl. & J. Br.-Bl. 1931 and *Adenostylion alliariae* Br.-Bl. 1926) or pioneer shrubby vegetation of alpine hygrophilous shrub communities (alliances *Salicion incanae* Aich., 1933 and *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993). Dominant scrub species in these communities are with *Salix elaeagnos* Scop., *Salix purpurea* L., *Hippophae rhamnoides* L., *Myricaria germanica* Desv., etc. [122, 123, 151, 152].

Palustrine communities that are connected with Sava Bohinjka and Sava Dolinjka rivers are diverse vegetation of bogs, mires, calcareous fens, and springs.

Limnogenic raised bogs on Pokljuka, Jelovica, and Olševa are developed on previous alpine lakes [149]. These actively peat-forming, ombrotrophic raised bogs belong to the alliance *Sphagnion magellanici* Pawl 1928 emend Moore 1968, within the class *Oxycocco-Sphagnetea* Br.-Bl. *et* Tx. 1943. The most important alpine-boreal species within the bogs are *Drepanocladus vernicosus* (Mitt.) Warnst., *Andromeda polifolia* L., *Drosera anglica* Hunds., *Drosera rotundifolia* L., *Oxycoccus palustris* Pers., *Carex pauciflora* Lightf., *Eriophorum vaginatum* L., *Scheuchzeria palustris* L., *Trichophorum cespitosum* (L.), and numerous *Sphagnum* species (*Sphagnum capillifolium* (Ehrh.) Hedw., *Sphagnum cuspidatum* Ehrh. *ex* Hoffm., *Sphagnum fallax* Klingg., *Sphagnum flexuosum* Dozy & Molk., *Sphagnum centrale* C. Jen., *Sphagnum fuscum* (Schimp.) Klinggr., *Sphagnum girgensohnii* Russ., *Sphagnum magelanicum* Brid., *Sphagnum palustre* L., *Sphagnum nemoreum* Scop., *Sphagnum papillosum* Lindb., *Sphagnum rubellum* Wilson., *Sphagnum russowii* Warnst., *Sphagnum tenellum* (Brid.) Bory).

Because of specific morpho-anatomical adaptations, *Sphagnum* mosses have an extraordinary ability to retain a huge amount of water. The class *Sphagnopsida*

Ochyra is distinguished from other members of the *Bryophyta* (*sensu stricto*) by its leaves possessing two types of cells—*photosynthetic cells* with chloroplasts and *hyaline* (colorless) cells that are dead at maturity. Hyaline cells have one or more pores (giving access to the environment) and can hold water. Waterlogged (anaerobic) conditions and low temperatures prevent decay processes of organic matter. Consequently, bogs accumulate partially decomposed organic mater and peat. Such conditions obstruct the renewal of mineral compounds. Bog species are adapted to oligotrophic conditions.

Besides, in true ombrotrophic bogs, *Sphagnum* species are frequent in spruce mires that belong to class *Vaccinio-Piceetea* Br.-Bl. 1939 and transitional mires (class *Scheuchzerio palustris-Caricetea nigrae* Tx. 1937). Transitional mires are widespread palustrine in a wider zone of alpine rivers Sava Dolinka and Sava Bohinjka [29–32, 149].

Calcareous fens are wetlands occupied by peat- or tufa-producing small-sedge and brown moss communities developed on soils permanently waterlogged, with calcareous water supply. Calciphile small sedges and other *Cyperaceae* usually dominate in these fen communities that belong to the alliance *Caricetalia davallianae* Br.-Bl. 1949.

Sava Bohinjka and Sava Dolinka collect water from numerous springs. Vegetation of alpine springs is represented by communities that belong to the class *Montio-Cardaminetea* Br.-Bl. *et* Tx. ex Klika *et* Hadač 1944. These communities may be divided into two main groups (orders *Montio-Cardaminetalia* Pawłowski et al. 1928 and *Cardamino-Chrysosplenietalia* Hinterlang 1992).

The alliance *Cratoneuron commutatum* W. Kock 1928 of the order *Montio-Cardaminetalia* Pawłowski et al. 1928 involves calcareous spring communities, commonly dominated by mosses. Such communities are numerous in the Julian Alps and Karavanke mountains [153, 154].

The alliance *Caricion remotae* Kästner 1941 of the order *Cardamino-Chrysosplenietalia* Hinterlang 1992 involves communities of muddy, shady soft-water springs in forested habitats from lowland up to the montane belt of Central and northwest Europe. These communities are numerous within the drainage area of Sava Bohinjka and Sava Dolinka.

3.2 Montainous Region

The confluence of the two rivers Sava Bohinjka and Sava Dolinka with an exceptional diversity of water and riparian habitats with wetlands, meanders, gravel beds, and many others.

Hydrological properties of the Sava River are heterogeneous. In wide valleys and within plain karst fields, the flow of the Sava River is relatively slow. However, in upper parts of the Sava River and in the Sava gorge (from the Sava village to Zidani Most), water flow is fast. Different hydrological conditions affect the type of deposited material. Therefore, the littoral and bottom of the Sava River in the mountainous region vary from large stone blocks (in the torrential parts of the watercourse and the gorge) to pebbly deposits, gravel and send. Such environmental conditions affected ecological differentiation of aquatic and littoral communities.

The main tributaries in the upper Sava River Basin are Kokra, Kamniška Bistrica, and Savinja (from the left side) and Sora (from the right). These rivers and the torrential parts of the Sava River (upper part of the river and the Sava gorge) are characterized by high flow velocity and rapid streams. Such situation is not favorable for development and establishment of macrophyte communities.

However, in slower parts of the Sava River and its tributaries Ljubljanica and Krka rivers, diverse aquatic communities are developed [155–159]. These communities belong to the alliances *Potamion* (Koch 1926) Libbert 1931, *Ranunculion fluitantis* Neuhäusl 1959, *Ranunculion aquatilis* Passarge 1964 (=*Callitricho-Batrachion* Den Hartog & Segal 1964) of the class *Potametea* Klika in Klika & Novák 1941 as well as alliances, *Hydrocharition morsus-ranae* Passarge 1996, and *Lemnion minoris* Tüxen ex O. Bolòs & Masclans 1955 of the class *Lemnetea* W. Koch *et* Tx. 1954.

Communities of alliances *Ranunculion fluitantis* Neuhäusl 1959 and *Ranunculion aquatilis* Passarge 1964 involve perennial, herbaceous, species-poor, aquatic macrophytic vegetation, colonizing limpid water in shallow, well-illuminated, and slow-flowing river stretches, composed by partially or totally submerged species, sometimes with emerging flowers. Communities of the alliance *Potamion* (Koch 1926) Libbert 1931 are represented by hydrophytic, afloat or submerged, rooted or not-rooted vegetation in mesotrophic and eutrophic slow-flowing, relatively deep waters.

The most important aquatic species of these communities are *Ranunculus trichophyllus* Chaix= *Batrachium trichophyllum* (Chaix) van den Bosch, *Ranunculus aquatilis* L, = *Batrachium aquatile* (L.) Dum., *Ranunculus fluitans* Lam.= *Batrachium fluitans* Wimm., *Ranunculus peltatus* Schrank, *Ranunculus penicillatus* (Dum.) Bab., *Batrachium circinatum* (Sibth) Spach, *Callitriche cophocarpa* Sendtner, *Sium erectum* =*Berula erecta* (Huds.) Coville, *Zannichellia palustris* L., *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L., *Najas marina* L., *Najas minor* All., *Ceratophyllum demersum* L., *Elodea canadensis* L. C. Rich., *Hippuris vulgaris* L., *Potamogeton crispus* L., *Potamogeton filiformis* Pers., *Potamogeton natans* L., *Potamogeton nodosus* Poir, *Potamogeton pectinatus* L., *Potamogeton perfoliatus* L., *Ranunculus circinatus* Sibith, *Potamogeton lucens* L., *Potamogeton praelongus* Wulf W. D. J. Koch, *Potamogeton zizii* Roth., *Polygonum amphibium* L., *Spirodela polyrhiza* (L.) Schleid, *Sagittaria sagittifolia* L., *Alisma lanceolatum* With., *Alisma plantago-aquatica* L., *Hottonia palustris* L., *Butomus umbellatus* L., *Hippuris vulgaris* L., etc.

Cinclidotus fontinaloides (Hedw.) P. Beauv., *Cinclidotus aquaticus* (Hedw.) B. S. G., *Rhynchostegium riparioides* (Hedw.) Card., and *Fontinalis antipyretica* L. are frequent bryophytes in these communities. Algae *Chara* spp. and *Nitella tenuissima* cover bottom of these rivers. The floating species *Lemna minor* L., *Lemna trisulca* L., *Spirodela polyrhiza* (L.) Schleid., *Nuphar lutea* (L). Sibith. Et

SM., and *Utricularia intermedia* Hayne are present in low abundance in habitats with slow water flow.

Helophytes *Glyceria fluitans* (L.) R. Br., *Mentha aquatica* L., *Myosotis palustris* (L.) Hill, *Iris pseudacorus* L., *Nasturtium officinale* R. Br., *Scirpus lacustris* L., *Sparganium emersum* Rehm, *Veronica anagallis-aquat*ica L., *Rumex hydrolapathum* Huds., *Oenanthe fistulosa* L., *Phalaris arundinacea* L., *Phragmites australis* (Cav.) Trin, *Plantago altissima* L., *Ranunculus flammula* L., *Ranunculus lingua* L., *Rorippa amphibia* (L.) Besser, *Rorippa sylvestris* (L.) Besser, *Schoenoplectus lacustris* (L.) Palla, *Senecio paludosus* L., *Teucrium scordium* L., *Typha angustifolia* L., *Veronica beccabunga* L., *Sium latifolium* L., *Sparganium emersum* L., *Sparganium erectum* L., *Eleocharis palustris* (L.) Roem. *et* Schult., *Equisetum palustre* L., *Galium palustre* L., *Gratiola officinalis* L., *Iris pseudacorus* L., *Juncus alpino- articulatus* Chaix, *Lycopus europaeus* L., *Lysimachia vulgaris* L., *Lythrum salicaria* L., *Mentha longifolia* (L.) Hudson, *Myosotis scorpioides* L., and *Nasturtium officinale* R. Br. occupy the littoral of Krka, Ljubljanica, and Ižica rivers and their tributaries.

Numerous intermittent or permanent karstic streams (Trbuhovica, Obrh, Stržen, Rak, Bloščica, Cerkniščica Pivka, and Unica) fed the Ljubljanica River. Intermittent watercourses (such as Mali Obrh, Martinjščica, Grahovščica, Goriški Potok, Žerovniščica, and Bloščica) have relatively low number of hydrophytes (aquatic plants). In permanent watercourses, the aquatic vegetation belongs to the alliances *Potamion* (Koch 1926) Libbert 1931, *Ranunculion fluitantis* Neuhäusl 1959, *Ranunculion aquatilis* Passarge 1964 (=*Callitricho-Batrachion* Den Hartog & Segal 1964) of the class *Potametea* Klika in Klika & Novák 1941. Communities of the alliances *Hydrocharition morsus-ranae* Passarge 1996 and *Lemnion minoris* Tüxen ex O. Bolòs & Masclans 1955 occupy Ižica River and the lower streams of the Ljubljanica and Krka rivers.

Littoral *forest vegetation* of the mountainous section of the Sava River and its tributaries is diverse. Šilc [123] described the riparian willow communities along the Sava, Krka, and Mirna rivers. These communities belong to the alliances *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993 and *Salicion albae* Soó 1930 of the class *Salicetea purpureae* Moor 1958.

The communities of the alliance *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993 occupy instable habitats of torrential waters, on coarse gravel, and sandy littoral. Dominating willows are *Salix eleagnos* Scop., *Salix triandra* L., *Salix purpurea* L., *Salix fragilis* L., and *Salix viminalis* L. Numerous species from scrub seral vegetation or marginal to broad-leaved woodland (class *Crataego monogynae-Prunetea spinosae* Tüxen 1962, order *Prunetalia spinosae* Tüxen 1952) are admixed to willows. The most important species of this group of plants are *Euonymus europaea*, *Sambucus nigra* L., *Rhamnus cathartica*, *Viburnum opulus*, *Cornus sanguinea*, *Clematis vitalba* L., *Crataegus monogyna* Jacq., *Lonicera caprifolium* L., *Berberis vulgaris* L., *Prunus spinosa* L., etc. Besides these species, *Alnus incana* (L.) Moench. also occurs occasionally. The most common species of the herb layer are *Phalaris arundinacea* L., *Juncus effusus* L., *Lamium maculatum* L., *Lysimachia vulgaris* L., *Mentha verticillata* L., *Myosotis* *sp.*, *Deschampsia cespitosa* (L.) P. B., *Eupatorium cannabinum* L., *Petasites hybridus* (L.) P. Gaertn., *Peucedanum verticillare* (L.) Koch ex DC., *Agrostis stolonifera* L., *Aegopodium podagraria* L., *Angelica sylvestris* L., *Carex appropinquata* Schumach., *Carex vulpina* L, *Chaerophyllum hirsutum* L., *Cirsium oleraceum* (L.) Scop., *Geranium robertianum* L., *Polygonum hydropiper* L., *Rorippa amphibia* (L.) Bess., *Rorippa sylvestris* (L.) Bess., *Rubus caesius* L., *Rumex crispus* L., *Senecio fuchsia* C. C. Gmel., *Succisella inflexa* Beck., *Urtica dioica* L., etc.

The communities of white willow (the alliance *Salicion albae* Soó 1930) are developed on fine gravel and sandy littoral, under the direct influence of streams just above the mean water level and are often flooded. The soil is structureless and only layers of sedimentation are found within it. In spite of the high quantity of organic residues (willow leaves, withered parts of tall herbs, river deposits), there is very little humus, since every year organic residues are covered by river sediments which hinder decomposition. Erosion also interrupts the development of the soil and vegetation.

Soil texture is an important factor for differentiation of willow communities. Physical properties of soil, such as maximum water capacity of the soil (the quantity of water that a certain soil type can retain), aeration, and water permeability, directly depend on soil structure. Communities within the alliance *Salicion incano-purpureae* Sillinger 1933 are developed on soils with coarse texture (high percentage of gravel). Communities of the alliance *Salicion albae* Soó 1930 are developed on soil with greater water capacity. The white willow communities are divided into three layers. The tree layer is dominated by the species *Salix alba* L., which is in places accompanied by other willows (*Salix purpurea* L., *Salix triandra* L.) and *Prunetalia* species: *Cornus sanguinea L.*, *Euonymus europaea* L., *Sambucus nigra* L., *Viburnum opulus* L. The herb layer is well developed and represented by tall herbs *Angelica sylvestris* L., *Phalaris arundinacea* L., *Urtica dioica* L., *Symphytum officinale* L., *Impatiens glandulifera* Royle, *Solidago gigantea* Aiton, *Heracleum sphondylium* L., *Petasites hybridus* (L.) Gaertner, etc.

Due to periodical floods deposit of organic material, and at the same time, a great deal of fertilizers from the nearby fields, these communities have numerous nitrophilous species which are syntaxonomically classified into the classes *Artemisia vulgaris* W. Lohmeyer, Preising & Tüxen *ex* von Rochow 1951 (*Artemisia vulgaris*, *Erigeron annuus*, *Echinocystis lobata*, *Cuscuta europaea*, *Eupatorium cannabinum* L., *Epilobium hirsutum*) and *Galio aparines-Urtica dioica* Passarge *ex* Kopecký 1969 (*Glechoma hederacea L.*, *Lamium maculatum L.*, *Galium aparine L.*, *Aegopodium podagraria*, *Rudbeckia laciniata*, *Galeopsis pubescens*, *Parietaria officinalis*, *Aristolochia clematitis L.*, *Chaerophyllum temulum*, *Cruciata laevipes*).

Hygrophilous species from helophytic vegetation (*Phragmito-Magnocaricetea*) and wet meadow vegetation (*Molinion*) are also frequent in these communities. Most important species of these groups are *Iris pseudacorus* L., *Carex gracilis* Curt., *Galium palustre* L., *Rorippa amphibia* (L.) Bess., *Mentha aquatica* L., *Veronica anagallis-aquatica* L., *Lycopus europaeus* L., *Ranunculus repens* L., *Lysimachia nummularia* L., *Rumex obtusifolius* L., *Mentha longifolia* (L.) Nath., *Barbarea vulgaris* R. Br., *Angelica sylvestris* L., *Cirsium oleraceum* (L.) Scop., *Lythrum salicaria* L., *Filipendulion ulmariae* (L.) Max., *Chaerophyllum hirsutum* L., etc.

In mountainous region, the Sava River flows through a gorge between the village of Sava and Zidani Most. The Sava River is torrential in this section and its level rapidly increases after heavy rain. Normally, there is a short period in May when the water level is low, and a longer period favorable for the development of the vegetation in the summer months from July to September [160]. Riparian vegetation in this section of the Sava River is represented by alpine ephemeral hygrophilous shrub communities with *Salix elaeagnos* Scop., *Salix purpurea* L., and *Hippophae rhamnoides* L. developed on gravel beds and alluvium.

Dolomite slopes above the Sava are overgrown with basophilic beech forests (Ostryo-Fagetum M. Wraber ex Trinajstić 1972, Arunco-Fagetum Košir 1962, Hacquetio-Fagetum Košir 1962) and also with acidophilous beech forests (Blechno-Fagetum sylvaticae Tüxen & Oberd. 1958). Forest communities of broad-leaved species (*Hacquetio-Fraxinetum* Marinček in Wallnöfer et al. 1993, *Veratro nigri-Fraxinetum* Dakskobler 2007, *Tilio cordatae-Aceretum platanoidis ostryetosum* Ž. Košir 1954) occupy hillside screes and the colluvium at the foot of slopes. The steepest, rockiest sites at the right bank of the Sava are overgrown with basophilic forests of Scots pine and black pine (*Genisto januensis-Pinetum sylvestris* Tomazic 1940), as well as forests of pubescent oak and hop hornbeam (*Querco pubescenti-Ostryetum carpinifoliae* Horvat 1938 and *Ostryo carpinifoliae-Fraxinetum orni* Aichinger 1933). Canyon and gorge forests are important ecosystems that represent significant biodiversity pools.

Riparian vegetation in the Sava River gorge is a mixture of helophytic communities (class *Phragmitetea* R. Tx. Et Prsg. 1942), wet grasslands (*Molinio-Arrhenatheretea R. Tx. 1937*), basophilic pine forests (*Erico-Pinetea* Ht. 1959), chasmophytic communities (*Asplenietea trichomanis* Br.-Bl. 1934. corr. Oberd. 1977), subalpine grasslands (*Elyno-Seslerietea* Br.-Bl. 1948), scrub and tall-herb vegetation in habitats moistened and fertilized by percolating water at high altitudes (*Mulgedio-Aconitetea* Hadač & Klika in Klika & Hadač 1944), thermophilic oak forests (*Quercetalia pubescentis* Br.-Bl. (1931) 1932), and thermophilic forest edges (class *Trifolio-Geranietea* T. Müller 1961).

Čarni [161] described communities of hygrophilous forest edges (alliances *Filipendulion* Segal 1966 und *Senecio fluviatilis* Tüxen 1967) within the Krka River Basin.

Lacustrine communities in the mountainous part of the Sava River are represented by *the vegetation of intermittent lakes* in the large karst region (Karst of Notranjska). Temporary lakes in Europe differ with respect to hydrology, geology, flooding season, duration of wet and dry phases, the source of feeding waters (e.g., subterranean water, running water, high precipitation level), chemistry of waters, geographic location, etc.

Turloughs are temporary lakes of the limestone areas, annually inundated mostly by groundwater via estavelles connecting to *underground water systems*, which rise and fall with high seasonal rainfall, and drain, usually in summer, supporting development of wetland vegetation [162, 163]. Most flood in the autumn and then dry up between April and July. Turloughs are mainly restricted to Ireland where they periodically occur on carboniferous limestone depressions in conditions of high levels of rainfall. Most turloughs dry out completely for a sufficient length of time during the growing season for their floors to be fully vegetated. The plant communities can be wet grassland or sedge-dominated swards, depending on the substrate moisture and its nutrient status. The vegetation mainly belongs to either the phytosociological alliance *Agropyro-Rumicion crispi* Nordh. 1940 (wet Boreo-Atlantic pioneer grassland communities of beaches and dunes, within the class *Plantaginetea majoris* R. Tx. et Prsg. in R. Tx 1950) or the sedge-dominated alliances *Caricion canescenti-nigrae* Nordhagen 1937 and *Caricion davallianae* Klika 1934 within the class *Scheuchzerio-Caricetea fuscae* [164]. As priority habitats in the EU Habitats Directive, most turloughs over 10 ha have Special Area of Conservation (SAC) protection status; many of them also have Special Protection Area (SPA) status under the Birds Directive [162].

Very shallow, the *Mediterranean temporary ponds* exist only in winter or late spring, with a flora mainly composed of Mediterranean therophytic and geophytic species belonging to the alliances *Isoetion* Br.-Bl. 1936, *Nanocyperion flavescentis* Koch ex Malcuit 1929, *Preslion cervinae* Br.-Bl. ex Moor 1937, *Agrostion salmanticae* Rivas Goday 1958, *Heleochloion* Br.-Bl. ex Rivas Goday and *Lythrion tribracteati* Rivas Goday & Rivas-Martínez ex Rivas Goday 1970 [9, 12, 165].

Temporary lakes in Slovenian karst are considered different to both turloughs and Mediteranean temporary lakes. Most temporary lakes of Slovenia are flooded in autumn and spring when the increased outflow of the water table is impeded by less permeable rocks. The catchment for the temporary lakes consists of multiple *superficial and underground watercources* from the Karst of Notranjska (or the Karst of the Ljubljanica River).

The Ljubljanica River (*sensus strictus*) is the right tributary of the Sava. Its drainage area includes most of the central part of Slovenia. In the past, the Ljubljanica River had flown on the surface as one river but later split into distinct surface watercourses due to karstic processes [166]. Recently, the Ljubljanica River (*sensu latus*) consists of numerous surface watercourses that flow subsequently through a series of karstic fields ("kraška polja") and disappear underground. These rivers are connected by groundwater passages. The main branch of this complex hydrological system is formed by the karstic streams Trbuhovica, Obrh (combines Mali and Veliki Obrh), Stržen, Rak, and Unica and the lowland Ljubljanica River (*sensus strictus*) that flows through the plain area, forming Ljubljana Moor ("Ljubljansko Barje"). The Pivka stream is a lateral branch that flows into the Rak stream underground.

Extreme water level fluctuations of these rivers form intermittent lakes. The temporary lakes of Slovenia occur around 300–500 m above sea level and are flooded in autumn and spring when the increased outflow of the water table is impeded by less permeable rocks.

Numerous intermittent lakes seasonally appear and disappear in the *Pivka River valley*. Palško jezero reaches 102.7 ha and Petelinjsko jezero 73.6 ha on an average full flood [167, 168]. Other temporary lakes (Jeredovce, Krajnikov dol, Petelinjsko jezero, Klenski dol, Radohovsko jezero, Malo Drskovško jezero, Veliko Drskovško jezero, Veliko Zagorsko jezero, Malo Zagorsko jezero, Kljunov ribnik, Veliki dol, Bačko jezero, Laneno jezero, Kalsko jezero, and Šembijsko jezero) range in size from 0.07 to 5.6 ha [169] described intermittent lakes in the Pivka valley.

The Pivka River runs through the valley among high karst plateaus of Nanos (1,313 m), Hrušica (1,264 m), Javorniki (1,268 m), low plateau Slavenski ravnik (600–700 m), Snežnik (1,796 m), and flysch hills that form a catchment area of the Reka River. It flows only intermittently aboveground until it reaches the impervious flysch near Postojna, where it disappears.

A seasonal flooding of the Pivka lakes creates special growing conditions for plant species as the floods last from some days to, in extreme circumstances, even to half a year. Some plants develop morphological and physiological adaptations to accept oxygen and carbon dioxide both from water or air [158]. The others can start their growth in water as true aquatic plants with submerged leaves and later continue on the surface as usual terrestrial species.

Periodic floods of intermittent lakes of the Pivka valley form strong gradients of environmental variables. Shallow estavelles and "ponors" (i.e., swallow holes in the alluvium) through which the basin fills and empties are flooded for the longest period. These habitats are the wettest parts of temporary lakes. Moisture decreases towards higher parts of intermittent lakes. The soil at marginal banks of lakes is shallow or even rocky, becoming thicker towards lower lying parts due to soil wash off from the margins. The washed soil accumulates in the lower parts of the lakes. Around the ponors, and deepest parts of temporary lakes, the washed soil forms mud dumps.

Such gradient of hydrological and soil conditions affected differentiation of vegetation in temporary lakes of the Pivka valley [163]. Margins of the lakes are surrounded by forests. The next zone is represented by scrub vegetation (class *Crataego monogynae-Prunetea spinosae* Tüxen 1962) and forest edge vegetation (class *Trifolio medii-Geranietea sanguine* Th. Müll. 1962). Purple willow inhabits the central parts and those parts that are flooded for a longer period. Dominating vegetation within the flooded area of temporary lakes is represented by dry and wet meadows. Dry meadows (*Festuco-Brometea* Br.-Bl. et Tüxen ex Soó 1947) are distributed at upper parts of temporary lakes, on shallow and stone soils. Dominant species of these xeric communities are *Dorycnium germanicum* (Gr.) Rouy., *Filipendula hexapetala* L., *Festuca ovina* L., *Trifolium montanum* L., *Allium carinatum* L., *Pimpinella saxifraga* L., *Briza media* L., *Koeleria pyramidata* (Lam.) Domin., *Galium verum* L., *Carex humilis* Leyss., and *Lotus corniculatus* L.

Species (*Centaurea jacea* L., *Oenanthe lachenalii* C. C. Gmel., *Inula salicina* L., *Ranunculus acris* L., *Phleum pratense* L., *Genista tinctoria* L., *Achillea millefolium* L., *Vicia cracca* L., *Valeriana officinalis* L., *Rumex acetosa* L., *Campanula glomerata* L., *Carex hirta* L., *Daucus carota* L., *Allium angulosum* L., *Plantago altissima* L., *Potentilla reptans* L., *Deschampsia cespitosa* (L.) P. B., *Gladiolus illyricus* Koch., *Carex panicea* L., *Gentiana pneumonanthe* L., *Agrostis stolonifera* L., *Solanum dulcamara* L., *Equisetum arvense* L., *Lysimachia vulgaris* L., *Filipendulion ulmariae* (L.) Max. *Plantago altissima* L. *Allium angulosum* L., *Potentilla reptans* L., etc.) dominate in more mesic communities.

Finally, the wettest sites that are flooded for the longest period occupy helophytes and hygrophylous plants *Galium palustre* L., *Eleocharis palustris* (L.) Roem. *et* Schult., *Rorippa sylvestris* (L.) Bess, *Juncus acutiflorus* Ehrh. ex Hoffm., *Ranunculus repens* L., *Carex elata* All., *Deschampsia cespitosa* (L.) P. B., *Carex panicea* L., *Solanum dulcamara* L., *Equisetum arvense* L., *Molinia caerulea* (L.) Moench, etc. Some species in these communities belong to the category of rare and endangeroud taxa (*Iris sibirica* L., *Allium angulosum* L., *Campanula glomera* L., *Clematis integrifolia* L., *Colchicum autumnale* L., *Gentiana pneumonanthe* L., *Gladiolus illyricus* Koch., *Pseudolysimachion longifolium* L, *Viola elatior* Fr. etc.). Due to relatively long dry season, aquatic plants are missing in temporary lakes of the Pivka valley. Intermittent lakes of the Pivka River resemble turloughs [163].

Contrary to these lakes, **Cerknica Lake**, the greatest temporary lake in the Karst of Notranjska, has extensive water, even in summer, and *supports aquatic plant communities*. Cerkniško polje is a depression enclosed by mountain range Javorniki Mts., Snežnik Mt., the Bloke plateau, and Loško polje.

The majority of *inflows* to the Cerknica Polje originate from its eastern and northeastern parts. Watercourses Žerovniščica, Martinjščica, Grahovščica, Žerovniščica, and Lipsenjščica emerge waters from the Bloke plateau. Waters from the Loško Polje (the Obrh stream) emerge in the karst springs Obrh, Okence, Cemun, Podpečmi, and several other small springs in the southeastern part of the Cerknica Polje. All of the springs merge into the Stržen stream, which is the major inflow to the Cerknica Polje. The Goriški Potok stream also emerges from draining waters of upper lying Loško Polje. The autogenic precipitation waters from the Snežnik and Javorniki Mts. emerge in several permanent and periodical karst springs at the southwestern rim of the Cerknica [170]. The *runoff* from the polje is a completely karst one, that is, underground. Lake Cerknica empties through numerous ponors—swallow holes in the alluvium at the bottom of the polje. The swallow holes in the center of the Cerknica Polje are hydraulically connected with the Ljubljanica river springs.

The intermittence in Lake Cerknica presents specific conditions that differ from flooded areas elsewhere [34]. Unlike other intermittent lakes, the permanent water bodies (i.e., water in deeper depressions and the Stržen stream with its tributaries) represent refugee habitats for numerous aquatic plants that persist in the lake even during the driest season. Cerknica Lake has permanent water bodies, even in summer, and *supports aquatic plant communities*. Such situation enables aquatic plants (e.g., *Chara* sp., *Potamogeton* sp., *Batrachium* sp., etc.) to complete their life cycle. Aquatic vegetation of the Cerknica Lake and its tributaries has been described in numerous articles [171, 172].

The most important helophytes in Lake Cerknica are *Phalaris arundinacea* L., *Phragmites australis* (Cav.) Trin, *Schoenoplectus lacustris* (L.) Palla, *Typha angustifolia* L., *Iris pseudacorus* L., *Veronica anagallis-aquatica* L., *Veronica beccabunga* L., *Butomus umbellatus* L., *Senecio paludosus* L., *Sium latifolium* L., *Sparganium emersum* L., *Sparganium erectum* L., *Teucrium scordium* L., *Plantago altissima* L., *Ranunculus flammula* L., *Ranunculus lingua* L., *Rorippa amphibia* (L.) Besser, *Rorippa sylvestris* (L.) Besser, *Mentha aquatica* L., *Mentha longifolia* (L.) Hudson, *Myosotis scorpioides* L., *Caltha palustris* L., *Eleocharis palustris* (L.) Roem. *et* Schult., *Equisetum palustre* L., *Galium palustre* L., *Gratiola officinalis* L., *Juncus alpino- articulatus* Chaix, *Lycopus europaeus* L., *Lysimachia vulgaris* L., *Lythrum salicaria* L., *Oenanthe fistulosa* L., etc. These species are adapted to excessive fluctuation of water level.

The extent and duration of floods at the Cerkniško polje create a hydrologic gradient that affects diversification of plant communities. Aquatic communities of permanent water bodies (alliances *Potamion* (Koch 1926) Libbert 1931 and *Callitricho-Batrachion* Den Hartog & Segal 1964) are replaced by emerged communities (*Pragmition* W. Koch 1926), helophytic communities of tall sedges (*Magnocaricion* W. Koch. 1926), calcareous fens (*Caricetalia davallianae* Br.-Bl. 1949), transitional mires on deep peats (*Rhynchosporion albae* Koch 1926), and wet meadows (*Molinion coerulae* W. Koch. 1926). Ilijanić [15] recorded associations *Scirpo-Phragmitetum* W. Koch 1926, *Caricetum elatae* W. Koch. 1926, *Caricetum gracilis* (Graebn. Et Hueck 1931) Tx. 1937, *Rhynchosporetum albae* W. Koch 1926, *Primulo-Schoenetum* (W. Koch 1926) Oberd. 1957 em 1962, *Deshampsio-Plantaginetum altissimae* Ilijanic 1977, and *Arrhenateretum medioeuropaeum* (Br. Bl. 1919) Oberd. 1952 of boggy, marshy, and grassy vegetation within the Cerknica Lake. Martinčič [173] described helophylous association *Rorippa amphibia-Eleocharis acicularis* on Cerknica Lake.

Due to morphophysiological adaptations and specific reproductive strategies, the amphibian plants are able to survive the alternation of floods and dry periods within intermittent lakes [174–178].

The most diverse *palustrine communities* of the mountainous segment of the Sava River are developed within the *Ljubljansko Barje* wetland. Seliškar [41, 179, 180] described vegetation of the wetland. Besides the aquatic communities that occur in Ljubljanica and Ižica rivers (communities of alliances *Potamion* (Koch 1926) Libbert 1931, *Callitricho-Batrachion* Den Hartog & Segal 1964, *Hydrocharition morsus-ranae* Passarge 1996, and *Lemnion minoris* Tüxen ex O. Bolòs & Masclans 1955), the vegetation of the Ljubljansko Barje wetland is represented by riparian forests (*Salicion albae* Soó 1930), hygrophilous forests (*Alno-Ulmion* Br.-Bl. *et* R. Tx. 1943), swamp forests (*Alnion glutinosae* (Malc. 1929) M. Dre. 1936), marshy helophyte communities (*Pragmition* W. Koch 1926, *Magnocaricion* W. Koch. 1926), calcareous fens (*Caricion davallianae* Klika 1934), and wet meadows (*Molinion coerulae* W. Koch. 1926, *Deschampsia cespitosa* Horvatić 1930, *Calthion* R. Tx. 1937 em. Bal.-Tul. 1978, *Filipendulenion* (Lohmeyer in Oberd. et al. 1967) Bal.-Tul. 1978). Fragments of boggy vegetation are distributed sporadically within the Ljubljansko Barje wetland [181]. Ljubljansko Barje is an important area for rare and endangered plants [182–184].

Diverse palustrine communities along the Krka valley are represented by wet meadows [133]. The most important associations of the *Molinion* Koch 1926 alliance are *Gentiana pneumonanthe-Molinietum litoralis* Ilijanic 1968 and *Junco* *conglomerati-Betonica officinalis* Zenik 2010. The communities of the *Calthion* Tüxen 1937 em. Balátová 1978 alliance are represented by associations *Angelico-Cirsietum oleracei* Tüxen 1937, *Scirpetum sylvatici* Ralski 1931, *Dactylorhiza majalis-Scirpetum georgiani* Zelnik 2004 and *Agrostio-Juncetum conglomerati* Šegulja 1974. Finally, the communities of the alliance *Deschampsia* Horvatić 1930 are represented by the association *Succisella inflexa-Deschampsia cespitosa* (Horvatić 1930) Ellmauer in Ellmauer & Mucina 1993.

3.3 Floodplain Region

East of the confluence of the Krapina River, the Sava River Basin is exposed to seasonal floodings. The floods occur generally in the spring, after snow melting, and in the autumn, after heavy rainfall. The heavy rainfalls during late autumn may cause high waters in the Sava tributaries. Due to specific topography (a wide peri-Pannonian lowland), the left tributaries of the Sava River (Krapina, Česma, Lonja, Pakra, Orljava, Bosut) are prone to floods. Excepting the Kupa River, the right tributaries of the Sava River flow through much smaller floodplains.

Floodplains are formed by the inundation process [185]. When floods go over the riverbank, the floodwater flows much slower, and the sediment it carries is quickly deposited as a smooth layer of mud. Repeated sedimentation of flood deposits forms a floodplain.

The rate of deposit sedimentation decreases with distance. Therefore, the greatest sedimentation occurs immediately, when river overflows its banks. As a result of such sedimentation pattern, a ridge beside the riverbanks (the Sava Trench) has been formed.

Floodplains act as natural flood-buffering systems. They represent large water retention areas capable of storing flood waves. Floodplains are important for natural water purification and the regeneration of groundwater resources [186]. Moreover, floodplains are important biodiversity hotspots [186, 187].

However, the wetland ecosystems along the Sava River floodplains are threatened, degraded, reduced, and significantly modified due to expansion of agriculture areas and because of development of a complex flood defense system that protects fertile agricultural land, settlement, and industrial facilities [188].

The flood defense system in the Croatia relies on five large lowland retention areas (Lonjsko Polje, Mokro polje, Kupčina, Zelenik, and Jantak), two basic water distribution facilities (Prevlaka and Trebež 1 sluices), and three relief canals (Odra, Lonja-Strug, and Kupa-Kupa).

In Bosnia and Herzegovina, on the right bank of the Sava River, the flood zones are divided into seven polders: Dubička ravan, Lijevče polje, Srbačko-Nožička ravan, Ivanjsko polje, Odžačka Posavina, Srednja Posavina, and Semberija. The polders are independently protected against floods by dykes, pump stations, and the system of canals (main boundary canals for external waters and the network of the main canal for collecting inland waters).

The flood defense line of Serbia consists of levees, dikes, sluices, and a dense network of canals and pumping stations.

Drainage of wetlands, development of dykes, and modifications of riverbanks resulted with a serious loss of wetlands. However, development of draining canals may have positive effects on biodiversity since the network of artificial canals represents a large refugee area for aquatic plants. Besides artificial canals, numerous artificial fishponds and lakes (Crna Mlaka; Lipovljani Sloboština/Vrbovljan; Prnjavor; Trnopolje; Saničani, a complex of carp fishponds at Jelas polje; Modrac lake; Živača; etc.) also represent important refugia for aquatic plants. Vegetation of the fishponds is represented by free-floating aquatic communities (alliances *Lemnion minoris* O. Bolòs & Masclans 1955, *Lemnion trisulcae* Hartog & Segal 1964, and *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 Bolós et Masclans 1955), submerged plant communities (*Potametea* R. Tx. et Preising 1942 and *Ceratophylletea* Den Hartog & Segal 1964), eutrophic sublittoral communities of rooting macrophytes (*Nymphaeion albae* Oberd. 1957), emerged littoral vegetation (alliance *Phragmition* W. Koch 1926), and helophyte vegetation (*Magnocaricion elatae* W. Koch 1926). The fishponds are temporarily dried. Such situation favors development of ephemeral dwarf-cyperaceous vegetation on muddy, periodically flooded habitats (alliance *Nanocyperion* W. Koch 1926 of the class *Isoëto-Nanojuncetea* Br.-Bl. *et* Tx. 1943). In further text we focused on important biodiversity hotspots along the Sava River floodplains.

The ***Krapina River*** valley is located in northwest Croatia, in the Zagorje region. Remnants of historically widespread lowland forests of *Quercus robur* and *Fraxinus angustifolia* are fragmentary distributed along the Krapina River. According to Stančić [54], the marshland vegetation along the Krapina River belongs to the class *Phragmito-Magnocaricetea* Klika in Klika et Novák 1941 and alliances *Phragmition* W. Koch 1926, *Magnocaricion elatae* W. Koch 1926 and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957.

The communities of alliance *Phragmition* W. Koch 1926 occupy comparatively deep water. They are poor in species. The hygrophylous species *Phragmites australis*, *Typha latifolia*, *Scirpus lacustris*, *Sparganium erectum*, *Glyceria maxima*, *Acorus calamus*, and *Rorippa amphibia* dominate in these communities. The most important *Phragmition* associations along the Krapina River are *Phragmitetum australis* Schmale 1939, *Typhetum latifoliae* Lang 1973, *Scirpetum lacustris* Chouard 1924, *Sparganietum erecti* Roll 1938, *Glycerietum maximae* Hueck 1931, *Acoretum calami* Schulz 1941, and *Oenantho-Rorippetum* Lohmeyer 1950.

The communities within the alliance *Magnocaricion elatae* W. Koch 1926 occupy shallow flooded habitats or moist habitats without surface water. Dominating species in these communities are *Carex elata*, *Carex acuta*, *Carex randalpina*, *Carex vesicaria*, *Carex vulpina*, *Carex riparia*, *Eleocharis palustris*, *Iris pseudacorus*, and *Phalaris arundinacea*. Associations that belong to the alliance *Magnocaricion elatae* W. Koch 1926 are *Caricetum elatae* W. Koch 1926, *Caricetum gracilis* Almquist 1929, *Caricetum vesicariae* Chouard 1924, *Caricetum* *vulpinae* Soó 1927, *Galio palustris-Caricetum ripariae* Balátová-Tuláčková in Balátová-Tuláčková et al. 1993, *Eleocharitetum palustris* Schennikov 1919, *Phalaridetum arundinaceae* Libbert 1931 and two communities that Stančić [54] denoted as the *Carex randalpina* community and the *Iris pseudacorus* community.

The alliance *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957 along the Krapina River contains two communities which grow on the banks of streams and other water bodies. They are affected by periodic floods and water flow.

Some species of these communities belong to the categories of rare and endangered taxa in these communities (*Hottonia palustris*, *Carex panicea*, *Carex riparia*, *Carex vesicaria*, *Fritillaria meleagris*, *Glyceria fluitans*, *Leersia oryzoides*, *Ophioglossum vulgatum*, *Poa palustris*, *Ludwigia palustris*, *Orchis laxiflora subsp. palustris*). Significant anthropogenic impact in the Krapina River valley is manifested in the presence of invasive plant species (*Solidago gigantea*, *Echinocystis lobata*, *Acorus calamus*, *Erigeron annuus*, and *Bidens frondosa*).

The armlet ***Savica*** has been separated from the Sava River in 1965, where the dyke was built. Today, the Savica wetland represents a complex of 12 small interconnected eutrophic lakes and surrounding habitats situated on the left riverbank of Sava within the Zagreb city area. The lakes are remnants of backwaters of the River Sava [189]. They are fed by precipitation and by cooling water from neighboring thermal power plant station.

According to Alegro et al. [189], the area of Savica is represented by a mosaic of more or less disturbed habitats around the lakes with single or small groups of trees and dense scrub between them. The most abundant tree species are *Salix alba*, *Populus nigra*, *Populus alba L.*, *Alnus glutinosa*, and *Robinia pseudoacacia*. The scrub consists mostly of *Cornus sanguinea L.*, *Prunus spinosa L.*, *Rosa canina*, *Crataegus monogyna Jacq.*, *Corylus avellana*, *Sambucus nigra*, *Salix purpurea*, *Ligustrum vulgare*, and some other species. This scrub belongs to the alliance *Carpino betuli-Prunion spinosae* (R. Tx. 1952) H. E. Weber 1974 and to ass. *Cornetum sanguinei* Kaiser 1930. Ruderal communities of herbaceous plants mostly belong to the alliance *Senecio fluviatilis* R. Tx. 1950 (*Convolvulion sepii* R. Tx. 1947). The most abundant species in this vegetation type are *Epilobium hirsutum*, *Epilobium tetragonum*, *Urtica dioica*, *Calystegia sepium*, *Solidago gigantea*, *Bidens frondosa*, *Helianthus tuberosus*, *Impatiens glandulifera*, *Lysimachia vulgaris*, and *Lysimachia punctata*.

Aquatic vegetation in lakes is represented by association *Myriophyllo-Nupharetum luteae* (W. Koch 1926) Hueck 1931, *Lemno-Spirodeletum polyrhizae* W. Koch 1954, and *Nymphoidetum peltatae* Bellot 1951. Communities of periodically flooded banks belong to the alliance *Nanociperion* W. Koch 1926. On the edges and banks of lakes, small patches of reed vegetation belonging to ass. *Phragmitetum australis* (Gams 1927) Schmale 1939 and ass. *Typhetum latifolia* (Soó 1927) Now. 1930 are developed.

The most important aquatic plants and helophytes in the Savica lakes and ponds are *Potamogeton natans* L.; *Lemna minor* L.; *Spirodela polyrhiza* (L.) Schleiden; *Alisma plantago-aquatica* L.; *Nymphoides peltata* (S. G. Gmelin) O. Kuntze;

Nuphar lutea (L.) Sm. in Sibith. et; *Myriophyllum spicatum* L.; *Berula erecta* (Huds) Coville; *Nasturtium officinale* R. Br.; *Phragmites australis* (Cav.) Trin. ex Steud. Trin. ex Steud.; *Typha latifolia* L.; *Sparganium erectum* L.; *Cyperus fuscus* L.; *Cyperus glomeratus* L.; *Cyperus serotinus* Rottb.; *Eleocharis palustris* (L.) Roem. et Schult.; *Scirpus sylvaticus* L.; *Carex elata* All.; *Carex hirta* L.; *Carex otrubae* Podp.; *Carex pendula* Huds.; *Carex pseudocyperus* L.; *Carex remota* L.; *Carex spicata* Huds.; *Polygonum aviculare* L.; *Polygonum mite* Schrank; *Polygonum persicaria* L.; *Rumex crispus* L.; *Rumex hydrolapathum* Hudson; *Rumex palustris* Sm.; *Humulus lupulus* L.; *Rorippa amphibia* (L.) Besser; *Petasites hybridus* (L.) P. Gaertn., B. Mey. et Schreb.; *Iris pseudacorus* L.; *Juncus compressus* Jacq.; *Juncus effusus* L.; *Juncus inflexus* L.; *Nasturtium officinale* R. Br.; *Humulus lupulus* L.; *Rorippa amphibia* (L.) Besser; *Rorippa sylvestris* (L.) Besser; etc.

Odra is a river in central Croatia. It flows from the Žumberak mountain, southwest of Zagreb eastwards through Turopolje region. The Odra River is 83 km long. Its confluence into the Kupa River is located near Odra Sisačka, just northeast of Sisak, also just before the Kupa joins the Sava River. The upper flow of Odra has been significantly altered by humans, by the digging of the 32 km long canal Sava-Odra-Sava, south of Zagreb, as a part of flood defense system. The region Turopolje and Črnec Polje covers a large area on alluvial deposits of gravel, sand, and clay. Due to periodic floods, eugley soils dominate within this region.

Extensive livestock farming is one of the most important measures in biodiversity and landscape conservation at the Odra River plain.

Climate-zonal forests of this area are represented by communities *Epimedio-Carpinetum* (Horvat 1938) Borhidi 1963 (*Querceto-Carpinetum croaticum* Horvat 1938), *Lonicero caprifoliae-Quercetum roboris* (Rauš 1971) Marinček 1994 s. lat. (*Carpino betuli-Qurcetum roboris* Anić 1959), and *Querco robori-Carpinetum illyricum* and *Querco petraeae-Carpinetum illyricum* Horvat et al. 1974. However, due to anthropogenic influence, these forests are fragmented.

Meadows are presented with several community types: *Deschampsia cespitosa* Horvatić 1930 is common within both in forest complexes and its edges, *Caricetum tricostato-vulpinae* Horvatić 1930 is present on wetlands, while *Bromo-Cynosuretum cristati* Horvatiić 1930 and *Arrhenatheretum elatioris* Br.-Bl. 1925 are present on somewhat raised and more permeable terrain. Near the villages, weed and ruderal communities are developed, *Lolio-Plantaginetum majoris* Beger 1930.

The wetland forests (*Leucoio-Fraxinetum angustifoliae* Glavač, 1959, *Genisto elatae-Quercetum roboris* Horvat 1938) are developed on the north riverside of the Odra River. The willow/poplar forest (*Salici-Populetum nigrae* (R. Tüxen 1931) Meyer Drees 1936) occupies riverbanks along the Sava River.

Herbaceous wetland communities are diverse, especially along the network of canals. Hulina [22–25] described aquatic and palustrine vegetation of the network of draining Sava-Odra channels, in the area of Turopolje and Črnec Polje. Floating species that dominate in aquatic vegetation of the Turopolje and Črnec Polje are *Berula erecta* (Huds) Coville, *Callitriche palustris* L., *Callitriche stagnalis* Scop., *Lemna minor* L., *Marsilea quadrifolia* L., *Nuphar lutea* (L) Sm., *Potamogeton* *natans* L., *Ranunculus circinatus* Sibth., *Marsilea quadrifolia* L., *Utricularia vulgaris* L., and *Wolffia arrhiza* (L.) Hork. ex Wimm.

Submerged plants of the aquatic vegetation are *Ceratophyllum demersum* L., *Elodea canadensis* Michx., *Lemna trisulca* L., *Spirodela polyrhiza* (L) Schleiden, *Myriophyllum spicatum* L., *Potamogeton crispus* L., *Potamogeton perfoliatus* L., *Potamogeton pusillus* L., *Zannichellia palustris* L., *Riccia fluitans* L., etc.

Helophyte communities belong to alliances *Phragmition* W. Koch 1926, *Magnocaricion elatae* W. Koch 1926 and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. The most important species of these communities are *Alisma plantago-aquatica* L., *Carex elata* All., *Carex rostrata* Stokes ex With., *Carex riparia* Curt., *Carex vesicaria* L., *Cyperus fuscus* L., *Eleocharis palustris* (L.) Roem. Sch., *Galium palustre* L., *Glyceria fluitans* (L) R. Br., *Iris pseudacorus* L., *Leersia oryzoides* (L.) Sw., Mentha aquatica L., *Nasturtium officinale* R. Br., *Oenanthe aquatica* (L.) Poir., *Phalaris arundinacea* L., *Phragmites communis* Trin., *Rorippa amphibia* (L) Besser, *Sagittaria sagittifolia* L., *Schoenoplectus lacustris* (L.) Palla., *Scirpus sylvaticus* L., *Sparganium erectum* L., *Typha angustifolia L.*, *Typha latifolia* L., *Veronica anagallis-aquatica* L., and *Veronica beccabunga* L.

Ruderal communities of the alliance *Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen are developed on muddy banks of draining channels [25].

Marekovici-Bukevje is an IPA site between Sava and Odra rivers. The most important species within the site are *Carex panicea* L., *Carex riparia* Curtis, *Cyperus longus* L., *Fritillaria meleagris* L., *Galium rubioides* L., *Gentiana pneumonanthe* L., and *Pseudolysimachion longifolium* (L.) Opiz.

The ***Kupa River*** is one of the largest Croatian rivers. It flows from Gorski Kotar towards NE, partly forming the border with Slovenia. Entering the lowland, it forms extensive basin almost completely covered with the complex of alluvial oak forests. Large lowland along the Kupa River is denoted as Kupa Basin (Pokupski Bazen).

The Kupa River source is located in the *Gorski Kotar*. This area represents a part of IPA (important plant areas) network in Croatia. The most important species in Gorski Kotar IPA site are *Campanula cespitosa* Scop., *Campanula cochlearifolia* Lam., *Cardamine kitaibelii* Becherer, *Cardamine waldsteinii* Dyer, *Centaurea fridericii* Vis., *Centaurea haynaldii* Borbas ex Vuk., *Cypripedium calceolus* L., *Ilex aquifolium* L., *Iris illyrica* Tomm., *Lilium bulbiferum* L., *Lilium carniolicum* Bernh. ex Koch, *Lonicera borbasiana* (Kuntze) Degen, *Myosotis suaveolens* Willd., *Pedicularis acaulis* Scop., *Peltaria alliacea* Jacq., *Thymus bracteosus* Vis. ex Benth., *Tofieldia calyculata* (L.) Wahlenb., *Typha shuttleworthii* Koch et Sond., *Eleocharis carniolica* Koch, *Polystichum illyricum* Borbas, etc.

Hygrophylous vegetation in this region is represented by alder woods that belong to the alliance *Alnion glutinosae* Malcuit 1929. The most important species of mixed alder woods (*Alnetum glutinoso-incanae* Br.-Bl. 1915) are *Alnus glutinosa* (L.) Gaertn., *Alnus incana*, *Viburnum opulus* L., *Rubus caesius* L., *Rubus hirtus* W. et K., *Carex remota* L., *Caltha palustris* L., *Valeriana dioica* L., *Filipendulion ulmariae* (L.) Max., *Lycopus europaeus* L., *Crepis paludosa* (L.) Moench, etc. Rare and endangered fern species *Matteuccia struthiopteris* (L.) Todaro fragmentary occurs within these forests. Fragments of transitional mires with *Carex echinata* Murray, *Carex flava* L., *Carex hostiana* DC., *Carex lepidocarpa* Tausch, *Carex panicea* L., *Carex serotina* Merat, *Cyperus flavescens* L., *Cyperus fuscus* L., *Drosera rotundifolia* L., *Rhynchospora alba* (L.) Vahl, *Eriophorum angustifolium* Honck., *Eriophorum latifolium* Hoppe, and *Eriophorum vaginatum* L. are sporadically distributed. Chasmophytic vegetation is developed on canyon habitats along the upper course of the Kupa River.

The area of *Pokupski Bazen* covers the lowland sector of the Kupa River. A number of smaller watercourses are flowing from adjacent hills of Žumberak, Plešivica, and Vukomeričke Gorice, entering the Kupčina River. It flows into artificial canal of Kupa-Kupa that cuts through Pokupski Bazen and the Kupa River in its SE part. Watercourses coming from Samoborsko Gorje are transferred into the Kupa-Kupa canal. Depressions in Pokupski Bazen are being flooded during abundant rainfall. The soil of such relief depressions is eugley, very heavy soil, saturated with water for the most part of the year. The area within the Pokupski Bazen is covered by aquatic vegetation marsh vegetation and alluvial wet forests.

Vukomeričke gorice is a large important plant area (IPA), within the Pokupski Bazen [190]. A large wetland with numerous watercourses, canals, and three fishponds (Draganici, Crna Mlaka, and Pisarovina) is located within the IPA site. *Crna Mlaka* is an area of extensive carp fishponds that represent important breeding and feeding site for number of wetland birds. Fishponds have been protected in 1980 as ornithological reserve. Since 1993, Crna Mlaka fishponds belong to internationally protected Ramsar sites.

Šegulja [42–48, 191, 192], Trinajstić and Šugar [193], and Stančić [54] described vegetation of *Pokupski Bazen*. The most important plants of the site are *Alopecurus aequalis* Sobol., *Alopecurus geniculatus* L., *Carex flava* L., *Carex lepidocarpa* Tausch, *Carex panicea* L., *Carex riparia* Curtis, *Carex vesicaria* L., *Cyperus flavescens* L., *Cyperus fuscus* L., *Cyperus michelianus* (L.), *Eleocharis carniolica* Koch, *Eleocharis ovata* (Roth) Roem. et Schult., *Fritillaria meleagris* L., *Gentiana pneumonanthe* L., *Glyceria fluitans* (L.) R. Br., *Glyceria plicata* (Fr.) Fr., *Hibiscus trionum* L., *Hottonia palustris* L., *Lilium martagon* L., *Lindernia procumbens* (Krock.) Philox, *Lythrum portula* (L.) D. A. Webb, *Marsilea quadrifolia* L., *Orchis coriophora* L., *Platanthera bifolia* (L.) Rich., *Scirpus mucronatus* L., and *Trapa natans* L.

Hygrophylous forests of the Pokupski Bazen are represented by communities *Frangulo-Alnetum glutinosae* Rauš, 1968; *Leucoio-Fraxinetum angustifoliae* Glavač, 1959; and *Genisto elate-Quercetum roboris* Horvat 1938.

Herbaceous palustrine communities along the Pokupski Bazen belong to alliances *Phragmition* W. Koch 1926; *Magnocaricion elatae* W. Koch 1926; *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd; and *Phalaridion arundinaceae* Kopecký 1961 [194]. Important plant species that form these communities are *Carex flava* L., *Carex lepidocarpa* Tausch, *Carex panicea* L., *Carex riparia* Curtis, *Carex vesicaria* L., *Cyperus flavescens* L., *Cyperus fuscus* L., *Cyperus michelianus* (L.) Link, *Eleocharis carniolica* Koch, *Eleocharis ovata* (Roth) Roem. et Schult., *Glyceria fluitans* (L.) R. Br., *Glyceria plicata* (Fr.) Fr., *Carex buekii* Wimm (syn. *Carex banatica* Heuff.), and *Scirpus mucronatus* L.

Diverse group of wet meadows and pastures belong to alliances *Calthion palustris* Tüxen 1937, *Juncion acutiflori* Braun-Blanq. *in* Braun-Blanq. & Tüxen 1952, *Molinion caeruleae* W. Koch 1926, *Deschampsia cespitosa* Horvatić 1930, *Alopecurion pratensis* H. Passarge 1964, and *Potentillion anserinae* Tüxen 1947. The most important wet meadow associations are *Potentilletum anserinae* Rapaics 1927, *Junco-Menthetum longifoliae* Lohm. 1953, *Rumici-Alopecuretum geniculati* Tx. 1950, *Trifolio-Agrostietum stoloniferae* Marković 1973, and *Agrostio-Juncetum conglomerati* Šegulja 1974.

Lacustrine ecosystems (eutrophic artificial fishponds of Crna Mlaka, Draganići, and Pisarovina) are represented by free-floating aquatic communities (alliances *Lemnion minoris* O. Bolòs & Masclans 1955, *Lemnion trisulcae* Hartog & Segal 1964, and *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 Bolós et Masclans 1955); submerged plant communities (*Potametea* R. Tx. et Preising 1942 and *Ceratophylletea* Den Hartog & Segal 1964); eutrophic sublittoral communities of rooting, leaf-floating macrophytes (*Nymphaeion albae* Oberd. 1957); and emersed helophyte vegetation (alliance *Phragmition* W. Koch 1926). The aquatic communities are *Lemno-Spirodeletum* W. Koch 1954, *Lemnetum trisulcae* Soó. 1927, *Myriophyllo-Nupharetum* W. Koch 1926, *Ceratophyllo-Potametum crispi* Horvatić et Micevski 1960, and *Hottonietum palustris* Tx. 1937.

Muddy riverbanks and borders of dried fishponds are covered by communities *Cyperetum flavescentis* W. Koch 1926 em. Aichinger 1933, *Ludwigietum palustris* and *Eleocharidi-Lindernietum* Pietsch 1973. These communities of dwarf helophytes, which belong to the alliance *Nanocyperion* Koch ex Libbert 1932, have important rare and endangeroud taxa (e.g., *Eleocharis ovata* (Roth) Roem. & Schult., *Cyperus flavrscens* L., *Ludwigia palustris* (L.) Elliott, etc.).

Ruderal, nitrophilous ruderal communities of periodically flooded habitats (*Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen and *Chenopodion rubri* (Tüxen ex Poli & J. Tüxen 1960) Kopecký) are sporadically distributed within the area.

A large wetland area ***Lonjsko polje*** is formed by Sava, Lonja, and Česma rivers and other smaller watercourses. Due to specific topography (the highest point of the area is 114 m a.s.l.), the wetland area is prone to floods and flooded water persists for relatively long period. Alluvial and fluvial processes formed numerous meanders, pools, ponds, and armlets. Floodplains Lonjsko polje (*sensu stricto*), Mokro polje, and Poganovo polje are protected as a Nature Park. Inside the Park "Lonjsko polje," there are ornithological reserves: Krapje đol, Rakita, and Dražiblato [195]. Since 1993, this area is an internationally protected Ramsar site. The nature park was included on the list of internationally Important Bird Areas in 1989 and has also been proposed for inclusion in the NATURA 2000 ecological network.

The Lonjsko polje floodplain is an important plant area [190]. The most important plants within the floodplain are *Alopecurus rendlei* Eig, *Baldellia ranunculoides* (L.) Parl., *Blysmus compressus* (L.) Panz. *ex* Link, *Carex acuta* L., *Carex panicea* L., *Carex riparia* Curtis, *Carex vesicaria* L., *Clematis integrifolia* L., *Cyperus fuscus* L., *Cyperus longus* L., *Cyperus michelianus* (L.) Link, *Equisetum hyemale* L., *Fritillaria meleagris* L., *Glyceria plicata* (Fr.) Fr., *Hibiscus trionum* L., *Hottonia palustris* L., *Hydrocotyle vulgaris* L., *Lindernia procumbens* (Krock.) Philcox, *Lythrum tribracteatum* Salzm. ex Spreng., *Marsilea quadrifolia* L., *Ophrys sphegodes* Mill., *Ranunculus lingua* L., *Orchis militaris* L., *Salvinia natans* (L.) All., *Stratiotes aloides* L., *Trapa natans* L., and *Wolffia arrhiza* (L.) Horkel ex Wimm.

Hydrological conditions (intensity and duration of flooding, groundwater level) and topography are main factors that affect floristic differentiation of riparian forest vegetation [37, 57, 65, 185, 196, 197].

Wetland woods of poplars and willows along the riverbanks (*Salicion albae* Soó 1930) are regularly and periodically flooded. These communities grow on river rims, on nutrient-rich soil because of the sedimentation of material. The willow coppices along the Sava, Lonja, and Česma rivers, canals, and pools are represented by the association *Galio-Salicetum albae* Rauš 1973. The dominant trees and shrubs of this community are *Salix alba* L. *Salix amygdaloides* Andersson, *Populus nigra* L., *Acer negundo* L., *Cornus sanguinea* L., and *Rubus caesius* L. The stratum of herbaceous plants is composed of *Phalaris arundinacea* L., *Galium palustre* L., *Rorippa amphibia* (L.) Besser, *Carex elata* All., *Iris pseudacorus* L., *Solanum dulcamara* L., *Rumex sanguineus* L., *Symphytum officinale* L., *Myosotis scorpioides* L., *Polygonum hydropiper* L., etc.

Compared to the forests of poplars and willows, the wetland forests of ash, black alder, and common oak that occupy topographic depressions are less frequently exposed to fluvial floods. However, the forests of ash (*Leucoio-Fraxinetum angustifoliae* Glavač 1959), black alder (*Frangulo-Alnetum glutinosae* Rauš 1968), and common oak (*Genisto elatae-Quercetum roboris* Horvat 1938.) are regularly inundated, because of specific climate conditions (high precipitation level) and particular structure of soil (clay soils with high water retention capacity).

The forests of common alder with buckthorn (*Frangulo-Alnetum glutinosae* Rauš 1968) are fragmentary distributed along old watercourse beds, on organogenic-marshy soil with a weak acid reaction. Most of the year, the groundwater level varies from 20 to 70 cm below soil surface. In the spring and autumn, the forest is regularly induded. Dominant species in these forests is *Alnus glutinosa* (L.) Gaertn. Other trees (*Fraxinus angustifolia* Vahl, *Ulmus laevis* Pall., *Ulmus minor* Mill., *Quercus robur* L.) occur less frequently. Shrub stratum is composed of *Acer campestre* L., *Rhamnus frangula* L., *Salix cinerea* L., *Viburnum opulus* L., *Sambucus nigra* L., etc. Dominant herbaceous species are *Carex elongata* L., *Carex vesicaria* L., *Carex riparia* Curt., *Hottonia palustris* L., *Glyceria fluitans* (L) R. Br., *Iris pseudacorus* L., *Lycopus europaeus* L., *Urtica radicans* Sw., *Mentha aquatica* L., *Rorippa amphibia* (L.) Besser, *Peucedanum palustre* (L.) Moench., etc.

The *Leucoio-Fraxinetum angustifoliae* Glavač 1959 forests of narrow-leaved ash and late snowflake are from the general use and economic point of view among the most important forest ecosystems in Croatia. They are distributed over about 30,000 ha in the riparian (inundated) areas of the Sava valley, the Kupa River Basin, and the Drava valley. The largest complexes are to be found in Lonjsko Polje Nature Park.

These forests occupy moisty topographic depressions and even pond borders. Groundwater level is high during most of the year. The forests are inundated during spring and autumn. Surface water persists for long time and during winter it usually freezes. Narrow-leaved ash is a very important tree species, because it thrives in adverse and mainly marshy conditions where other tree species of tree cannot grow.

Forests of common oak and broom, *Genisto elatae-Quercetum roboris* Horvat 1938, occupy habitats a few meters above the normal water level. They are either periodically inundated, with the flood lasting for a short time, or else they are out of the reach of flood waters, but in that case they occupy habitats with high groundwater level.

The common oak and broom forest is divided into subassociations *Genisto elatae-Quercetum roboris caricetosum brizoides* Horvat 1938, *Genisto elatae-Quercetum roboris caricetosum remotae* Horvat 1938, *Genisto elatae-Quercetum roboris carpinetosum betuli* Glavač 1961, and *Genisto elatae-Quercetum roboris aceretosum tatarici* Rauš, 1973. The first three subassociations are developed within the Lonjsko polje nature park.

The subassociation *Genisto elatae-Quercetum roboris caricetosum remotae* Horvat 1938 is developed on clay soil. Average level of groundwater during vegetation period is 150 cm [198]. The subassociation *Genisto elatae-Quercetum roboris caricetosum brizoides* Horvat 1938 represents a transitional phase towards the oak and hornbeam forest on the ridge. During vegetation period, the average groundwater level of these forests is 200 cm. The subassociation with hornbeam (*Genisto elatae-Quercetum roboris* Horvat 1938 *carpinetosum betuli* Glavač 1961) is relatively less frequent type of forests, and it represents a transition to the association *Carpino betuli-Quercetum roboris* Rauš 1969.

The oak and hornbeam forest (*Carpino betuli-Quercetum roboris*, Rauš 1969) occupy habitats outside the range of inundation. Hornbeam tolerates short-lasting transient floods but not standing water or a high level of groundwater. It appears only on relatively high elevation, on habitats where the groundwater level is below 300 cm. Dominating trees of these communities are *Quercus robur* L. and *Carpinus betulus* L. Less frequent trees that occur in these forests are *Acer campestre* L., *Fraxinus angustifolia* Vahl, *Tilia cordata* Mill., etc. *Cornus sanguinea* L., *Crataegus monogyna* Jacq., *Euonymus europaeus* L., *Corylus avellana* L., *Ulmus minor* Mill., and other species form the shrub stratum of the community. Stratum of herbaceous plants is diverse. The most frequent herbaceous plants in the community are *Stellaria holostea* L., *Veronica montana* L., *Carex brizoides* L., *Euphorbia amygdaloides* L., *Lysimachia nummularia* L., *Polygonatum multiflorum* (L.) All., *Circaea lutetiana* L., *Galeopsis tetrahit* L., *Brachypodium sylvaticum* (Huds.) Beauv., etc. The association *Carpino betuli-Quercetum roboris* Rauš 1969 is differentiated into subassociations *typicum* Rauš 1973, *fagetosum* Rauš 1973, *quercetosum cerris* Rauš 1969, and *tilietosum tomentosae* Rauš 1969.

Tall herbaceous communities along hygrophylous forest edge (*Convolvulion sepii* Tx. 1947, *Filipendulion* Segal 1966, *Senecio fluviatilis* R. Tx. 1947 1950 em. 1967) are represented by associations *Glycyrrhizetum echinatae* Slavnić, 1951 and *Echinocystetum lobatae* Gaži-Baskova et al. 1979. Dominant species of these communities are *Glycyrrhiza echinata* L., *Althea officinalis* L., *Senecio erraticus* Bertol., *Asclepias syriaca* L., *Euphorbia lucida* W. et K., *Urtica dioica* L., *Echinocystis lobata* (Michx) Torrey & A. Gray, *Rudbeckia laciniata* L., *Impatiens glandulifera* Royle, and *Helianthemum tuberosum* Garsault. Many of these species belong to the group of allochtonous neophytes.

Large meadows of the Lonjsko polje are represented by communities *Trifolio-Agrostietum stoloniferae* Marković 1973 and *Rorippo sylvestris-Agrostetum stoloniferae* (Moor 1958) Oberd. *et* Mull. 1961.

Fragmentary distributed hay meadows belong to communities *Deschampsia cespitosa* Hayek ex Horvatič 1930, *Bromo-Cynosuretum cristati* Horvatić 1930, *Arrhenatheretum elatioris* Tx. 1937, and *Agrostio-Hordeetum secalini* Ilijanić 1959.

Marsh vegetation (alliances *Phragmition* W. Koch 1926, *Magnocaricion elatae* W. Koch 1926, and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957) is represented by communities *Scirpetum lacustris* Schmale 1939, *Phragmitetum australis* Schmale 1939, *Typhetum angustifoliae* Pignatti 1953, *Glycerietum maximae* Slavnić 1956, *Acoro-Glycerietum maximae* Slavnić 1956, and *Acoretum calami* Schultz 1941 [193, 195].

Aquatic vegetation (classes *Lemnetea* R. Tx. 1955 and *Potametea* Klika et Novak 1941, and *Ceratophylletea* Den Hartog & Segal 1964 and *Stratiotetea* Den Hartog et Segal 1964) is represented by associations *Lemno-polyrhizae* Koch 1954, *Spirodelo-Salvinietum natantis* Slavnić 1956, *Lemnetum trisulce* Den Hartog 1964, *Hydrochariti-Stratiotetum* Westoff 1941, and *Myriophyllo-Nupharetum* W. Koch 1926 [193, 195, 199].

Muddy riverbanks and borders of dried fishponds are covered by community *Cyperetum flavescentis* W. Koch 1926 em. Aichinger 1933 and *Eleocharidi-Lindernietum* Pietsch 1973.

Important species of palustrine and aquatic vegetation within the Lonjsko polje nature park are *Alopecurus rendlei* Eig, *Baldellia ranunculoides* (L.) Parl., *Blysmus compressus* (L.) Panz. ex Link, *Carex acuta* L., *Carex panicea* L., *Carex riparia* Curtis, *Carex vesicaria* L., *Clematis integrifolia* L., *Cyperus fuscus* L., *Cyperus longus* L., *Cyperus michelianus* (L.) Link, *Equisetum hyemale* L., *Fritillaria meleagris* L., *Glyceria plicata* (Fr.) Fr., *Hibiscus trionum* L., *Hottonia palustris* L., *Hydrocotyle vulgaris* L., *Lindernia procumbens* (Krock.) Philcox, *Lythrum tribracteatum* Salzm. ex Spreng., *Marsilea quadrifolia* L., *Ophrys sphegodes* Mill., *Ranunculus lingua* L., *Orchis militaris* L., *Salvinia natans* (L.) All., *Stratiotes aloides* L., *Trapa natans* L., and *Wolffia arrhiza* (L.) Horkel ex Wimm.

Sunjsko polje borders with Lonjsko polje. Large wetland area between the Sunja Sava and Una rivers includes large wet grasslands, flooded forests, marshland, and aquatic vegetation. According to Croatian legislative, the area is protected as an important landscape. Moreover, the Sunjsko polje is important plant area [190]. The group of endangered plants within the protected area are *Alopecurus rendlei* Eig., *Carex riparia* Curtis, *Carex vesicaria* L., *Glyceria fluitans* (L.) R. Br., *Hottonia* *palustris* L., *Lythrum tribracteatum* Salzm. *ex* Spreng., *Marsilea quadrifolia* L., and *Stratiotes aloides* L.

Wetland forests within the Sunjsko polje area are represented by willow-poplar woods (*Galio-Salicetum albae* Rauš 1973 and *Salici albae-Populetum nigrae* (R. Rx. 1931) Meyer Drees 1936.), the wetland forests of ash, black alder, and common oak (*Leucoio-Fraxinetum angustifoliae* Glavač 1959, *Frangulo-Alnetum glutinosae* Rauš 1968, *Carici elongatae-Alnetum*, and *Genisto elatae-Quercetum roboris* Horvat 1938.)

Marshland vegetation (alliances *Phragmition* W. Koch 1926, *Magnocaricion elatae* W. Koch 1926, and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957) is represented by communities *Phragmitetum australis* Schmale 1939, *Typhetum angustifoliae* Pignatti 1953, *Glycerietum maximae* Slavnić 1956, *Caricetum elatae* Koch 1926, *Caricetum acutae* Tx 1937, *Caricetum ripariae* Máthé et Kovács 1959, *Caricetum vesicariae* Chouard 1924, and *Acoro-Glycerietum maximae* Slavnić 1956.

Aquatic vegetation in pools, ponds, and old armlets is represented by communities of vegetation classes *Lemnetea* R. Tx. 1955 and *Potametea* Klika et Novak 1941, and *Ceratophylletea* Den Hartog & Segal 1964 and *Stratiotetea* Den Hartog et Segal 1964. Muddy riverbanks and borders of dried pools and ponds are covered by communities of the alliance *Nanocyperion* W. Koch 1926. Wet meadows are represented by communities *Trifolio-Agrostetum stoloniferae* Marković 1973 and *Deschampsia cespitosa* Horvatić 1930.

Bardača wetland covers 3.500 ha, near the estuary of the Vrbas River. The wetland encompasses artificial 11 fishponds that are supplied by water from several lowland rivers inclduing Matura, Stublaja, and Brzaja rivers. Since 2007, Bardača is an internationally protected Ramsar site.

Kovačević [200], Kovačević, and Stojanović [201] described aquatic and wetland vegetation within the Bardača wetland.

Plant communities of the wetland are ordered along moisture gradient from aquatic vegetation (Potametea Tx. et Prsg. 1942 and Lemnetea Tüxen ex O. Bolòs & Masclans 1955), over littoral (Phragmition communis Koch 1926), and other marsh vegetation (Phalaridion arundinaceae Kopecký 1961, Oenanthion aquaticae Hejný ex Neuhäusl 1959 and Sparganio-Glycerion Br.-Bl. et Sissing) to tall-sedge vegetation (Magnocaricion elatae Koch 1926, Magnocaricion gracilis Géhu 1961). This zonation is typical of vegetation of aquatic and marshland habitats. Temporary dried littoral belt is covered by Isoëto-Nanojuncetea *Br.-Bl.* et *Tüxen 1943*, *Bidention tripartiti* Nordh. 1940, and *Chenopodium murale* Br.-Bl. 1931 em. O. Bolos 1967 communities.

Aquatic vegetation is represented by associations *Salvinio-Spirodeletum polyrrhizae* Slavnić 1958, *Ceratophylletum demersi* (Soó 1927) Hild. 1956, *Myriophyllo-Potametum* Soó 1934, *Najadetum marinae* Fukarek 1961, *Nympheetum alboluteae* Nowinski 1928, *Nymphaeetum albae* Vollmar 1947, *Hydrochari-Nymphoidetum peltatae* Slavnić 1956, *Nymphoidetum peltate* (Allorge 1922) Oberd. et Müller 1960, *Trapetum natantis* Müller et Görs 1960, *Scirpo-Phragmitetum* W. Koch 1926, *Typhetum angustifoliae* Pign 1953, and

Sparganietum erecti Roll 1938. The most important species of these communities are *Alisma plantago-aquatica* L., *Sagittaria sagittifolia* L., *Rorippa amphibia* (L.) Bess., *Butomus umbellatus* L., *Ceratophyllum demersum* L., *Carex gracilis* Curt., *Carex hirta* L., *Heleocharis palustris* (L.) R. Br., *Schoenoplectus lacustris* (L.) Palla, *Myriophyllum spicatum* L., *Hydrocharis morsus-ranae* L., *Vallisneria spiralis* L., *Iris pseudacorus* L., *Juncus effusus* L., *Lycopus europaeus* L., *Mentha aquatica* L., *Lemna gibba* L., *Lemna minor* L., *Lemna trisulca* L., *Spirodela polyrhiza* (L.) Schl., *Wolffia arrhiza* (L.) Wimm., *Utricularia vulgaris* L., *Marsilea quadrifolia* L., *Nymphoides peltata* (Gmel.) Ktze., *Najas marina* L., *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Glyceria maxima* (Hartm.) Hol., *Phragmites communis* Trin., *Typhoides arundinacea* (L.) Mnch., *Polygonum amphibium* L., *Rumex hydrolapathum* Huds., *Potamogeton crispus* L., *Potamogeton fluitans* Roth., *Potamogeton natans* L., *Potamogeton perfoliatus* L., *Ranunculus circinatus* (Sibth.) Spach., *Salvinia natans* (L.) Allioni, *Sparganium simplex* Huds., *Trapa natans* L., *Typha angustifolia* L, and *Typha latifolia* L.

Weed and ruderal vegetation is consisted of associations: *Polygono- Bidentetum tripartitae* (W. Koch 1926) Lohm. 1950, *Lolio-Plantaginetum majoris* Beger 1930, *Panico-Galinsogetum* Tüxen et Becker 1942, *Polygonetum avicularis* Gams 1927, *Arctio-Artemisietum vulgaris* (Tüxen 1942) Oberdorfer et al. 1967, etc.

Wetland meadows are represented by communities of alliances *Agropyro-Rumicion* Nordh. 1940 and *Deschampsia cespitosa* Horvatić 1930.

Forest vegetation is represented by willow and poplar communities (*Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931), swamp forests (*Alnion glutinosae* Malcut 1929), and temporary flooded forests (*Alno-Quercion roboris* Horvat 1938). The most important types of hygrophilous forests are *Genisto elatae-Quercetum roboris* Horv. 1938, *Leucojo-Fraxinetum angustifoliae* Glavač 1959, *Salici-Populetum* (R. Tüxen 1931) M. Drees 1936, and *Populetum nigrae-albae* Slavnić (1942) 1952.

A flooded wetland ***Dvorina-Gajna*** is located between the Sava River and the east dyke near Slavonski Brod. The wetland is a mosaic complex of grasslands, old armlets, canals, and numerous temporary ponds developed after the flood. As a biodiversity hotspot, the Dvorina-Gajna wetland is nominated as an important plant area. Moreover, one part of the wetland is protected as a Special Ornithological Reserve since 1988.

Aquatic vegetation in deeper ponds and depressions is represented by communities *Myriophyllo-Nupharetum* W. Koch 1926, *Lemno-Spirodeletum polyrrhizae* W. Koch 1954, and *Spirodelo-Salvinietum* Slavnić 1950. Shallow waters and littoral occupy *Nanocyperion* Koch ex Libbert 1932 communities. Rare ferns *Marsilea quadrifolia* and *Salvinia natans* occur in the aquatic communities.

Littoral and marsh vegetation (alliances *Phragmition* Koch 1926, *Oenanthion aquaticae* Hejny ex Neuhausl 1959 and *Sparganio-Glycerion* Br.-Bl. et Sis. 1942.) is represented by communities *Phragmitetum australis* Soó 1927, *Typhetum latifoliae* G. Lang 1973, *Glycerietum fluitantis* Eggler 1933, *Oenantho aquaticae-Rorippaetum amphibiae* Lohm. 1950, and *Scirpetum lacustris* Schmale 1939. Tall-sedge vegetation (*Magnocaricion elatae* Koch 1926, *Magnocaricion gracilis* Gehu 1961) is represented by the community *Caricetum ripariae* Knapp *et* Stoffers 1962.

Hygrophylous forests of willows (*Salicion albae* Soó 1930) poplars (*Populion albae* Br.-Bl. 1931) and pedunculate oak (*Alno-Quercion roboris* Horvat 1938) are fragmentary distributed, mainly along the Sava Riverbanks.

Diverse meadow communities dominate within the Dvorina-Gajna wetland. Anthropogenic pastures and meadows (class *Molinio-Arrhenatheretea* Tüxen 1937) are developed on relatively deep, fertile soils throughout Europe. This class includes secondary mesic and wet grasslands on nutrient-rich soils. They have developed due to regular mowing or grazing on sites of deciduous, mixed, or coniferous forests. Grasses, sedges, and perennial herbs dominate in these communities. Annuals are rare due to strong competition of tall perennial species. Moisture is the main environmental gradient responsible for variation in species composition of Central European meadows.

Mesic meadows and pastures (*Arrhenatherion elatioris* Luquet 1926, *Cynosurion cristati* Tx. 1947, *Polygono bistortae-Trisetion flavescentis* Br.-Bl. et Tüxen ex Marschall 1947, *Poion alpinae* Oberd. 1950) occupy habitats on well-drained, relatively fertile mineral soils.

Wet meadows and pastures occur on permanently or temporary flooded habitats, mainly on organogenic, mineral-rich soils. This group of communities includes wet meadows on persistently wet habitats, often in the littoral zone of water bodies or in flooded alluvia (*Calthion palustris* Tüxen 1937, *Juncion acutiflori* Braun-Blanq. *in* Braun-Blanq. & Tüxen 1952), occasionally wet meadows (*Molinion caeruleae* W. Koch 1926), periodically flooded meadows (*Deschampsia cespitosa* Horvatić 1930, *Cnidion dubii* Bal.-Tul. 1966, *Alopecurion pratensis* H. Passarge 1964), and nitrophilous, periodically flooded meadows and pastures on trampled (compressed) soil (*Potentillion anserinae* Tüxen 1947).

The wet meadows and pastures within the Dvorina-Gajna wetland (and generally along the Sava River floodplains) belong to different alliances [16, 17, 135, 202]. Due to different successional stages, variable hydrological conditions (frequency and duration of flooding, groundwater level), and different managing modes (intensity and frequency of mowing and grazing), floristic composition of these communities is unstable and variable. Such situation prevents unambiguous delimination of associations and higher syntaxonomic units. Nevertheless, the most frequent associations of wet meadows and pastures within the Dvorina-Gajna wetland are *Trifolio-Agrostetum stoloniferae* Marković 1973, *Rorippo-Agrostetum* s*tolonifera* Oberd. *et* Mull. 1961, and *Succisella inflexa-Deschampsia cespitosa* Horvatic 1930. Due to abandonment of extensive agriculture, the grazing intensity is significantly reduced and such situation induces succession of pastures. Recently, the invasive species *Amorpha fruticosa* L. covers a large part of the meadow.

Communities within the Dvorina-Gajna wetland have numerous species that belong to the category of rare and endangered taxa (*Alopecurus aequalis* Sobol., *Carex riparia* Curtis, *Clematis integrifolia* L., *Cyperus fuscus* L., *Cyperus glomeratus* L., *Glyceria fluitans* (L.) R. Br., *Hibiscus trionum* L., *Iris illyrica*

Tomm., *Lythrum tribracteatum* Salzm. ex Spreng., *Marsilea quadrifolia* L., *Peltaria alliacea* Jacq., *Ranunculus lingua* L., *Ranunculus traunfellneri* Hoppe, *Rhinanthus rumelicus* Velen., *Salvinia natans* (L.) All., etc.).

A large ***Spačva*** wetland is formed by Sava, Virovi, Spačva, and Studva rivers and numerous canals and temporary flooded ponds. The wetland area is prone to floods. The wetland is partly protected. Two forest reserves are protected since 1975, i.e., Lože and Radiševo. Two areas are protected since 1999 as important landscapes: Virovi and Spačva. The whole site is included in the Croatian ecological network as an important bird area and important plant area.

Different types of forest communities dominate within the wetland area [36, 37, 203]. Coastal floodplain forests of poplars and willows (*Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931) are regularly and periodically flooded. The most important willow and poplar communities within the Spačva wetland are *Salici-Populetum nigrae* (R. Tx. 1931) Meyer Drees 1936, *Galio palustri-Salicetum albae* Rauš 1973, and *Populetum nigrae-albae* Slavnić 1952. Temporary flooded forests of pedunculate oak, black alder, and narrow-leaved ash (alliance *Anlo-Quercion roboris* Horvat 1938) and swamp forests of black alder (alliance *Alnion glutinosae* Malcut 1929) are developed on temporary induded habitats and topographic depressions with long-lasting floods. The most important associations of these forests are *Leucojo-Fraxinetum angustifoliae* Glavač 1959, *Frangulo-Alnetum glutinosae* Rauš 1968, and *Genisto elatae-Quercetum roboris* Horvat 1938. Topographically higher habitats, outside the flooding zone, occupy forests of common hornbeam and pedunculate oak (alliance *Erythronio-Carpinion betuli* (Horvat 1938) Marinček in Walnöfer, Mucina et Grass 1993, *Carpinion betuli* Issler 1931). Dominating association of these forests is *Carpino betuli-Quercetum roboris*, Rauš 1969. The forest vegetation within the Spačva region is endangered by the change of water regime, especially by the reduction of underground water level.

Tall herbaceous communities along the hygrophylous forest edge (*Convolvulion sepii* Tx. 1947, *Filipendulion* Segal 1966, *Senecio fluviatilis* R. Tx. 1947 1950 em. 1967) occur mainly within the zone of willow and poplar forests.

Aquatic vegetation is represented by floating and submerged communities of open water habitats and sublittoral and littoral communities.

The most important communities of freely floating plants (alliances *Lemnion* W. Koch et Tx. 1954, *Hydrocharition* Rübel 1933, and *Utricularion vulgaris* Pasarge 1964) are *Lemno-Spirodeletum polyrrhizae* W. Koch 1954, *Lemnetum trisulcae* Knapp et Stoffers 1962, *Spirodelo-Salvinietum* Slav. 1950, *Riccietum fluitantis* Slav. 1956, *Hydrocharitetum morsus-ranae* Van Langendock 1935, and *Lemno-Utricularietum vulgaris* Soó. The association *Ceratophylletum demersi* Hild 1956 (alliance *Ceratophyllion demersi* Hartog & Segal *ex* H. Passarge 1996) involves widespread communities of submerged plants.

Sublittoral communities of rooted, leaf-floating plants (alliances *Nymphaeion albae* Oberd. 1957 and *Potamion pectinati* (W. Koch 1926) Libbert 1931) are represented by associations *Potamogetonetum pectinati* Carstensen 1955, *Myriophyllo-Nupharetum* W. Koch 1926, *Nymphaeetum alboluteae* Nowinski 1928, and *Trapetum natantis* Muller et Gors 1960.

Littoral communities (alliances *Phragmition* W. Koch 1926 and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957) are included in associations *Phragmitetum australis* Schmale 1939, *Typhetum angustifoliae* Pignatti 1953, *Glycerietum maximae* Slavnić 1956, *Acoro-Glycerietum maximae* Slavnić 1956, *Acoretum calami* Schultz 1941, *Scirpo-Phragmitetum* W. Koch 1926, *Typhetum latifoliae* G. Lang 1973, and *Glycerietum fluitantis* Eggler 1933.

These communities have numerous species that belong to the group of rare and endangered taxa: *Acorus calamus* L., *Alisma plantago-aquatica* L., *Bolboschoenus maritimus* (L.) Palla, *Butomus umbellatus* L., *Callitriche stagnalis* Scop., *Ceratophyllum demersum* L., *Chlorocyperus glomeratus* (L.) Hay., *Elodea canadensis* L. C. Rich., *Glyceria fluitans* (L.) R. Br., *Glyceria maxima* (Hartm.) Holmb, *Hippuris vulgaris* L., *Hydrocharis morsus-ranae* L., *Lemna gibba* L., *Lemna minor* L., *Lemna trisulca* L., *Marsilea quadrifolia* L., *Mentha aquatica* L., *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L., *Najas marina* L., *Nuphar lutea* (L.) Sibth. & Sm., *Nymphaea alba* L., *Nymphoides peltata* Kuntze, *Oenanthe aquatica* (L.) Poiret, *Phragmites australis* (Cav.) Trin. ex Steud, *Polygonum amphibium* L., *Potamogeton crispus* L., *Potamogeton natans* L., *Potamogeton pectinatus* L., *Ranunculus aquatilis* L., *Ranunculus circinatus* Sibth, *Riccia fluitans* L., *Ricciocarpus natans* L., *Rorippa amphibia* (L.) Besser, *Sagittaria sagittifolia* L., *Salvinia natans* (L.) All., *Schoenoplectus lacustris* (L.) Palla, *Sium latifolium* L., *Sparganium erectum* L., *Spirodela polyrhiza* (L.) Schleid, *Stratiotes aloides* L., *Trapa natans* L., *Typha angustifolia* L., *Typha latifolia* L., *Utricularia vulgaris* L., *Veronica anagallis-aquatica* L., and *Wolffia arrhiza* (L.) Horkel ex Wimm.

The largest and the most preserved forests of pedunculate oak (*Quercus robur*) in Serbia are located in the alluvial pane of the Bosut and Studva rivers [204]. This area is protected as the ***Morović-Bosut*** Nature Park. The Bosut and Studva rivers and a few smaller watercourses flow through the park, forming a large wetland. The vegetation of Morović-Bosut and Spačva wetlands is essentially the same. Coastal floodplain forests of poplars and willows (*Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931) in the Morović-Bosut wetland are developed along the watercourses. A large percent of these forests is represented by intensively managed poplar plantations. Temporary flooded forests of pedunculate oak, black alder, and narrow-leaved ash (alliance *Anlo-Quercion roboris* Horvat 1938) and swamp forests of black alder (alliance *Alnion glutinosae* Malcut 1929) are dominating forest types within the wetland. At drier habitats, these forests are replaced by the community *Carpino betuli-Quercetum roboris*. The most frequent communities of flooded forests within the Nature Park belong to the association *Fraxino angustifoliae-Quercetum roboris* Jov. *et* Tomić 1979 (*Leucojo-Fraxinetum angustifoliae* Glavač 1959) and *Carici remotae-Fraxinetum angustifoliae* B. Jov. et Tom. At relatively dry habitats, on ridges, these communities are replaced by the association *Carpino-Fraxino-Quercetum roboris caricetosum remotae* Mišić 1974. According to the International code of phytosociological nomenclature [205], the name *Carpino-Fraxino-Quercetum roboris* Jov. et Tom. 1979 is not valid, since "the name of an association or of a syntaxon of higher rank is formed from the validly published scientific name(s) of one or two of the plant species or infraspecific taxa" (Art. 10). Such principle of syntaxonomic nomenclature may be questioned since the "polydominant" (ecotone) communities frequently occur in wetlands and in canyons and gorges [206–209].

The association *Saliceto cinereae-Fraxinetum angustifoliae* B. Jov. *et* Tom. occupies induded habitats in deep depressions.

The ***Zasavica stream*** is located in northern Mačva, in the area between Sava and Drina rivers. Two streams, Prekopac and Jovača, form more than 33 km long Zasavica watercourse that flows southwest-northeast and runs into Sava near Mačvanska Mitrovica. According to widely accepted assumptions, the Zasavica stream represents a residue (a lateral branch) of the Drina River estuary. There are several underground springs that supply Zasavica with freshwater (from the Drina and Sava rivers and by gravitational water from the Cer Mountain). The water regime in the site is highly dependent on the dam and pumping station located at the mouth of Zasavica into Sava River. Zasavica creates six large meanders along its course. Complex riparian ecosystems along the Zasavica are protected as a special nature reserve, since 1997. Since 2006, the Zasavica Reserve is designated as a Ramsar site.

The vegetation of the Zasavica Reserve includes flooded forests, swamps, seasonally flooded meadows, and sedge marshes [210–214].

Mixed willow-poplar forests (*Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931) are fragmentary distributed along shores of ponds, rivers, and canals. Intensively managed poplar plantations occupy much larger area. Swamp forests of black alder (alliance *Alnion glutinosae* Malcut 1929) are developed on temporary induded habitats and topographic depressions. Pioneer communities of swamp willow *Salix cinerea* L. (*Saliceto cinereae-Fraxinetum angustifoliae* Jov. et Tom. 1979, *Salicetum cinereae* Zol. 1931) are initial stages of succession from herbaceous to forest vegetation. Small fragments of the association *Fraxino angustifoliae-Quercetum roboris* Jov. et Tomić 1979 (*Genisto elatae-Quercetum robori* Horv. 1938. subass. *fraxinetosum* Glav. 1959) are sporadically distributed within the area.

Aquatic vegetation is represented by communities of alliances *Charion vulgaris* (Krause *ex* Krause & Lang 1977) Krause 1981 (vegetation of submerged stonewort swards of oligotrophic and mesotrophic water bodies), *Lemnion minoris* O. Bolòs & Masclans 1955, and *Lemnion trisulcae* Hartog & Segal 1964 (free-floating duckweed communities of still, eutrophic waters), *Ceratophylletea* Den Hartog & Segal 1964 (submerged vegetation), *Nymphaeion albae* Oberd. 1957 (eutrophic vegetation of floating-leaved rooting macrophytes), and *Hydrocharition morsus-ranae* Rübel *ex* Klika *in* Klika & Hadač 1944 (eutrophic vegetation of free-floating macrophytes in nutrient-rich waters).

Vesić et al. [213] recorded nine species of submerged stonewort in Zasavica Reserve: *Nitella mucronata* (A. Braun) Miq. in H. C. Hall 1840 emend. Wallman 1853, *Chara globularis* Truill. 1799, *Chara vulgaris* L. 1753, *Chara contraria* A. Braun ex Kütz. 1845, *Nitella capillaris* (Krocker) J. Groves et Bullock-Webster

1920, *Nitella syncarpa* (Truill.) Chevall. 1827, *Nitella confervacea* (Bréb.) A. Braun ex Leonh. 1863, *Tolypella intricata* (Trentepohl ex Roth) Leonhardi 1863, extremely rare species of algal flora of the Balkan Peninsula, and *Tolypella prolifera* (Ziz *ex* A. Braun) Leonhardi 1863.

Lemna trisulca L. and *Utricularia vulgaris* L. dominate in the association *Lemno-Utricularietum vulgaris* Soo (1928) 1938. Sublittoral communities of white water lily and yellow pond lily (*Nymphaeetum alboluteae* Nowinski 1928) are widely distributed within the Zasavica watercourse. The communities *Hydrocharo-Nymphoidetum peltatae* Slavnić 1956, *Potamogeto pusilli-Ceratophylletum demersi* Jank. 1974, *Hottonietum palustris* Tx. 1937, and *Nymphaeto-Stratiotetum aloidi* Jank. 1974 are sporadically distributed in the Zasavica stream, Jovača canal, and Ribnjača pond. The most frequent species within these communities are *Lemna gibba* L., *Lemna minor* L., *Lemna trisulca* L., *Riccia fluitans* L., *Alisma plantago-aquatica* L., *Ceratophyllum demersum* L., *Ceratophyllum submersum* L., *Butomus umbellatus* L., *Callitriche palustris* L., *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L., *Najas marina* L., *Najas minor* All., *Nuphar lutea* (L.) Sibth. & Sm., *Nymphaea alba* L., *Nymphoides peltata* (S. G. Gmelin) O. Kuntze, *Sagittaria sagittifolia* L., *Salvinia natans* (L.) All., *Potamogeton acutifolius* Link in Roemer et Schultes, *Potamogeton crispus* L., *Potamogeton lucens* L., *Potamogeton pectinatus* L., *Potamogeton pusillus* L., *Potamogeton trichoides* Cham. & Schlecht., *Urtica kioviensis* Rogow., *Utricularia australis* R. Br., *Utricularia vulgaris* L., *Wolffia arrhiza* (L.) Horkel ex Wimer, *Zannichelia palustris* L., *Spirodela polyrhiza* (L.) Schleiden, and *Stratiotes aloides* L.,

Marshland vegetation involves communities of the alliances *Phragmition* W. Koch 1926, *Magnocaricion elatae* W. Koch 1926, and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957.

The communities of emersed (sub)littoral communities are represented by associations *Acoro-Glycerietum maximae* Hueck. 1931 and *Scirpo-Phragmitetum* W. Koch 1926. Dominating species in these communities are *Acorus calamus* L., *Juncus compressus* Jacq., *Lycopus europaeus* L., *Lysimachia nummularia* L., *Lythrum salicaria* L., *Mentha aquatica* L., *Carex pseudocyperus* L., *Carex vulpina* L., *Glyceria maxima* (Hartman) Holomberg, *Hippuris vulgaris* L., *Iris pseudacorus* L., *Oenanthe aquatica* (L.) Poiret in Lam., *Phragmites australis* (Cav.) Trin. ex Stendei, *Polygonum amphibium* L., *Ranunculus circinatus* Sibth., *Ranunculus lingua* L., *Rumex hydrolapathum* L., *Scirpus lacustris* L., *Sium latifolium* L., *Solanum dulcamara* L., *Sparganium emersum* Rehmann, *Sparganium erectum* L., *Typha angustifolia* L., and *Typha latifolia* L.

Zasavica Reserve is the only remaining refuge area in Serbia for globally threatened species *Aldrovanda vesiculosa* L. Distribution of this species in Serbia covered the Sava River region (it was recorded in Obedska Bara and Makiš ponds near Belgrade). Janković and Stevanović [101] assumed that this species disappeared from Serbia. However, Stanković [215] detected a small population of *Aldrovanda vesiculosa* L. within the special nature reserve "Zasavica."

Other rare and endangered taxa in Zasavica Reserve are *Hippuris vulgaris* L., *Lindernia palustris* Hartm., *Ranunculus lingua* L., *Urtica kioviensis* Rogow., *Hottonia palustris* L., *Achillea asplenifolia* Vent., *Dryopteris carthusiana* (Vill.) H. P. Fuchs, *Stratiotes aloides* L., *Thelypteris palustris* (Schott) subsp. *palustris*, *Salvinia natans* (L.) All., *Stratiotes aloides* L., *Trapa natans* agg., *Butomus umbellatus* L., *Schoenoplectus triqueter* (L.) Palla, etc.

Distribution of *Stratiotes aloides* L. in Serbia is limited only on the lowland area of Pannonian Plane. *Urtica kioviensis* Rogow is relict species of the postglacial period. It has been recorded near Čelarevo village on the Danube bank, in Koviljski Rit, Kovinski Rit, and in Obedska Bara near Kupinovo (Obradović, Panjković-Matanović, and Igić, 1991). *Utricularia australis* R. Br. is very rare in flora of Vojvodina. It has been recorded in Obedska Bara [216].

Obedska Bara is a residue of old meander of the Sava River. The arch-shaped permanent pond Obedska Bara is located between villages Kupinovo and Obreža. Two canals (Vok and Revenica) connect the pond with the Sava River. Obedska Bara is one of the best preserved complex of wetland ecosystems, including aquatic plant communities, marshes, flooded forests, and meadows. Oxbows and mostly overgrown old meanders are the most outstanding landscape features. Obedska Bara is located in the alluvial plane, in southern Srem.

A large wetland area of Obedska Bara has been under protection since 1874, when it was proclaimed to be imperial hunting ground by the Habsburg Empire authorities. The conservation status of the area has been updated and modified several times. Since 1994, Obedska Bara is a special nature reserve. Considering international legislative, Obedska Bara is protected as a Ramsar site, since 1997. Moreover, it is an important plant area (IPA) and important bird area (IBA).

Different types of forest communities dominate within the wetland area [204, 217–221]. Coastal floodplain forests of poplars and willows (*Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931) are regularly and periodically flooded. The most important willow and poplar communities within the Obedska Bara Reserve are *Populetum nigrae-albae* Slavnić 1952. and *Salici albae-Populetum nigrae* (R. Tx. 1931) Meyer Drees 1936 (*Saliceto-Populetum* Rajevski 1953).

Temporary flooded forests of pedunculate oak, black alder, and narrow-leaved ash (alliance *Anlo-Quercion roboris* Horvat 1938) and swamp forests of black alder (alliance *Alnion glutinosae* Malcut 1929) are developed on temporary induded habitats and topographic depressions with long-lasting floods. The most important associations of these forests are Ulmeto-Fraxineto-Quercetum roboris Mišić 1974 (Leucojo-Fraxinetum angustifoliae Glavač 1959) and Fraxinetum oxycarpae Mišić 1974. They occupy long humid topographic depressions. *Fraxinus angustifolia* Vahl., subsp. *oxycarpa* (Willd.) Fukarek is frequent in these forests. However, introduced species *Fraxinus pennsylvanica* Marh. also occurs in the communities.

Topographically higher habitats outside the flooding zone occupy forests of common hornbeam and pedunculate oak (alliance *Erythronio-Carpinion betuli*

(Horvat 1938) Marinček in Walnöfer, Mucina et Grass 1993, *Carpinion betuli* Issler 1931). Dominating association of these forests is *Carpineto-Fraxineto-Quercetum roboris* Mišić 1974 (*Carpino betuli-Quercetum roboris*, Rauš 1969) and *Quercetum roboris* Mišić 1974.

Aquatic vegetation is represented by floating and submerged communities of open water habitats and sublittoral and littoral communities [216, 222, 223].

The most important communities of freely floating plants (alliances *Lemnion* W. Koch et Tx. 1954, *Hydrocharition* Rübel 1933, and *Utricularion vulgaris* Pasarge 1964) are *Potameto pusilli-Ceratophylletum demersi* Jank. 1974 and *Ceratophylleto-Myriophylletum verticilati* Jank 1974. Dominating species of these communities are *Ceratophyllum demersum* L., *Myriophyllum verticillatum* L., *Potamogeton pusillus* L., *Uticularia vulgaris* L., and *Lemna trisulca* L. Leaf-floating species *Hydrocharis morsus-ranae* L., *Nymphoides flava* Hill, *Nymphaea alba* L., and *Salvinia natans* (L.) All. occur sporadically within the zone of submerged vegetation.

Sublittoral communities of rooted, leaf-floating plants (alliance *Nymphaeion albae* Oberd. 1957) are represented by associations *Nymphaeto-Stratiotetum aloidi* Jank. 1974, *Hydrocharideto-Nymphoidetum peltatae* Slavnić 1953, and *Nymphaetum alboluteae* Nowinski 1928, which is divided into subassociations *nymphaeetosum* (Timar) Karpati (in deep water) and *nupharetosum* (Timar) Karpati in shallow sublittoral zone. Dominating flotant species in these communities are *Nymphoides flava* Hill, *Nymphaea alba* L., *Salvinia natans* (L.) All., *Hydrocharis morsus-ranae* L., *Lemna minor* L., *Potamogeton natans* L., and *Stratiotes aloides* L. Submerged species that frequently occur in these communities are *Ceratophyllum demersum* L., *Myriophyllum verticillatum* L., *Myriophyllum spicatum* L., *Potamogeton fluitans* Roth., *Potamogeton crispus* L., etc.

Littoral communities (alliances *Phragmition* W. Koch 1926 and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957) involve associations *Scirpo-Phragmitetum* W. Koch 1926, *Acoretum calami* Schultz 1941, *Oenantho-Rorippetum* Lohmeyer 1950, and *Phragmiteto-Salicetum cinerei* Gigov. The most important species that belong to the groups of aquatic plants and helophytes are *Hydrocharis morsus-ranae* L., *Lemna trisulca* L., *Alisma plantago-aquatica* L., *Ceratophyllum demersum* L., *Ceratophyllum submersum* L., *Salvinia natans* (L.) All., *Spirodela polyrhiza* (L.) Schleiden, *Stratiotes aloides* L., *Nymphaea alba* L., *Oenanthe aquatica* (L.) Poiret in Lam., *Phragmites australis* (Cav.) Trin. ex Stendei, *Polygonum amphibium* L., *Polygonum hydropiper* L., *Rorippa amphibia* (L.) Besser, *Rumex hydrolapathum* Hudson, *Urtica kioviensis* Rogow., *Utricularia vulgaris* L., *Scirpus lacustris* L., *Solanum dulcamara* L., *Echinochloa crus-galli* (L.) P. B., *Glyceria maxima* (Hartman) Holomberg, *Lythrum salicaria* L., *Sparganium erectum* L., *Thelypteris palustris* Schott, *Typha angustifolia* L., etc. In 1915, the rare species *Aldrovanda vesiculosa* L. was discovered near Kupinovo, in the Obedska Bara Special Nature Reserve. However, since 1977 this species disappeared from this area [224].

Marshy vegetation is represented by the association *Phalaridetum arundinaceae* Labb, which occupies habitats with high level of groundwater and the association *Caricetum vulpinae-ripariae* R. Jov. which inhabits deep depressions with long-lasting floods.

Halophyte vegetation occupies salty ponds (alliances *Rupion maritimae* Br.-Bl. 1931 and *Bolboshoenion maritimi* Hejny). The most important associations of the halophyte vegetation within the Obedska Bara Reserve are *Parvipotamo-Zanichellietum pedicellatae* Soó (1934) 1962 and *Bolboschoenetum maritimi-continentale* Soó (1927) 1957.

The most frequent communities of wet meadows belong to the associations *Trifolio-Agrostietum stoloniferae* Marković 1973 and *Poo-Alopecuretum pratensis* R. Jov. 1957 [225].

Large complexes of wetland forests (Crni lug. Makiš, Ada Ciganlija, Ada Huja, Veliko Ratno ostrvo) are distributed within the (sub)urban area of Belgrade town, along the shores of Sava and Danube rivers [137–139, 226–234].

The process of alluvial sedimentation at the confluence of the Sava River resulted with a unique geomorphological formation of two river islands (Veliko and Malo Ratno ostrvo). The islands have been created by an underwater dune that emerged in the sixteenth century. The Island ***Veliko Ratno Ostrvo*** is protected as a landscape of outstanding features under the Serbian nature protection legislative. Despite large human impact, high percentage of the island is covered with temporary flooded forests. Due to human impacts and variable water table conditions, the forest communities have unstable structure, prone to successional change.

The most abundant tree species in wetland forests on the island are *Salix alba* L., *Populus alba* L., *Populus nigra* L., *Fraxinus excelsior* L., *Fraxinus pennsylvanica* Marshall, *Quercus robur* L., and *Ulmus minor* Miller. Other less frequent trees are *Morus nigra* L., *Acer campestre* L., and *Prunus cerasifera* Ehrh.

The scrub consists mostly of *Cornus sanguinea* L., *Crataegus monogyna* Jacq., *Prunus spinosa* L., *Corylus avellana* L., *Sambucus nigra* L., and other species. The willows, poplars, elms, and other trees and scrubs are covered with thickly intertwined stems of lianas *Vitis vinifera* subsp. *sylvestris* (C. C. Gmelin) Hegi, *Humulus lupulus* L., *Echinocystis lobata* (Michx) Torrey & A. Gray, and *Clematis vitalba* L.

The stratum of herbaceous plants is diverse. The most frequent species in this stratum are *Typha angustifolia* L., *Scirpus lacustris* L., *Stachys palustris* L., *Symphytum officinale* L., *Solanum dulcamara* L., *Solanum nigrum* L., *Rubus caesius* L., *Rumex conglomeratus* Murray, *Rumex crispus* L., *Rumex obtusifolius* L., *Rumex stenophyllus* Ledeb., *Chenopodium album* L., *Bidens tripartita* L., *Sambucus ebulus* L., *Lycopus europaeus* L., *Lysimachia vulgaris* L., *Lythrum salicaria* L., *Malva sylvestris* L., *Polygonum amphibium* L., *Polygonum aviculare* L., *Polygonum hydropiper* L., *Polygonum lapathifolium* L., *Polygonum mite* Schrank, *Polygonum persicaria* L., *Mentha aquatica* L., *Mentha longifolia* L., *Rorippa amphibia* (L.) Besser, *Rorippa sylvestris* (L.) Besser, etc. The invasive alien plants *Echinocystis lobata* (Michx) Torrey & A. Gray, *Amorpha fruticosa* L., *Xanthium strumarium* subsp. *italicum* (Moretti) D. Löve, and *Reynoutria japonica* Houtt. are frequent in the willow-poplar forests on the Veliko Ratno ostrvo. Despite unstable structure and high anthropogenic influence, the forests of the Veliko Ratno ostrvo are important seminatural ecosystems. Tall trees of these forests are nesting sites for many rare and endangered bird species such as the white-tailed eagle.

Floodplains along the estuaries of Kupa, Una, Vrbas, Bosna, and Drina rivers are meliorated and transformed into large complexes of arable land and orchards. Due to high anthropogenic pressure, the wetland vegetation of these regions is reduced significantly. Small fragments of riparian forests (alliances *Salicion albae* Soó 1930, *Populion albae* Br.-Bl. 1931), flooded forests (*Anlo-Quercion roboris* Horvat 1938), swamp forests (*Alnion glutinosae* Malcut 1929), marshy vegetation (alliances *Phragmition* W. Koch 1926 and *Sparganio-Glycerion fluitantis* Br.-Bl. et Siss. in Boer 1942, nom. inv. Oberd. 1957, *Magnocaricion elatae* W. Koch 1926, and *Caricion gracilis* Neuhäusl 1959) are degraded by meliorative activities and development of flood defense systems for urban and industrial facilities and arable land.

Pioneer and ruderal communities (alliances *Nanocyperion* W. Koch 1926, *Oenanthion aquaticae* Heijný *ex* Neuhäusl 1959, *Bidention tripartitae* Nordhagen 1940 em. Tüxen in Poli & J. Tüxen, and *Chenopodion rubri* (Tüxen ex Poli & J. Tüxen 1960) Kopecký), as well as seminatural tall-herb riparian communities (alliances *Convolvulion sepii* R. Tüxen 1947 *in* Oberd. 1957 and *Filipendulion ulmariae* Segal 1966) are developed along riverbanks.

Contrary to floodplain sectors, the mountainous sectors of Kupa, Una, Vrbas, Bosna, and Drina rivers are less exposed to anthropogenic influence. The riparian zone of the mountainous sectors of these rivers occupies alder forests (Alnenion glutinoso-incanae Oberd. 1953 communities, of the alliance Alno-Ulmion Braun-Blanq. & Tüxen ex Tchou 1948), willow shrubs (alliances Salicion incanae Aich., 1933 and Salicion eleagno-daphnoidis (Moor 1958) Grass 1993) and tall-herb and scrub communities of montane-subalpine riverine gravel terraces (Adenostylion alliariae Br.-Bl. 1926).

Numerous canyons and gorges of upper sectors of Bosna and Drina rivers and their tributaries represent significant biodiversity hotspots of endemo-relic vegetation [208, 209, 235–244].

Karst springs in Igman Mountain represent the source of the ***Bosna River***. Canyons of Bosna River and their tributaries (Misoča, Stavnja, Lašva, Stupčanica, Krivaja, Usora Spreča) are refugial habitats for endemo-relic pine forests (the syntaxonomic class *Erico carneae-Pinetea nigrae-sylvestris* Horvat 1959), hornbeam forests (alliance *Ostryo carpinifoliae-Carpinion orientalis* Horvat 1954 of the class *Quercetea pubescentis* Doing-Kraft ex Scamoni & Passarge 1959), and saxatile vegetation (classes *Asplenietea trichomanis* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberd. 1977 and *Thlaspietea rotundifoliae* Br.-Bl. 1948).

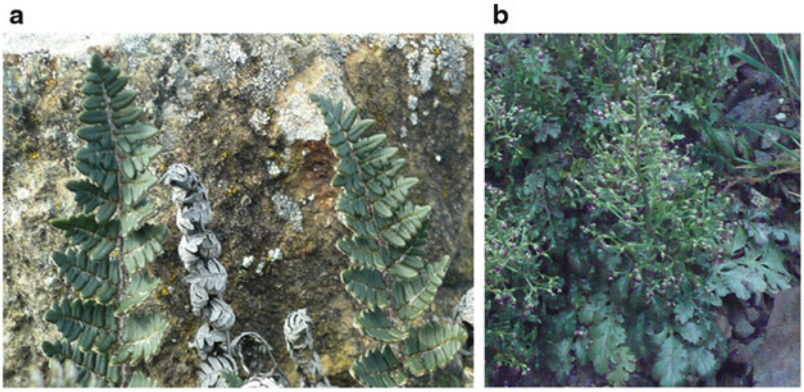

Fig. 2 Important serpentinophytes in canyons and gorges of Bosna and Drina rivers and their tributaries. (**a**) *Notholaena marantae* (L.) Desv (**b**) *Scrophularia tristis* K. Maly

The syntaxonomic class *Erico carneae-Pinetea nigrae-sylvestris* Horvat 1959 is inadequately denoted as *Erico-Pinetea* Horvat 1959 *nom. inval.* (art. 2d, 5), or *Erico carneae-Pinetea nigrae-sylvestris* Horvat 1959 s. syn. [9]. The communities of the class *Erico carneae-Pinetea nigrae-sylvestris* Horvat 1959 involve forests of Balkan relict woods of Scots pine (*Pinus sylvestris*) and black pine (*Pinus nigra*) on ultramafic rocks and dolomites (*Fraxino orni-Ericion carneae* Horvat 1958) and central and southern Balkan open *Pinus nigra* woods on calcareous substrates (*Fraxino orni-Pinion nigrae* Em (1972) 1978).

The canyons of the Bosna River and its tributaries are located within the ophiolitic complex of old volcanic ultramafic rocks (periodite, serpentinite, dunite, amphibolite, piroxen). The serpentine soils are characterized by low levels of the essential plant elements (nitrogen, phosphorus, potassium, and calcium), as well as high levels of iron, magnesium, and manganese, and toxic levels of chromium, cobalt, and nickel [245–251]. Due to the "serpentine stress" (toxic effects of heavy metals, nutrient shortages, and droughts), most plant species avoid serpentine soils. A small percent of serpentine-tolerant taxa has evolved morpho-anatomical and physiological adaptations that allow them to survive in extremely unfavorable conditions.

Strong selective pressures of serpentine soil and spatial isolation of serpentine regions resulted with high percent of endemic serpentinophyte taxa in the Balkans, Scandinavian Peninsula, Britain, Ural, California, etc. The serpentine flora in Balkan Peninsula has been investigated by Krause and Ludwig [252, 253], Krause and Klement [254], Krause et al. [255], Ritter-Studnička [256], Babalonas [257, 258], Tatić and Veljović [259], Pavlova et al. [260], and Pavlova [261].

The most important endemic serpentinophytes in canyons and gorges of Bosna and Drina rivers and their tributaries (Fig. 2) are *Halacsya sendtneri* (Boiss.) Doerfl., *Potentilla visianii* Pančić., *Scrophularia tristis* K. Maly, *Sesleria latifolia* (Adam.) Degen var. *serpentinica* Deyl., *Alyssum markgrafii* O. E. Schulz., *Linaria concolor* Gris. f. *rubioides* (Vis. et Panc.) Maly, *Potentilla rupestris* L. var. *mollis* (Panč.) A. et G., *Polygonum albanicum* Jav., *Euphorbia glabriflora* Vis., *Potentilla opaca* Jusl. f. *malyana* (Borb.) Hayek, *Cytisus heuffelii* Wierzb. var. *maezeius* K. Maly, *Asplenium cuneifolium* Viv., *Notholaena marantae* (L.) Desv., *Fumana bonapartei* Maire et Petitm., *Haplophyllum boissierianum* Vis. et Panč., *Gypsophila spergulaefolia* Gris. f. *Serbica* Vis. et Panč., etc.

Drina River is the most important and the largest tributary of the Sava River. Drina River is created of two rivers: Piva and Tara River, originating from Montenegro, with confluence on the location "Šćepan Polje." The most important tributaries of the Drina River are Janja, Drinjača, Žepa, Prača, Bistrica, Sutjeska, Piva, Jadar, Lim, Rzav, Ćehotina, and the Tara River.

Upper course of the Drina River is a torrential section from Šćepan Polje to the Lim River mouth. In its middle course (the section from Lim River mouth to Zvornik), the Drina River is a large, fast-flowing river. Due to high incination (fall or elevation difference of 161 m along 174 km), this section of the Drina River has significant hydroenergy potential, used for construction of hydropower plants Višegrad, Bajina Bašta, and Zvornik. In its lower course, Drina River is getting all characteristics of a large lowland river.

High biodiversity and presence of many endemic and rare species are the main characteristics of paleo-endemic communities in numerous gorges and canyons of the Drina River and its tributaries. These characteristics may be explained by the stability of environmental conditions within canyon habitats, in both recent and historical terms.

Both human-induced and climate-induced disturbances of habitats are minimized in canyons [208, 244]. The canyon habitats are economically inefficient and expensive for exploitation. This prevented human-induced disturbance of vegetation in canyons and gorges that are located in central parts of the Balkan Peninsula.

Moreover, the significant climate-induced disturbances that occurred during the repeated glaciations and interglacial periods are low in canyons. The specific orography of canyons and gorges modifies radiant energy of insolation, increases air humidity, and attenuates hygrothermic extremes. Due to such microclimate conditions, canyons and gorges have represented refugia for many tertiary species that had migrated southwards during glacial periods.

Refugial habitats along the Drina River and its tributaries represent a valuable pool of endemic taxa. The most important paleo-endemic species in this region is steno-endemic Serbian spruce *Picea omorika* (Pančić) Purkyne (Fig. 3).

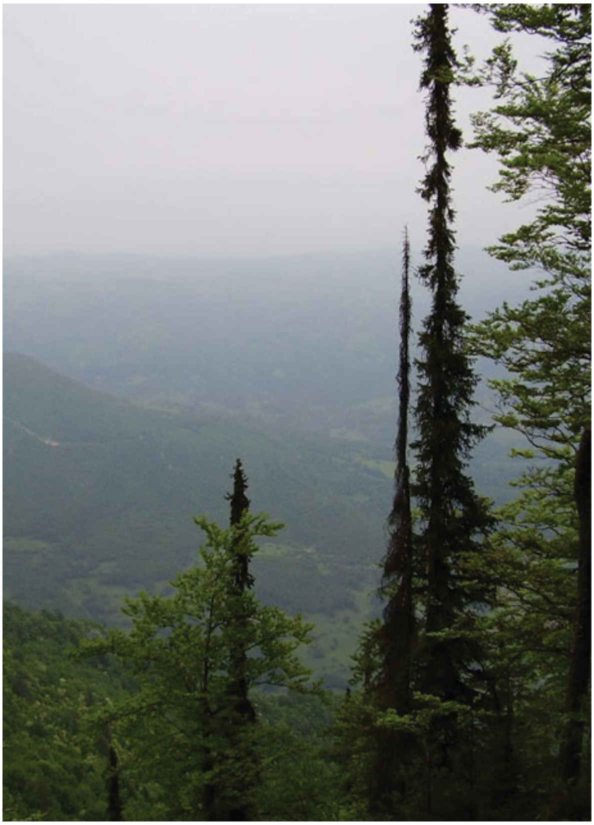

Fig. 3 *Picea omorika* (Pančić) Purkyne in the Drina River canyon

Another very important tertiary relic is oap hornbeam (*Ostrya carpinifolia* Scop.). Distribution of *Ostrya carpinifolia* comprises Apennines, Tyrol, western parts of the Balkan Peninsula, Asia Minor, and Lebanon [262–264]. The closest aliens of this species are distributed in East Asia and North as well as Central America [265]. Such distribution clearly reflects a tertiary disjunction of the genus *Ostrya* Scop. Due to a broad ecological tolerance, the black hornbeam *(Ostrya carpinifolia* Scop.) (co)dominates in extremely different communities. Considering the Balkan Peninsula, these communities may be included in five syntaxonomic orders: *Quercion ilicis* Br.-Bl. 1931, *Ostryo-Carpiaion orientalis* Horv. 1954, *Quercion frainetto* Horv. 1954, *Ostryo-Fagion* Borh. 1963, and *Orno-Ericion* Horv. 1959 [57, 63, 265]. Regardless on a great ecological plasticity, especially with respect to light and soil conditions, the black hornbeam is a thermophilous species which occurs mainly in habitats with increased air humidity [209, 266, 267].

Forests of oap hornbeam are widely distributed in canyons along the Drina River and its tributaries. These forests belong to the thermophilous deciduous forests of the order *Quercetalia pubescentis* Klika 1933. Čarni et al. [268] divided thermophilous deciduous forests in the northwestern part of Southeastern Europe into alliances *Quercion pubescenti-sessiliflorae* Br.-Bl 1932, *Aceri tatarici-Quercion* Zólyomi *et* Jakucs 1957, *Quercion confertae* Horvat 1954, *Quercion petraeae-cerris* (Lakušić *et* Jovanović 1980) Čarni et al. 2009, *Syringo-Carpinion orientalis* Jakucs 1959, *Carpinon orientalis* Horvat 1954, and *Fraxino orni-Ostryion carpinifoliae* Tomažič 1940. The group of thermophilous forests dominated by *Ostrya carpinifolia* (*Fraxino orni-Ostryion carpinifoliae* Tomažič 1940) is found in the inner part of the mountain chains along the Adriatic coast at higher altitudes showing some similarities to the vegetation of the *Erico-Pinetea* Horvat 1959.

In continental parts of the Southeastern Europe, *Ostrya carpinifolia* forms complex extrazonal forests, mainly in canyons and gorges. A polydominant structure and biogeographic complexity are main characteristics of these forests. Polydominancy of these communities is a consequence of both the richness of phanerophytes and high evenness or equitability of species importance values. Karadžić et al. [209] recorded 30 different trees and shrubs in these forests. In various combinations with other trees (*Juglans regia* L., *Fraxinus ornus* L., *Carpinus betulus* L., *Carpinus orientalis* L., *Quercus cerris L.*, *Quercus petraea*, *Quercus pubescens*, *Fagus sylvatica* L., *Tilia platyphyllos* Scop., etc.), the black hornbeam forms mosaic-like patters with a large proportion of species with overlapping distribution. Some of phanerophytes have very restricted distribution (endemic taxa) and/or low population densities (rare and endangered species), such as *Rhamnus saxatilis* Jacq., *Rhamnus fallax* Boiss., *Frangula rupestris* (Scop.) Schur., *Chamaecytisus leiocarpus* (Kern.) Rothm., *Spiraea media* Fr. Schm., *Cotinus coggygria* Scop., *Cotoneaster tomentosa* (Ait.) Lindl., *Daphne alpina* L., *Euonymus verrucosa* Scop., *Staphylea pinnata* L., *Viburnum lantana* L., etc.

Herbaceous plants also belong to the group of endemic and/or endangered species. Most important among them are *Campanula secundiflora* Vis. & Pančić, *Lathyrus binatus* Pančić, *Centaurea derventana* Pančić, *Hieracium waldsteinii* Tausch, *Melampyrum heracleoticum* Boiss. & Orph., *Melampyrum hoermannianum* K. Maly, *Minuartia bosniaca* (G. Beck) K. Maly, *Centaurea grisebachii* (Nyman) Form., *Cerastium decalvans* Schlosser & Vuk., *Hieracium gymnocephalum* Griseb. ex Pant., *Onosma stellulata* Waldst. & Kit., *Stachys anisochila* Vis. & Pančić, *Erysimum linariifolium* Tausch, *Euphorbia subhastata* Vis. & Pančić, *Athamanta turbith* (L.) Brot. subsp. *haynaldii* (Borbas & Uechtr.) Tutin, *Dianthus petraeus* Wald. *et* Kit., *Polygala murbeckii* Deg., *Arabis procurrens* Wald. *et* Kit. etc. (Fig. 4).

Fig. 4 Endemic species in canyons and gorges of the Drina River and its tributaries. (**a**) *Lathyrus binatus* Pančić, (**b**) *Athamanta turbith* (L.) Brot. subsp. *haynaldii* (Borbas & Uechtr.) Tutin, (**c**) *Corydalis ochroleuca* Koch subsp *leiosperma* (Conr.) Hayek, and (**d**) *Onosma stellulata* Waldst. & Kit.

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Zooplankton Community Along the Sava River

Aleksandar M. Ostojić, Ivana D. Radojević, and Anita Galir Balkić

Abstract The aim of this study was to examine for the first time the composition of zooplankton community along the greatest part of the Sava River flow. Eighty two zooplankton taxa were collected at the Sava River in September of 2012, 7 Rhizopoda, 8 Ciliophora, 57 Rotifera, 7 Cladocera, 2 Copepoda, and 1 Bivalvia. The number of zooplankton species found at sampling sites varied between 2 and 30. The most diverse group was Rotifera, which comprised 69 % of the total number of recorded taxa. The abundance of zooplankton was low and the abundance of individual zooplankton communities varied between 1 and 36 ind/L, and these results are in accordance with the results of previous works. The similarity indices (Sørensen's and Jaccard's) between the localities studied were rather low, despite relatively close distances between them. The probable reason was that the sites were localized at the sections of the river characterized by different environmental factors.

Keywords Community structure • Diversity • Large rivers • Sava River • Zooplankton

1 Introduction

Zooplankton communities are more commonly studied in lake than in river ecosystems [1]. One of the reasons is that, as a rule, the composition and abundance of zooplankton are poorer in running waters. Also, in the upper flows, where the velocity is high and the depth low, typical zooplankton species are often absent. Plankton, in large rivers, is only important when residence time allows enough time for growth and reproduction [2]. Zooplankton communities can only develop in rivers exceeding the length of about 500–700 km, because species growth requires a certain time period. The life span of rotifers is 12 days, and they can reach their

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peak reproductive level in about 3.5 days, while cladocerans and copepods have longer and similar life spans (approximately 50 days). There are differences between their peaks; cladocerans require 14–15 days while copepods require 24 days [3]. When river flow is high and water-level fluctuations are large and without the necessary depth, zooplankton will be destroyed due to frictions against the bank, riverbed, and plants [4]. Therefore, well-developed zooplankton communities of large rivers are usually concentrated in lower flows, where the water flow is slower and the depth higher and where the macrophyte vegetation is dense (it is used as a shelter from predators).

The Sava River, even though the largest river in the territory of former Yugoslavia, has rarely been studied as far as zooplankton is concerned. Zooplankton studies refer to the artificial lakes formed on its course [5–9] and the stagnant waters along the riverbanks [10], or it was the subject of saprobiological analysis [11–17]. There are very few data about the composition and abundance of zooplankton in the river itself. Such data are obtained only from the part flowing through Serbia [18].

The present study includes, for the first time, the greatest part of the Sava River flow. The results of the analysis represent a significant contribution to the knowledge of species composition of zooplankton in the Sava River, as well as of the changes in the composition and abundance of zooplankton along its flow.

2 Materials and Methods

A one-time hydrobiological investigation of the Sava River was carried out in September of 2012. Samplings were conducted at 14 sampling sites (Slovenia, (1) Hrastnik, (2) below the dam HPP Vrhovo, (3) below the dam Blanca, (4) Krško, downstream the bridge, (5) Brežice, bridge; Croatia, (6) Rugvica, (7) Lukavec Posavski, (8) Mlaka, (9) Slavonski Brod, (10) Štitar; Serbia, (11) mouth of the river Bosut, (12) Sremska Mitrovica, (13) Jarak, (14) Makiš).

Qualitative zooplankton samples were collected using a plankton net (Ø40 cm) of mesh size 40 μ. A Carl Zeiss light microscope was used for the identification of zooplankton, at 100–400× magnification. Quantitative samples (10 L) were collected using 1-L Ruttner hydrobiological bottles (below water surface) and then filtered across a plankton net. The collected material was quantitatively transferred to sample storage bottles. The samples were fixed immediately with 4 % formalin. The sample volume was adjusted to 100 mL storage bottles. For quantitative analysis of zooplankton, Utermöhl's inverted microscope method was applied. Sedimentation chambers, 50 mL in volume, were used for counting individuals. The animals were counted in the whole sample. Qualitative and quantitative analyses of the collected material were performed in the laboratory at the Institute of Biology and Ecology of the Faculty of Science, University of Kragujevac. Qualitative analysis was carried out down to species level or to genus level where it was impossible to identify the species. Specimens were determined using the identification keys by Bartoš [19], Dussart [20], Flössner [21], Hofrat and Ottendorfer [22], Koste [23], and Šramek-Hušek et al. [24].

Similarity among the samples was calculated using the Sørensen index (SI), $SI = 2c/(a + b)$, where a and b are the number of species in samples a and b, respectively, and c is the number of species shared by the two samples [25]. As control we used the Jaccard index (C_j) [26], $C_j = c/(a + b - c)$, where a is the number of species present in one sample, b is the number of species present in the other sample, and c is the number of species present in both samples.

Hierarchical cluster analysis was conducted to show the relationship between the sampling points and taxa [27]. Cluster method represents average linkage between groups. For binary data (presence-absence), the Jaccard measure was used. The analysis was performed using the SPSS package. The result was presented as a cluster dendrogram.

3 Results

During the qualitative analysis of the zooplankton samples, from the Sava River, 82 taxa were identified: 7 Rhizopoda (9 % of all identified zooplankton taxa), 8 Ciliophora (10 %), 57 Rotifera (69 %), 7 Cladocera (9 %), 2 Copepoda (2 %), and 1 Bivalvia (1 %) (Table 1). Apart from these, the samples also included representatives of groups typical for benthos and periphyton and which were, by water currents, flown into planktons: Nematoda, Gastrotricha, Oligochaeta, Tardigrada, and Ostracoda.

The number of zooplankton species found at sampling sites varied between 2 and 30. The greatest diversity was recorded within the samples from Slovenia and the lowest within the samples from Serbia (Fig. 1). Specifically, the highest numbers of taxa were recorded in locations 1 (beneath the dam of the hydropower plant of Vrhovo in Slovenia) and 6 (Rugvica in Croatia), 30 taxa each, and the lowest in location 14 (Makiš in Serbia), only 2 taxa (Table 1). Rhizopoda were more diverse in localities 1 and 2 (5 taxa each), while they were not represented by any taxon in locality 14. Ciliophora exhibited the greatest diversity in localities 1 and 6 (5 taxa each), and no taxon was recorded in locality 11. Rotifera were the most diverse group. The greatest number of taxa was recorded in locality 6 (21 taxa) and the lowest in locality 14 (only 1 taxon). For both Cladocera and Copepoda, the number of taxa by localities was very low, with only one or two taxa per location recorded at all. However, the larval stages of Copepoda were recorded in almost all samples, except in localities 4, 5, 11, and 14. In addition to these common zooplankton taxa, the larval stages of *Dreissena polymorpha* (Bivalvia) were recorded in localities 3, 5, and 8. Interestingly, not a single sample from localities in Serbia contained Cladocera, adult Copepoda, and larvae *D. polymorpha*.

The most dominant according to number of taxa, in almost all locations, is Rotifera, with usually more than 50 % of the recorded taxa (Fig. 2). Its greatest dominance is recorded in locations 11 (75 %) and 6 (70 %), and only in three

Table 1 Qualitative composition of zooplankton in the Sava River, September 2012

	Slovenia					Croatia				Serbia				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Rhizopoda														
Arcella sp.	+	+	+	+	+	+	+		+	+		+	+	
Centropyxis aculeata (Ehrenberg, 1832)	+					+			+					
Cyphoderia ampulla (Leydi, 1879)	+		+	+	+	+								
Cyphoderia sp.		+												
Difflugia corona (Wallich, 1864)		+												
Difflugia sp.	+	+	+	+	+	+	+	+	+		+	+		
Euglypha sp.	+	+	+	+	+		+							
Ciliophora														
Epistylis sp.	+		+		+	+								
Paramecium caudatum (Ehrenberg, 1833)						+								
Paramecium sp.	+	+	+				+			+		+		
Stylonychia sp.	+					+	+		+			+		
Tintinnidium fluviatile (Stein, 1863)		+					+	+	+			+	+	+
Tintinnopsis lacustris (Entz, 1901)	+	+	+		+	+	+		+			+	+	
Tokophrya sp.			+	+										
Vorticella sp.	+	+			+	+			+					
Rotifera														
Anuraeopsis fissa (Gosse, 1851)						+								
Ascomorpha saltans (Bartsch, 1870)										+				
Bdelloidea	+	+	+		+	+	+		+	+	+	+		+
Brachionus calyciflorus (Pallas, 1766)												+		
Brachionus diversicornis var. *homoceros* (Daday, 1883)	+													
Brachionus falcatus (Zacharias, 1898)							+							
Brachionus forficula (Wierzejski, 1891)						+	+	+		+		+		
Cephalodella catalina (Muller, 1786)	+	+	+									+	+	
Cephalodella gibba (Ehrenberg, 1830)			+						+					

(continued)

Table 1 (continued)

	Slovenia					Croatia				Serbia				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cephalodella tenuior (Gosse, 1886)						+								
Cephalodella sp.		+	+		+				+	+				
Colurella adriatica (Ehrenberg, 1831)		+												
Colurella colurus (Ehrenberg, 1830)	+	+	+		+									
Colurella obtusa (Gosse, 1886)						+	+		+	+		+		
Colurella uncinata (Muller, 1773)					+									
Colurella uncinata bicuspidata (Ehrenberg, 1832)						+								
Epiphanes macroura (Barrois and Daday, 1894)						+								
Euchlanis deflexa (Gosse, 1851)							+							
Euchlanis dilatata (Ehrenberg, 1832)					+							+	+	
Euchlanis dilatata lucksiana (Hauer, 1930)						+								
Filinia brachiata (Rousselet, 1901)		+												
Filinia longiseta (Ehrenberg, 1834)	+	+												
Filinia passa (Muller, 1786)							+							
Keratella cochlearis (Gosse, 1851)	+	+	+	+		+	+	+	+		+	+	+	
Keratella cochlearis tecta (Gosse, 1851)	+	+	+	+		+	+	+				+		
Keratella irregularis (Lauterborn, 1898)	+													
Keratella quadrata (Müller, 1786)						+								
Keratella testudo (Ehrenberg, 1832)	+					+								
Lecane (L.) flexilis (Gosse, 1886)	+													
Lecane (L.) inermis (Bryce, 1892)	+	+												

(continued)

Table 1 (continued)

	Slovenia					Croatia				Serbia				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Lecane (L.) luna (Müller, 1776)						+				+				
Lecane (L.) nana (Murray, 1913)			+				+		+					
Lecane (L.) tenuiseta (Harring, 1914)			+											
Lecane (M.) bulla (Gosse, 1851)	+	+				+	+							
Lecane (M.) closterocerca (Schmarda, 1859)		+				+	+							
Lecane (M.) hamata (Stokes, 1896)							+							
Lecane (M.) lunaris (Ehrenberg, 1832)	+	+	+			+								
Lecane (M.) piriformis (Daday, 1905)	+													
Lecane (M.) quadridentata (Ehrenberg, 1830)												+		
Lepadella patella (Müller, 1773)	+		+			+	+						+	
Monommata caudata (Myers, 1930)									+					
Monommata dentata (Wulfert, 1940)										+				
Mytilina mucronata (Müller, 1773)							+							
Notholca squamula (Müller, 1786)						+								
Platyias quadricornis (Ehrenberg, 1832)									+					
Polyarthra dolichoptera (Idelson, 1925)	+	+	+								+			
Polyarthra vulgaris (Carlin, 1943)				+										
Rotaria neptunia (Ehrenberg, 1830)		+												
Synchaeta sp.		+				+						+		
Testudinella patina (Hermann, 1783)						+								
Trichocerca brachyura (Gosse, 1851)							+							

(continued)

Table 1 (continued)

	Slovenia					Croatia				Serbia				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Trichocerca cylindrica (Imhof, 1891)		+												
Trichocerca elongata (Gosse, 1886)						+								
Trichocerca longiseta (Schrank, 1802)	+						+							
Trichocerca pussila (Jennings, 1903)		+			+	+								
Trichocerca similis (Wierzejski, 1893)									+					
Trichocerca stylata (Gosse, 1851)		+												
Cladocera														
Alona affinis (Leydig, 1860)			+		+									
Alona costata (Sars, 1862)		+												
Bosmina coregoni (Baird, 1857)	+													
Bosmina longirostris (O.F. Müller, 1776)	+	+			+									
Camptocercus rectirostris (Schödler, 1862)								+						
Chydorus sphaericus (O.F. Müller, 1776)							+	+						
Pleuroxus uncinatus (Baird, 1850)			+											
Copepoda														
Acanthocyclops sp.									+					
Bryocamptus sp.				+			+		+	+				
Nauplius calanoida	+	+						+	+	+		+		
Nauplius cyclopoida	+	+	+			+							+	
Nauplius harpacticoida		+												
Copepoda cyclopoida							+	+				+		
Bivalvia														
Dreissena polymorpha			+		+			+						
Total taxa	29	30	23	9	16	30	25	8	18	10	4	16	7	2
Abundance (ind/L)	32	1	4	2	8	12	16	11	10	2	6	30	36	–

Sampling points—Slovenia, (1) Hrastnik, (2) below the dam HPP Vrhovo, (3) below the dam Blanca, (4) Krško, downstream the bridge, (5) Brežice, bridge; Croatia, (6) Rugvica, (7) Lukavec Posavski, (8) Mlaka, (9) Slavonski Brod, (10) Štitar; Serbia, (11) mouth of the river Bosut, (12) Sremska Mitrovica, (13) Jarak, (14) Makiš

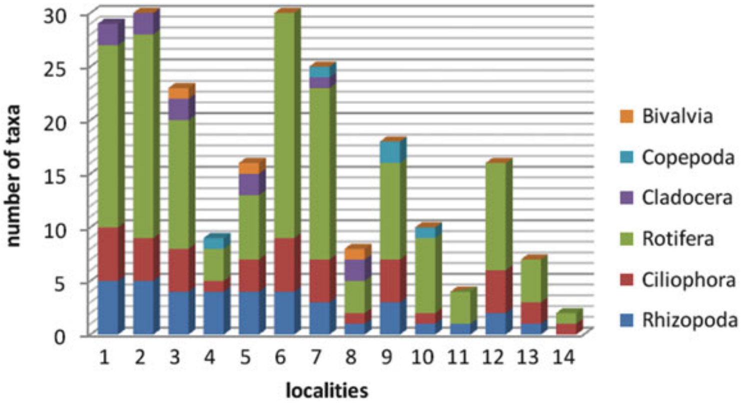

Fig. 1 The number of zooplankton species found at sampling points 1–14 (see Sect. 2)

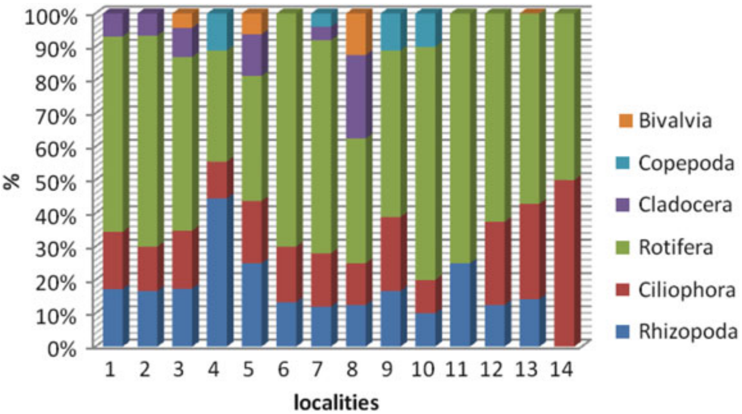

Fig. 2 Percentage of recorded zooplankton taxa by sampling points in the Sava River

localities (4, 5, and 8) is their recorded presence below 50 %. The only two localities where Rotifera were not the most diverse group are locality 4, where the presence of recorded Protozoa was 56 % (Rhizopoda 45 %, Ciliophora 11 %), and locality 5, where the presence of recorded Protozoa was 44 % (Rhizopoda 25 %, Ciliophora 19 %).

The greatest number of recorded taxa is found periodically, in smaller number of localities. Several taxa were recorded in more than 50 % of localities (Table 1): *Arcella* sp., *Difflugia* sp., *Tintinnidium fluviatile*, *Tintinnopsis lacustris*, *Keratella cochlearis*, *Keratella tecta*, and Philodinidae.

As far as the abundance of zooplanktons is concerned, it was very low and did not exceed 40 ind/L in any of the localities (Table 1). The abundance of individual zooplankton communities varied between 1 and 36 ind/L. The lowest individual numbers were measured in sampling point 2 (only 1 ind/L). The highest individual numbers were registered in sampling point 1 (32 ind/L) and two localities in Serbia (sampling point 12–30 ind/L, sampling point 13–36 ind/L). At the same time, it is interesting that in quantitative samples from sampling point 14 (Makiš, Serbia), not a single representative of zooplankton was recorded.

Since the abundance of recorded zooplanktons was very small, only the data obtained by the analysis of qualitative samples were used to analyze the similarity indices. Based on the common taxa, the Sørensen (Table 2) and Jaccard similarity indices were calculated (Table 3). The obtained data show a few similarities between the researched localities. The values higher than 50 % for Sørensen index of similarity and the values higher than 30 % for Jaccard index of similarity were recorded only while comparing sampling point 1 with sampling points 2, 3, and 6, sampling point 3 with sampling point 5, sampling point 9 with sampling point 12, and sampling point 12 with sampling point 13 (Tables 2 and 3). The tables also show that sampling points 4 and 14 do not have common taxa.

Figure 3 represents a cluster dendrogram of researched sampling points 1–14 (see Sect. 2) based on qualitative analysis of zooplankton communities. Two separate groups of clusters are noticeable in Fig. 3. The first group connects sampling points 1 and 2 with sampling points 3 and 5, as well as sampling point 6. The second group connects sampling points 7 and 12 with sampling points 9, 8, and 10. According to the qualitative composition of zooplankton, the lowest connection showed the sampling sites 13 and 4 and 11 and 14, respectively.

4 Discussion

The zooplankton composition identified in the Sava River during the present study in terms of higher taxonomic groups has also been recorded in previous studies, but without lists of species [14–17]. Nevertheless, the number of recorded taxa (82) was much higher than the previously obtained results. In the past, researches did not include the whole river flow of the Sava River, not even its greatest part. Thus, Djurkovic et al. [18] recorded 42 taxa in the Sava River, but only in the Serbian part.

The dominance of Rotifera and Protozoa according to the number of taxa was also previously recorded [18]. The explicit dominance of Rotifera (69 % of total number of taxa, Fig. 2) is in accordance with the well-known fact that in large rivers they have a much higher number of taxa and much greater density than Cladocera and Copepoda [28] and that the contribution of rotifers in zooplankton communities often exceeds 70 % [29].

In Rotifera group there are several genera represented with higher number of species: *Brachionus*, *Cephalodella*, *Colurella*, *Euchlanis*, *Filinia*, *Keratella*, *Lecane*, and *Trichocerca* (Table 1). This corresponds with the data of Djurkovic et al. [18], as well as with the data recorded by Gulyás [4], who researched zooplankton of the Danube and several of its tributaries (Sava included). The dominance of the representatives of these genera was also recorded in other European rivers [29–33].

Table 2 Sørensen's similarity index (SI) (1–14 sampling points, see Table 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.57	0.58	0.26	0.44	0.50	0.44	0.16	0.34	0.15	0.24	0.36	0.28	0.06
2			0.40	0.25	0.38	0.39	0.39	0.21	0.29	0.20	0.23	0.43	0.26	0.12
3				0.44	0.58	0.37	0.42	0.26	0.39	0.24	0.30	0.41	0.33	0.08
4					0.32	0.25	0.35	0.35	0.30	0.21	0.31	0.32	0.25	–
5						0.34	0.25	0.17	0.35	0.23	0.20	0.32	0.26	0.11
6							0.43	0.21	0.37	0.24	0.17	0.43	0.21	0.06
7								0.37	0.46	0.34	0.21	0.47	0.31	0.15
8									0.23	0.11	0.33	0.42	0.27	0.20
9										0.39	0.27	0.50	0.32	0.20
10											0.14	0.38	0.11	0.17
11												0.30	0.18	0.33
12													0.52	0.22
13														0.22

Table 3 Jaccard's similarity index (C_j) (1–14 sampling points, see Table 1)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.41	0.41	0.15	0.29	0.33	0.37	0.09	0.20	0.08	0.14	0.22	0.16	0.03
2			0.32	0.14	0.24	0.19	0.24	0.11	0.17	0.11	0.13	0.27	0.15	0.06
3				0.28	0.39	0.23	0.26	0.15	0.24	0.14	0.17	0.26	0.20	0.04
4					0.19	0.14	0.21	0.21	0.17	0.12	0.18	0.19	0.14	–
5						0.21	0.14	0.09	0.21	0.13	0.11	0.19	0.15	0.06
6							0.27	0.11	0.23	0.14	0.09	0.27	0.18	0.03
7								0.22	0.30	0.21	0.12	0.37	0.19	0.08
8									0.13	0.06	0.20	0.26	0.15	0.11
9										0.22	0.16	0.31	0.19	0.11
10											0.08	0.24	0.06	0.09
11												0.18	0.10	0.20
12													0.35	0.13
13														0.13

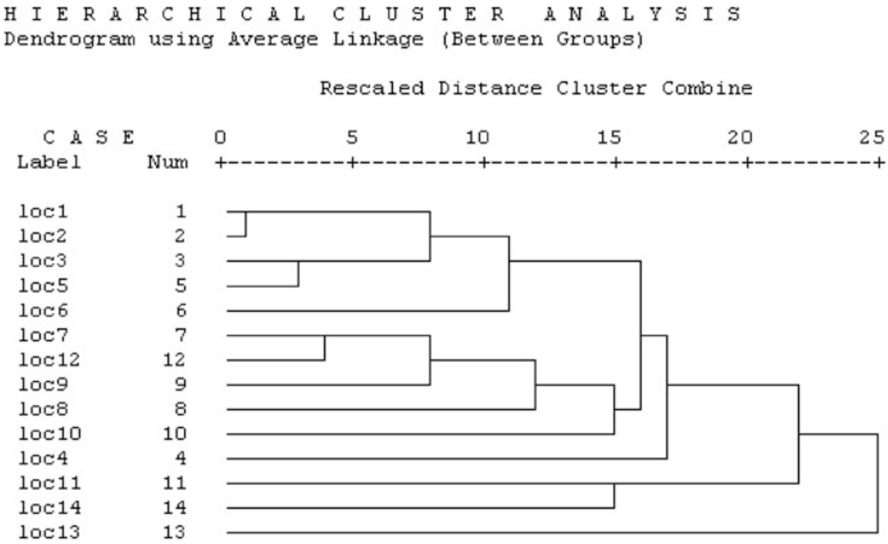

Fig. 3 Cluster analysis of researched sampling points 1–14 (see Sect. 2) based on qualitative analysis of zooplankton communities in the Sava River. Cluster dendrogram showing groupings of sampling points based on qualitative analysis of zooplankton communities

It is rather interesting that among the planktonic crustaceans no high individual number of species was found. Their poor diversity might be caused by suspended solids [4], plenty of which were found during this research (see chapter Climate Projections for the Sava River Basin). Thorp and Mantovani [34] hypothesize that rotifers indirectly benefit from river turbidity because their food competitors (Cladocera) and predators (e.g., cyclopoid copepods and visually feeding fish) are relatively more susceptible to suspended sediments. The second reason is fish predation [29]. Jack and Thorp [35] report that fish are selective in feeding, preying on more often larger planktonic Crustacea, which makes their influence on Rotifera rather small.

The occurrence of larval stages of invasive species *Dreissena polymorpha* is the appearance which was also previously identified [16–18]. Its spread upstream of the confluence of the Sava and the Danube is facilitated by the fact that the Sava is navigable for most parts of its flow, which made possible for other invasive species, such as some Pontocaspian amphipods, to colonize habitats at even few hundreds of kilometers away from the confluence of the Sava and the Danube [36].

The composition and abundance of zooplankton are influenced by a number of factors, both abiotic, physical (light), chemical (nutrient concentrations), and hydrological (current velocity and discharge), and biotic, phytoplankton production and fish predation [29]. This should be taken into account when analyzing the differences in the composition and production of zooplankton in individual sections of rivers.

During the research period, the qualitative composition of zooplankton of the Sava varied according to localities. The notably smaller number of taxa recorded in Serbia (18) than in Slovenia (53) and Croatia (52) is most probably the consequence of stronger anthropogenic influence as well as of higher water level.

Even though it would be expected that the highest diversity and abundance were in the lower course of the river, the most diverse compositions of zooplankton were recorded in the upper and middle courses of the Sava River. There are differences even between localities with greatest numbers of recorded species (localities 2 and 6 with 30 recorded taxa each); in locality 2 the riverbank is arranged, rocky, and with no vegetation, while locality 6 includes developed macrophytic vegetation. Riparian zones with well-developed vegetation represent a refuge mainly for species of zooplankton larger than planktivorous fishes. However, the riparian zone represents, at the same time, a refuge for fish fry that feed on zooplankton [37, 38]. Therefore, the specific situations are possible, which are recorded in our study at sampling point 2, that the greatest diversity occurs in localities with a vegetation-free riparian zone and a concrete riparian zone [39]. The same authors report that the precise causes of the greater abundance of zooplankton in rivers with vegetation-free riparian zone are difficult to explain. Cluster analysis of localities, regarding qualitative compositions of zooplankton, showed the highest connection between localities 1 and 2 (Fig. 3). Both localities are characterized by a rocky riverbed, but they differ according to the speed of the river flow (sampling point 1, high speed of river flow; sampling point 2, low speed of river flow). These results suggest that a terrain type of riverbed can have greater influence on the similarities of zooplankton composition than the speed of the river flow.

In Slovenia, the greatest diversity was recorded in localities placed beneath the dams (Table 1), which is in accordance with the authors arguing that the reservoirs could influence the species composition of planktons downstream of dams [30]. Dam reservoirs, like lakes, change the hydrological and ecological conditions in flowing water and are a valuable source of zooplankton in rivers [29]; however, the relative abundance of macro- and microzooplankton in rivers decreases downstream of dams [40]. In the Slovenian part of the Sava River, the lowest abundance was at sampling stations (2 and 4) downstream of dams (Table 1). The lowest diversity of zooplankton in Slovenia was recorded in locality 4 (Krško). According to the data of Dobnikar Tehovnik [41], the water bodies with the highest amount of absorbable organic halogen compounds (AOX) were determined on the lower Sava, where the main source was the direct industrial outflow from the VIPAP VIDEM KRŠKO factory. Apart from that, the water flow in this locality is very fast compared to localities placed above this one. Nevertheless, even in fast parts of the river flow, the zooplankton community can be more diverse than in sampling points downstream, which was the case with sampling point 1 (Hrastnik—29 taxa, Table 1). The situation that the plankton community can be more diverse in upper river parts is also argued by some other authors [32].

In the Croatian part of the Sava River, the greatest number of species of zooplankton was recorded in locality 6 (Rugvica), which is placed some 20 km downstream of Zagreb. It is possible that the construction of water purification plant in Zagreb [36] contributed to the reduction of pollution and better conditions for zooplankton development. The smallest number of species was recorded in locality 8 (Mlaka) in the conditions of high water level and with smallest values of oxygen saturation during the research (only 64 %).

The lowest diversity was recorded in Serbian part, whereas the number of recorded taxa (18) is significantly smaller compared to the previous researches (42—[18]). The worst situation throughout the whole research was recorded in sampling point 14 (Makiš), where only two taxa were identified. This specific locality is the one with a very strong anthropogenic influence. The samples were taken next to restaurants and clubs on rafts, where the organic contamination is high. As for the species composition of zooplankton, it is in accordance with the results of Djurkovic et al. [18], with that difference that in this research no adult specimens of planktonic Crustacea were recorded nor were the larvae of *Dreissena polymorpha*.

Even though one of the localities lies immediately behind the confluence of the Bosut and the Sava (locality 11), the influence of this tributary to the composition of zooplankton of the Sava cannot be seen. The detailed research of Rotifera and planktonic Crustacea in the Bosut River and its tributaries, namely, the Spačva and Studva, showed a very diverse zooplankton community. In the Bosut with its tributaries, 14 species of Protozoa, 62 species of Rotifera, 23 species of Cladocera, and 12 species of Copepoda were recorded [42–45]. A considerably higher number of species were recorded in the Kolubara River, one of Sava's tributaries, even 91 species of Rotifera, 6 Cladocera, and 7 Copepoda [46]. Because of the hydrological (e.g., water discharge) and biological (e.g., available food, predators, riparian zone) conditions, the tributaries bring small amounts of zooplankton to the main river channel. The fact that tributaries often do not have greater influence to the main river was also noted by Czerniawski et al. [29].

Low numeral values of zooplankton in the Sava (2–36 ind/L) are in accordance with the results of other authors. During the research of the Sava quality, it was recorded that the production of zooplankton varied by localities in 2002 in the range of 2–137 ind/L [16] and in 2003 in the range of 6–91 ind/L [14, 17]. Gulyás [4] reported the numeral value in the Sava to be 11,220 ind/m^3 (11.22 ind/L), while Djurkovic et al. [18] reported higher values, ranging from 40,320 to 107,360 ind/m^3 (40.32–107.34 ind/L).

The water quality of the Sava through the Serbian part has been influenced by a variety of point and nonpoint pollution sources from both municipal and industrial facilities, as well as agricultural land runoff [15]. The most damaging polluting materials come from metal and metal works, chemicals, textile, leather, pulp and paper, and food industrial discharges, which could lead to reduced zooplankton abundance. In our research we have not recorded any representatives of zooplankton at the sampling point 14 (Makis) where there are several sources of pollution.

As far as planktonic communities in rivers are concerned, it is common for maximal diversities of phytoplankton and zooplankton not to match and that diversity and/or production of zooplankton is generally higher in localities placed downstream of the localities where the diversity of phytoplankton is at its highest

[4]. In this research as well, it can be noticed that the community of phytoplankton in the Sava River has a different spatial distribution from the community of zooplankton (see chapter Algal Communities along the Sava River). The greatest diversity of phytoplankton was recorded in locality 4 (Krško), while the lowest diversity of zooplankton in Slovenia was recorded in the same locality. It can also be noticed that there is not an overlap between the maximal productions of phytoplankton and zooplankton.

The similarity indices between the localities studied were rather low, despite relatively close distances between them. The probable reason was that the sites were localized at the sections of the river characterized by different environmental factors [29].

These results are consistent with the views of Lair et al. [47] which showed the complexity of the processes active in the regulation of potamoplankton and the extent to which they are naturally induced or caused by human impact. The influence of abiotic parameters was most pronounced on cladoceran communities, while rotifers were the least affected [39]. In the Serbian part of the Sava River (most polluted part), we have not recorded a single representative of cladocerans (Table 1).

Since our research was a one-time survey, it is difficult to provide general conclusions regarding the composition, production, and succession of zooplankton communities. However, even these results are in accordance with the remarks of Czerniawski et al. [29], which stated that the greatest influence on the zooplankton communities have physicochemical factors including temperature, conductivity, and content of inorganic nutrients; however, another factor determining the physicochemical factors and zooplankton communities is the water residence time. It can be concluded that, despite a large number of works related to zooplankton in rivers, our understanding of the environmental factors controlling their composition, production, and distribution is limited [40].

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Aquatic Macroinvertebrates of the Sava River

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Abstract The objective of this chapter is to present the data on aquatic macroinvertebrate communities along the Sava River, based on investigation performed during 2011 and 2012 at 12 sampling sites within the sector between Vrhovo (Slovenia) and Belgrade (confluence to the Danube). During our study 227 macroinvertebrate taxa were recorded in the Sava River. Having in mind that upper stretch of the Sava River was not covered by this work (alpine and subalpine stretch), as well as based on the review of previous works on the macroinvertebrate fauna of the Sava River, more than 300 species will be confirmed for the Sava River. The data on the distribution of aquatic macroinvertebrates revealed five different stretches—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava. Physical habitat degradation, pollution and pressure caused by biological

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invasions were found to be the main factors of endangerment of aquatic macroinvertebrate fauna diversity. There is an obvious need for further investigation of the Sava River in order to complete the data on aquatic macroinvertebrates and to provide the basis for accurate assessment of environmental status of the river.

Keywords Aquatic macroinvertebrates • Sava River • Community structure • Species richness

1 Introduction

Aquatic macroinvertebrates are diverse group of organisms that spent their entire (e.g. aquatic worms, leeches, molluscs or crustaceans) or a part of life cycle (e.g. some orders of insects, such mayflies or caddis flies) in water. The term macroinvertebrates describes animals that have no backbone and can be seen with the naked eye. In general, the group comprises species larger that than 0.5 mm (could be collected by mesh with opening size of 0.5 mm). Smaller animals that pass through such a sieve are called meiozoobenthos. In regard to size, aquatic macroinvertebrates include small organisms such as tiny aquatic worms (Oligochaeta) or different insect larvae, but also some species that could be larger than 10 cm, such as freshwater mussels (Bivalvia: Unionidae) or crayfish species (Crustacea: Decapoda).

Other names are also commonly used for this group of animals, such as macrozoobenthos or macrozoobenthon. We prefer to use the formulation aquatic macroinvertebrates rather than other mentioned terms which denote that organisms live on the bottom of water bodies, which is not the case. The group also includes animals that live on the aquatic vegetation, submerged objects or water surface.

Aquatic macroinvertebrates comprise different taxonomic assemblages and it is not taxonomic, but ecological group. In some habitats aquatic macroinvertebrates occur in a great variety of species and in large quantities, and thus, this group plays an important role in energy cycling and mass balance in aquatic ecosystems and is represented with wide scale of functional feeding guilds. Macroinvertebrates inhabit all types of waters, from fast-flowing mountain streams of different sizes to large lowland rivers, lakes and ponds. They play an important role in maintaining ecosystem health, as they are consumers of organic matter, and thus help to remove nutrients from water systems. They also provide a food source for a variety of predators such as invertebrates, fish, amphibians and birds.

The aim of this paper is to present the diversity of macroinvertebrate communities of the Sava River. Also, attention was focussed to nonindigenous taxa, since mass occurrence of invasive alien species could significantly influence native biodiversity and could disturb the functionality of aquatic ecosystems.

2 Previous Investigations

Despite importance of the Sava as large transboundary river, macroinvertebrate communities of its main course have not been systematically studied recently. The most comprehensive research of macroinvertebrates of the Sava River was carried out by Matoničkin et al. [1]. The investigation was performed in period 1966–1975 on 41 sampling sites covering the entire length of the Sava River, including the Sava Dolinka and Sava Bohinjka (the Sava River is formed on the place of confluence of those two rivers). The authors [1] provided extensive biocenological and saprobiological analyses. Also, Matoničkin et al. [1] presented the literature review on the investigation of the Sava River and main tributaries up to 1970s and concluded that only the results of taxonomical investigations limited to individual taxa groups are available. Since the comprehensive study of Matoničkin et al. [1], published results concerning macroinvertebrates of the Sava were mostly restricted to limited stretches of the river [2–11]. Recently, Paunović et al. [12] presented the results of investigation on macroinvertebrate community along 622 km of the Sava River, between Martinska Ves (downstream Zagreb) and confluence to the Danube. The most comprehensive study of macroinvertebrates that involved the Sava River Basin in Slovenia was provided by Urbanič [13].

Based on the review of previous investigation, we can conclude that still limited information is available on aquatic macroinvertebrate communities along the Sava River. The comparable high-quality data is necessary not only for research purposes but also for design of proper management of water resources within the basin area.

3 Study Area

The detailed description of the Sava River Basin is provided in Simić et al. [14] of this volume. The Sava flows from the mountain region in Slovenia to the lowlands of Croatia, Bosnia and Herzegovina and Serbia and confluences of the Danube in Belgrade (river km 1171). It is the largest tributary of the Danube. Due to the different influences along the course caused by diverse surroundings (relief, geological substrate, altitude, bad slope and climate), this mighty river is heterogeneous concerning overall environmental conditions. Due to the geographic position, diverse climate, petrographic and pedological variety and orographic characteristics, the Sava River Basin is one of the most complex regions in Europe concerning the distribution of plants and animals [15]. Consequently, the investigation on the distribution of aquatic macroinvertebrates along the Sava River is complex issue.

4 Material and Methods

The overview of aquatic macroinvertebrates of the Sava River was performed based on recent investigations in 2011 and 2012. In addition, the literature data were used to complement our survey data.

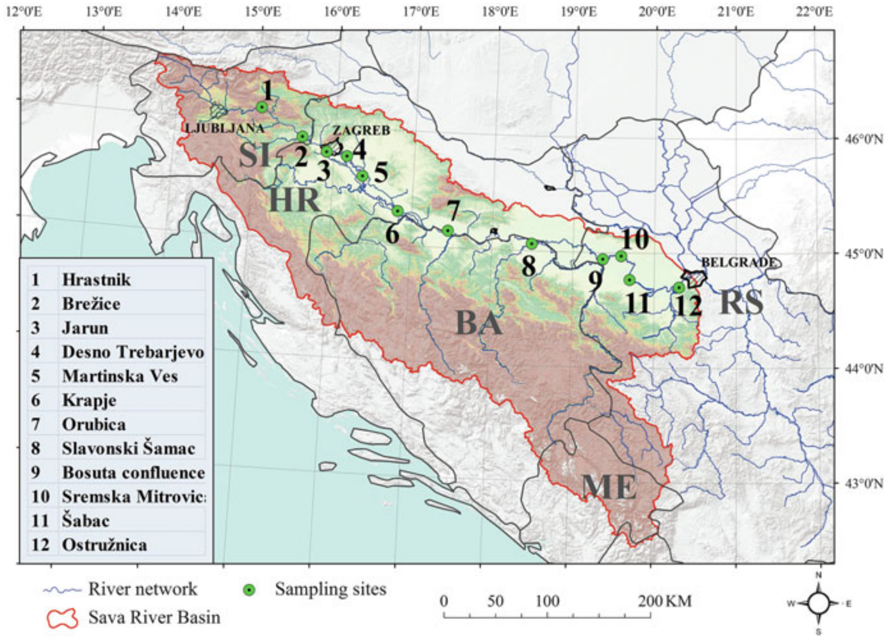


Fig. 1 Sampling sites along the Sava River—2011 and 2012 surveys

Macroinvertebrate sampling was performed during September (low water conditions) 2011 and 2012 at 12 sampling sites (Fig. 1). Low-water condition period was selected since most microhabitats on river bank are available for sampling in that period and in order to get comparable data with recent investigations on the Danube that were performed in same period of the year (Joint Danube Survey 1, 2 and 3 [16–18], and AquaTerra Danube Survey—[19–22]).

Samples were collected using hand nets (mesh size 500 μm) on the area of 0.0625 m^2, in a shallow bank region (up to the depth of 1.5 m), from all available types of substrate (stones, gravel, sand, mud, as well as from artificial structures—groynes, longitudinal dykes and revetments). During the material collection, the relative contribution to each microhabitat was taken into the consideration and the number of samples collected from particular microhabitat within each reach corresponds to the relative contribution of this microhabitat to the substrate of the assessed river reach (10 % = 1 sample). The fauna attached to stone surfaces was collected with tweezers and, if necessary, scraped with a brush. Freediving was also performed to collect mussels.

Approximate length of investigated reach at each sampling site was 100 m of the shore region.

Qualitative (number of taxa) composition and quantitative composition (relative abundance) of macroinvertebrate community were discussed. Relative abundance was analysed as the mean number of taxa in ten replicate samples and expressed as percentage participation of each taxa group.

Asterics software Version 3.3.1. [23] was applied for calculating community structure in regard to saprobic preference, substrate type, river zonation and feeding-type composition, while the autecological data are used from AQEM [23].

5 Results and Discussion

5.1 Qualitative, Quantitative and Functional Analyses of Macroinvertebrate Community

Based on the examined material collected during 2011 and 2012 survey, 227 macroinvertebrate taxa were recorded in the Sava River, within the sector of investigation (Tables 1 and 2).

Aquatic insects were found to be the principal component of the community with 157 recorded species. Among insects, order Diptera (true flies) was characterised by larger number of identified species (70) with 52 recorded taxa belonging to family Chironomidae (chironomids or nonbiting midges). Insect's orders Trichoptera (caddis flies), Coleoptera (beetles) and Ephemeroptera (mayflies) were also found to be important element of the macroinvertebrate community in regard to taxa richness with 35, 23 and 15 identified species, respectively.

Considerable taxa richness was recorded among molluscs (27—Gastropoda 19 and Bivalvia 8) and annelids (24—Oligochaeta 18, Hirudinea 5 and Polychaeta 1). Based on our results, other macroinvertebrate groups of the investigated stretch of the Sava River contain less species.

Analysis of the molluscs fauna along the Sava in regard to relative abundance are *Theodoxus danubialis* (33.82 %) and *Lithoglyphus naticoides* (33.12 %), followed by *Bithynia tentaculata* (8.05 %) and *Esperiana daudebartii acicularis* (7.59 %), while percentage participation of the other taxa in the mollusc community was significantly lower.

Bivalves *Corbicula fluminea* and *Unio pictorum*, together with two snail species *Lithoglyphus naticoides* and *Bithynia tentaculata*, were the most frequent representatives of molluscs on investigated stretch.

It is important to emphasise that stable population of freshwater mussel *Unio crassus* (Fig. 2) was found in the middle and part of the lower stretch of the Sava River—sites 5–10. The species is included in Annexes 2 and 4 of the EU Habitat Directive and is considered as rare and endangered species in many European countries according to IUCN classification [24, 25] This fact indicates the importance of the Sava River in respect to protection of *U. crassus*.

The number of recorded taxa per locality (Fig. 3) varied between 28 (Brežice, sampling site 2) and 106 (Martinska Ves, sampling site 5). Considerable taxa richness was detected for sites: Orubica (site 7, 86 taxa) and Jarun (site 3, 81 taxa).

During our investigations, the change of macroinvertebrate community related to alter of general river type is recorded. Beside the above-mentioned change in the total number of recorded taxa, the change along the river continuum is also illustrated by other community patterns. Thus, the decrease of the number of mayflies (ordo

Table 1 The list of recorded macroinvertebrate taxa

Spongillidae Gen. sp.
Nematoda
Turbellaria
Dugesia lugubris (Schmidt, 1861)
Dugesia tigrina (Girard, 1850)*
Planaria torva (Müller, 1774)
Polycelis tenuis (Ijima, 1884)
Oligochaeta
Branchiura sowerbyi (Beddard, 1892)*
Eiseniella tetraedra (Savigny, 1826)
Embolocephalus velutinus (Grube, 1879)
Enchytraeidae
Isochaetides michaelseni (Lastockin, 1936)
Limnodrilus claparedeanus (Ratzel, 1868)
Limnodrilus hoffmeisteri (Claparède, 1862)
Limnodrilus udekemianus (Claparède, 1862)
Nais bretscheri (Michaelsen, 1899)
Nais communis (Piguet, 1906)
Nais elinguis (Müller, 1774)
Ophidonais serpentina (O.F. Müller, 1773)
Potamothrix hammoniensis (Michaelsen, 1901)
Propappus volki (Michaelsen, 1916)
Psammoryctides barbatus (Grube, 1861)
Stylaria lacustris (Linnaeus, 1767)
Stylodrilus heringianus (Claparède, 1862)
Tubifex tubifex (Müller, 1774)
Hirudinea
Glossiphonia complanata (Linnaeus, 1758)
Erpobdella octoculata (Linnaeus, 1758)
Erpobdella lineata (O. F. Müller, 1774)
Helobdella stagnalis (Linnaeus, 1758)
Piscicola geometra (Linnaeus, 1761)
Polychaeta
Hypania invalida (Grube, 1860)*
Gastropoda
Acroloxus lacustris (Linnaeus, 1758)
Borysthenia naticina (Menke, 1845)
Bithynia tentaculata (Linnaeus, 1758)
Esperiana daudebartii acicularis (A. Ferussac, 1823)
Esperiana esperi (A. Ferussac, 1823)
Ferrissia clessiniana (Jickeli, 1882)
Gyraulus albus (Müller, 1774)
Gyraulus laevis (Alder, 1838)

(continued)

Table 1 (continued)

Gyraulus crista (Linnaeus, 1758)
Holandriana holandrii (Pfeiffer, 1828)
Lithoglyphus naticoides (Pfeiffer, 1828)
Physella acuta (Draparnaud, 1805)*
Planorbis planorbis (Linnaeus, 1758)
Radix auricularia (Linnaeus, 1758)
Radix labiata (Rossmässler, 1835)
Theodoxus danubialis (C. Pfeiffer, 1828)
Theodoxus fluviatilis (Linnaeus, 1758)
Viviparus acerosus (Bourguignat, 1862)
Valvata cristata (O. F. Müller, 1774)
Bivalvia
Corbicula fluminea (O. F. Müller, 1774)*
Dreissena polymorpha (Pallas, 1771)*
Sinanodonta woodiana (Rea, 1834)*
Sphaerium rivicola (Lamarck, 1818)
Pisidium sp.
Unio crassus (Philipsson, 1788)
Unio pictorum (Linnaeus, 1758)
Unio tumidus (Philipsson, 1788)
Crustacea
Isopoda
Asellus aquaticus (Linnaeus, 1758)
Amphipoda
Corophium curvispinum (Sars, 1895)*
Dikerogammarus haemobaphes (Eichwald, 1841)*
Dikerogammarus villosus (Sowinsky, 1894)*
Gammaridae
Mysidae
Decapoda
Astacus leptodactylus (Eschscholtz, 1823)
Orconectes limosus (Rafinesque, 1817)
Odonata
Calopteryx splendens (Harris, 1782)
Coenagrionidae Gen. sp.
Cercion lindeni (Sélys, 1840)
Coenagrion mercuriale (Charpentier, 1840)
Gomphus flavipes (Charpentier, 1825)
Gomphus vulgatissimus (Linnaeus, 1758)
Ischnura elegans (Vander Linden 1820)
Onychogomphus forcipatus (Linnaeus, 1758)
Platycnemis pennipes (Pallas, 1771)
Pyrrhosoma nymphula (Sulzer, 1776)

(continued)

Table 1 (continued)

Ephemeroptera
Baetis fuscatus (Linnaeus, 1761)
Baetis lutheri (Müller-Liebenau, 1967)
Baetis rhodani (Pictet, 1843)
Baetis vernus (Curtis, 1834)
Brachycentrus subnubilus (Curtis, 1834)
Caenis luctuosa (Burmeister, 1838)
Cloeon dipterum (Linnaeus, 1761)
Cloeon simile (Eaton, 1870)
Cloeon sp.
Ephemera danica (Müller, 1764)
Ephemerella sp.
Heptageniidae
Heptagenia sulphurea (Müller, 1776)
Heptagenia sp.
Torleya major (Klapálek, 1905)
Neuroptera
Sisyra fuscata (Fabricius, 1793)
Trichoptera
Athripsodes albifrons (Linnaeus, 1758)
Athripsodes sp.
Ceraclea fulva (Rambur, 1842)
Ceraclea sp.
Cheumatopsyche lepida (Pictet, 1834)
Cyrnus trimaculatus (Curtis, 1834)
Ecnomus tenellus (Rambur, 1842)
Ecnomus sp.
Holocentropus stagnalis (Albadra, 1864)
Holocentropus sp.
Hydropsyche angustipennis (Curtis, 1834)
Hydropsyche bulgaromanorum (Malicky, 1977)
Hydropsyche contubernalis (McLachlan, 1865)
Hydropsyche exocellata (Dufour, 1841)
Hydropsyche fulvipes (Curtis, 1834)
Hydropsyche pellucidula (Curtis, 1834)
Hydropsychidae spp.
Hydropsyche sp.
Hydroptila vectis (Curtis, 1834)
Hydroptila sp.
Leptoceridae
Lepidostoma hirtum (Fabricius, 1775)
Mystacides sp.
Neureclipsis bimaculata (Linnaeus, 1758)

(continued)

Table 1 (continued)

Oecetis notata (Rambur, 1842)
Oecetis sp.
Polycentropodidae
Polycentropus flavomaculatus (Pictet, 1834)
Psychomyia pusilla (Fabricius, 1781)
Psychomyia sp.
Rhyacophila sp.
Setodes punctatus (Fabricius, 1793)
Trichoptera Gen. sp.
Tinodes pallidulus (McLachlan, 1878)
Tinodes sp.
Collembola
Collembola
Coleoptera
Dytiscidae
Dryopidae Gen. sp. Lv.
Elmidae
Elmis aenea (Müller, 1806)
Esolus angustatus (Müller, 1821)
Hydrophilidae
Hydrophilus sp.
Hydroporus sp. Lv.
Hemerodromia unilineata Zetterstedt, 1842
Limnius volckmari (Panzer, 1793)
Oulimnius troglodytes (Gyllenhal, 1827)
Oulimnius tuberculatus (Müller, 1806)
Oulimnius sp.
Orectochirus villosus (Müller, 1776)
Macronychus sp. Ad.
Normandia nitens (Müller, 1817)
Noterus sp.
Patambus sp.
Pomatinus substriatus Ad. (Müller, 1806)
Potamophilus acuminatus (Fabricius, 1772)
Polycentropodidae Gen. sp.
Riolus cupreus (Müller, 1806)
Stenelmis canaliculata (Gyllenhal, 1808)
Diptera
Athericidae
Atherix ibis (Fabricius, 1789)

(continued)

Table 1 (continued)

Antocha sp.
Ceratopogonidae
Chaoboridae
Chelifera sp.
Ephydridae
Hemerodromia unilineata (Zetterstedt, 1842)
Ibisia marginata (Fabricius, 1781)
Micronecta sp.
Micronecta scholtzi (Fieber, 1860)
Oxycera sp.
Stratiomyidae
Scatella sp.
Chironomidae
Ablabesmyia longistyla (Fittkau, 1962)
Beckidia zabolotzkyi (Goetghebuer, 1938)
Dicrotendipes nervosus (Staeger, 1839)
Demicryptochironomus vulneratus (Zetterstedt, 1838)
Cricotopus gr. sylvestris sensu (Hirvenoja, 1973)
Cricotopus trifascia (Edwards, 1929)
Cricotopus triannulatus agg. sensu (Moller Pillot, 1984)
Cricotopus bicinctus (Meigen, 1818)
Cryptochironomus sp.
Cryptotendipes sp.
Conchapelopia melanops (Meigen, 1818)
Cladotanytarsus spp.
Cladopelma gr. laccophila
Chironomus spp.
Harnischia sp.
Lipiniella araenicola (Shilova, 1961)
Microchironomus tener (Kieffer, 1918)
Micropsectra bidentata (Goetghebuer, 1921)
Microtendipes pedellus agg. sensu (Moller Pillot, 1984)
Nanocladius dichromus (Kieffer, 1906)
Nanocladius bicolor agg.
Orthocladius (*Orthocladius*) spp.
Parametriocnemus stylatus (Spaerck, 1923)
Paratanytarsus dissimilis (Johannsen, 1905)
Paratanytarsus austriacus (Kieffer, 1924)
Paratendipes nubilus (Meigen, 1830)
Procladius sp.

(continued)

Table 1 (continued)

Parachironomus frequens (Johannsen, 1905)
Parachironomus gr. arcuatus
Paralauterborniella nigrohalteralis (Malloch, 1915)
Paratendipes albimanus (Meigen, 1818)
Paratrichocladius rufiventris (Meigen, 1830)
Phaenopsectra sp.
Polypedilum nubeculosum (Meigen, 1804)
Polypedilum cultellatum (Goetghebuer, 1931)
Polypedilum convictum (Walker, 1856)
Polypedilum scalaenum (Schrank, 1803)
Polypedilum albicorne (Meigen, 1838)
Potthastia gaedii (Meigen, 1838)
Pseudochironomus prasinatus (Staeger, 1839)
Rheotanytarsus spp.
Rheopelopia sp.
Rheocricotopus chalybeatus (Edwards, 1929)
Rheocricotopus effusus (Walker, 1856)
Stictochironomus maculipennis (Meigen, 1818)
Synorthocladius semivirens (Kieffer, 1909)
Thienemanniella majuscula (Edwards, 1924)
Tvetenia discoloripes (Goetghebuer and Thienemann, 1936)
Tanypus punctipennis (Meigen, 1818)
Tanytarsus spp.
Thienemanniella majuscula (Edwards, 1924)
Xenochironomus xenolabis (Kieffer, 1916)
Empididae
Hexatoma sp.
Simuliidae
Tipula sp.
Heteroptera
Aphelocheirus aestivalis (Fabricius, 1794)
Micronecta sp.
Neuroptera
Sisyra fuscata (Fabricius, 1793)
Hydracarina
Hydrachnidia Gen. sp.
Bryozoa
Plumatellidae

Table 2 Number of species per taxa group

Group	No. of taxa
Phylum Porifera192978_Talapatra	1
Phylum Bryozoa	1
Phylum Nematoda	1
Phylum Platyhelminthes	
Class Turbellaria	4
Phylum Annelida	24
Oligochaeta	18
Hirudinea	5
Polychaeta	1
Phylum Mollusca	27
Gastropoda	19
Bivalvia	8
Phylum Arthropoda	
Subphylum Crustacea	7
Class Arachnida	
Hydracarina	1
Class Collembola	1
Class Insecta	157
Odonata	10
Ephemeroptera	15
Neuroptera	1
Trichoptera	35
Coleoptera	23
Diptera	70
Diptera: other than Chironomidae	18
Diptera: Chironomidae	52
Heteroptera	2
Neuroptera	1

Fig. 2 *Unio crassus* collected from the Sava River in Sremska Mitrovica (site 10) (photo by Paunović 2012)

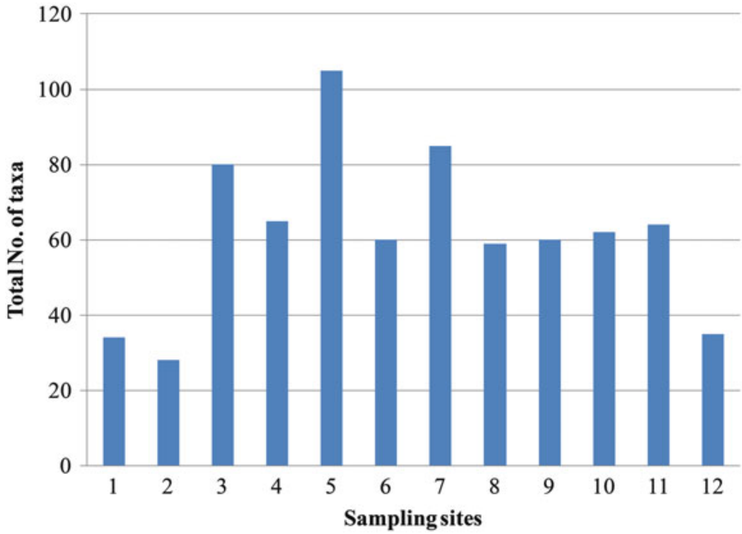


Fig. 3 Number of recorded taxa per locality

Ephemeroptera) and caddis flies (ordo Trichoptera) taxa along the watercourse (Fig. 4) clearly reflects change in the overall character of the river. Those insect orders are generally characterised by occurrence of higher number of species in the middle and upper stretches of the rivers in comparison to lower stretches [26]. Flat worms, Turbellaria, were detected on the sites 1–8. The number of taxa among the groups that are characteristic for large lowland rivers (aquatic worms, Oligochaeta; bivalves, Bivalvia; snails, Gastropoda; true flies, Diptera; and dragonflies and damselflies, Odonata) is larger at sites 3–12 in comparison to sites 1 and 2.

Lithoglyphus naticoides (Mollusca: Gastropoda) and *Limnodrilus hoffmeisteri* (Annelida: Oligochaeta) were found to be the most frequent and abundant species within the investigated stretch. Aquatic worms *Potamotrix hammoniensis* and *Psammoryctides barbatus* were also recorded along the entire sector of investigation.

In regard to quantitative composition of the macroinvertebrate community, gradual changes were also detected along the Sava River, with the similar pattern as detected for qualitative composition (Fig. 5). Thus, the general decline of percentage participation of caddis flies (Trichoptera) and Turbellaria in the total macroinvertebrate community was observed from upper to lower stretch. Further, the increase of percentage participation of aquatic worms (Oligochaeta) and molluscs (Gastropoda and Bivalvia) was recorded within the sites 4–12 in comparison to sites 1–3.

According to ecological classification of taxa in regard to saprobic valence of Moog [27], beta-mesosaprobic taxa are the most numerous with 23.75 % in respect to the total number of identified species. Almost 15 % of the recorded taxa could be characterised as typical for rivers with high organic load (alpha-mesosaprobic and polysaprobic indicators). Only 2.59 % of recorded taxa could be characterised as sensitive to organic pollution (xeno- and oligosaprobic indicators). For the rest of

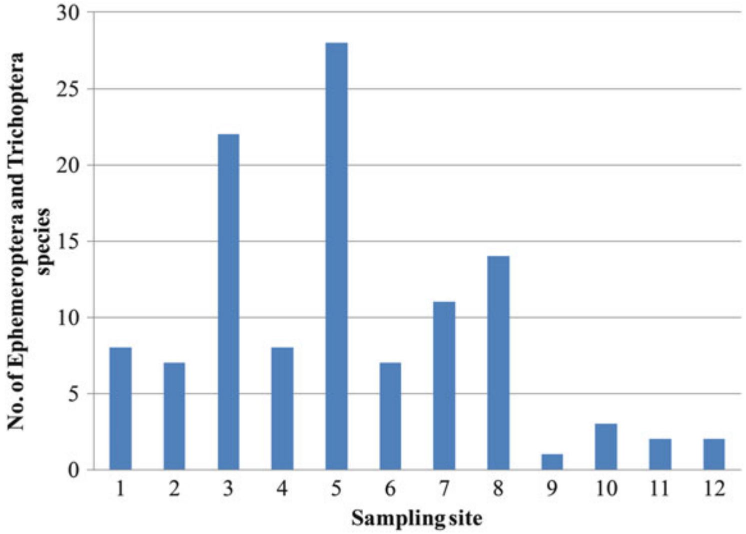


Fig. 4 Number of mayfly (Ephemeroptera) and caddis fly (Trichoptera) species at sampling sites

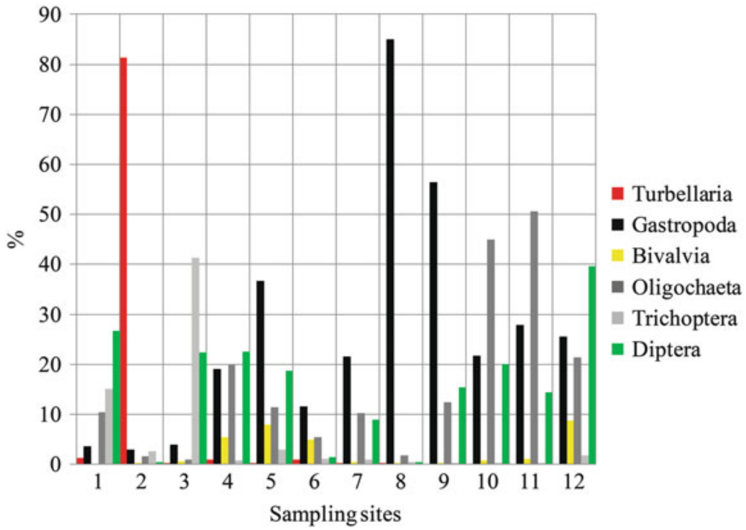
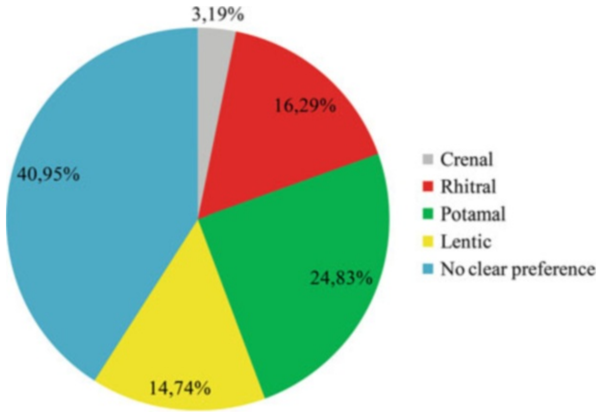


Fig. 5 Percentage participation of the main faunistic groups in the total macroinvertebrate community at sampling sites

the species (52.59 %), there is no data to classify them in regard to saprobic tolerance [23]. This finding indicates that organic pollution is a significant pressure that influences the macroinvertebrate community along the investigated stretch.

In regard to a preferred zone within the river continuum (longitudinal zonation), the greatest proportion of recorded species (24.83 %) is characteristic for the lower river stretches (hypopotamal, epipotamal, metapotamal)—potamal species [23, 26,

Fig. 6 Proportion of species with different preferences to particular zone of the river continuum



27] (Fig. 6). The rest of the taxa prefer lentic zones (standing water) (14.74 %) or fast-flowing stretches (rhitral zone—16.29 %). Small amount of taxa is characteristic for source region of the river (Crenal), while information about preferred zone for smaller number of registered species is not available (9.3 %).

The majority of the identified species (19.96 %) are adapted to the river bed consisted of gravel and stones [23, 27], while 16.90 % of the total number of taxa is characteristic for substrate types typical of large lowland rivers (substrate types pelal, psammal and argillal). For other identified species, there is not enough information to determine clear preference for particular substrate type [23].

In regard to functional feeding types, the greatest part of recorded species belongs to functional groups characteristic to be dominant in the lower stretches of the rivers (Fig. 7)—gatherers/collectors (25.40 %) and filtrators (11.10 %) [26]. Grazers/scrapers and shredders that are typically dominant in the middle and upper stretches of the rivers [26] are also characterised with significant proportion in the total number of recorded species—17.80 and 3.50 %, respectively.

For 13.40 % of the taxa, feeding preference is unknown [23].

Analyses of overall species composition in regard to saprobic, feeding and bottom preference, as well as specific zone within river continuum, illustrate that investigated stretch is diverse in respect to environmental conditions. The change of relative abundance of the main taxa groups and functional analyses provided the information on changes of the community along the watercourse.

The domination of organisms adapted to fine substrate (silt, sand and clay) was recorded for sites 4, 5 and 9–12 (Fig. 8), which indicates gradual change of the river type along the watercourse.

Gradual change of macroinvertebrate community along the watercourse was also identified by functional analyses of saprobic groups and feeding preference (Figs. 9 and 10).

Thus, percentage participation of organisms that are adapted to high organic load (species typical for polysaprobic conditions) increases in downstream direction,

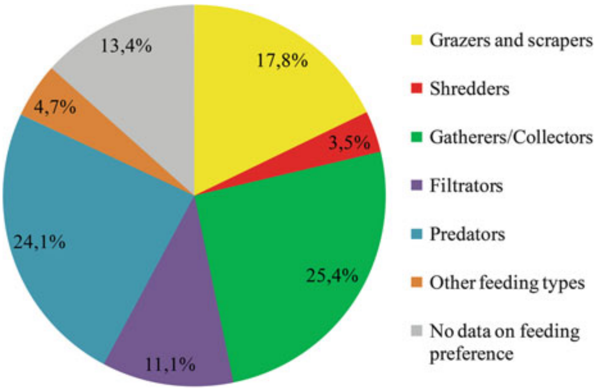


Fig. 7 Proportion of species with characteristic feeding preference

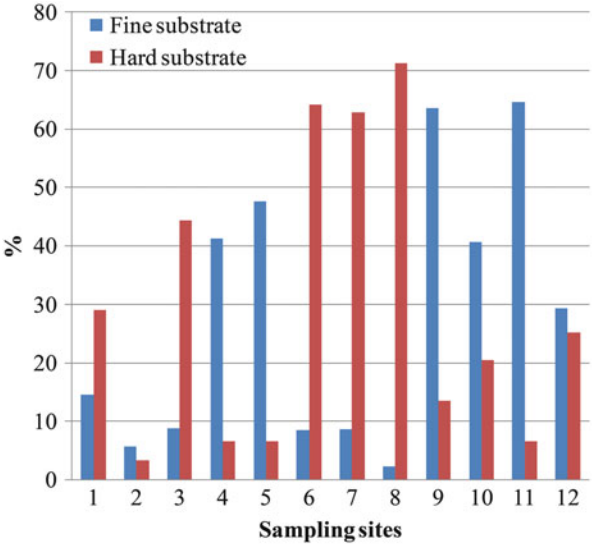


Fig. 8 Percentage participation of organisms that prefer fine (silt, clay and sand) and hard substrate (gravel and stone) type in the total macroinvertebrate density

while the share of beta-mesosaprobic organisms increases from site 2 to site 9 and then decreases (sites 10–12) (Fig. 9).

The change of functional feeding group percentage participation is presented at Fig. 10. In respect to feeding preference, gatherers/collectors and filter feeders (groups characteristic for the lower stretches of the rivers [26]) are dominant at sites 9–12, while the share of grazers/scrapers and shredders (groups characteristic for the middle and upper stretches of the river) is larger at the sites 1–8.

During our study, a significant number of species were detected (227), in comparison to previous investigations. Thus, Matoničkin et al. [1] reported 143 macroinvertebrate species for longer stretch of the Sava River, with domination of insects (69 species). Matoničkin et al. [1] also reported 27 species of aquatic worms (Oligochaeta), eight species of leeches (Hirudinea) and 21 species of

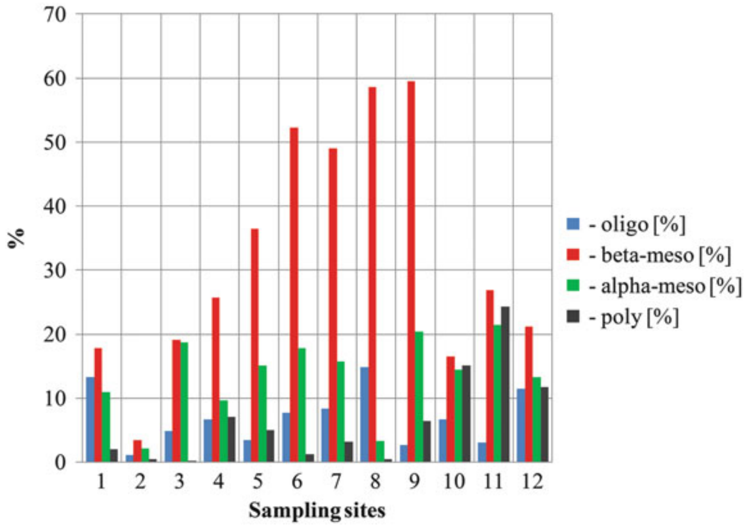


Fig. 9 Percentage participation of saprobic groups at sampling sites

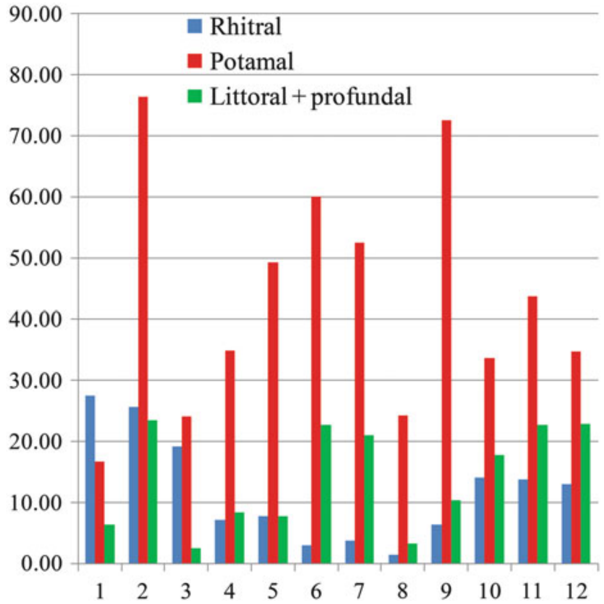


Fig. 10 Percentage participation of functional feeding groups at sampling sites

molluscs (15 snails and six bivalves). Having in mind that their research comprised the upper stretch of the Sava River, which was not covered by our investigation, it is expected that they identified 16 species of stoneflies (Plecoptera), while in the material collected during our study, those insects were not present. A total of 98 macroinvertebrate taxa were found during the investigation on a cobble substrate in the lower rhitron section of the Sava River at four different sampling sites [7]. Paunović et al. [10] reported 63 macroinvertebrate species for lower stretch of the Sava River, but this study did not comprise the analysis of nonbiting midges (Chironomidae).

Having in mind the above-mentioned investigations, and the fact that this study did not provide information on the diversity within the stretch upstream Hrastnik, which is different in respect to overall environmental conditions, the total number of macroinvertebrate taxa of the Sava River is much higher and we could expect more than 300 species to be found. The additional number of species is expected primarily among aquatic insects—stoneflies (Plecoptera), mayflies (Ephemeroptera) and caddis flies (Trichoptera)—but also within other macroinvertebrate groups that include species characteristic for fast water and hard bottom substrate.

During the 9-year study on the artificial substrates in the middle stretch of the Sava River, Mihaljević et al. [8] reported Chironomidae and Oligochaeta as the dominant groups, which is in accordance with the results of our study for the middle section of the Sava River.

High species richness of the Sava River could be revealed based on the comparison with the investigation of other large river within the Danube River Basin. Thus, during the AquaTerra Danube Survey (ADS) in the sector between Klosterneuburg (Austria, 1,942 river km) and Vidin-Calafat (Bulgaria-Romania, 795 river km), 89 macroinvertebrate taxa were detected [19] with molluscs as a dominant group in macroinvertebrate community with regard to species richness (35 taxa). Altogether 107 macroinvertebrate taxa were found during 2001 International Tisa Survey [28] that covered 744 km of the river.

Molluscs were also found to be one of the principal components of the macroinvertebrate community of the Sava River in its middle and lower stretch [1, 11, 12, 29], as well as in our study.

Molluscs and oligochaetes constitute two of the largest groups of invertebrates in regard to the number of identified species, as well as in regard to relative abundance, especially in large lowland rivers [20, 21, 30–32].

5.2 Sectioning of the Sava River Based on Aquatic Macroinvertebrates

Qualitative, quantitative and functional analyses clearly show the gradual changes along the watercourse.

For accurate discussion on the sectioning of the river, more research effort is needed. The proper typology, based on basic natural characteristics of water types, is an important activity which presents the basis for effective water management and monitoring of ecological status, as proposed by Water Framework Directive (WFD; WFD [33]). Grouping of similar rivers is a prerequisite to following the river-type-specific approach of the WFD. Thus, the classification of river types, as relatively homogeneous ecological systems, implies similar associated biological communities. The concept offered in the WFD in regard to typology is complex, because it demands the water classification in functional entities, characterised by the array of common features that could be described by biological traits from one side, but from the other side, the system should be simple enough to be applicable for an effective management, which includes monitoring, as well [22].

Based on the presented data on macroinvertebrate communities, the border between distinctive stretches of the Sava River could be between sites 8 (Slavonski Šamac) and 9 (the Bosut confluence). In a particular stretch, the Sava River became the typical large lowland river, after receiving several larger tributaries (the Bosna and Drina Rivers). The change occurs in the bottom substrate as well [34, 35] from substrate dominated by gravel and sand to this dominated by sand, with different proportion of silt and clay. Based on the preliminary study of macroinvertebrates along the longitudinal profile, the additional border between river types could be positioned upstream Zagreb, since the change of macroinvertebrate community structure is also observed at sites 3 and 4, in comparison to sites 1 and 2. Part of the recorded changes are consequence of anthropogenic pressures that are evident in the area (damming of the Sava River in Slovenian stretch, influence of settlements and water regulation structures), which makes the analyses in regard to river typology complex.

In regard to the upper stretch, Urbanič [13] identified the mouth of the Ljubljanica River (confluence of the Sava downstream Ljubljana) as the natural border between typical alpine watercourses belonging to ecoregion 4 (Alps [36]) and subalpine waters belonging to ecoregion 5 (Dinaric western Balkan [36]). Further, Urbanič [13] indicated that the border between ecoregions 5 (Dinaric western Balkan [36]) and ecoregion 11 (Pannonian plain [36]) is at elevation of about 200 m (Kraško-Brezinska Kotlina plain or between settlements Radeče and Zidani Most).

Based on the previous discussions on findings of Urbanič [13], as well as data presented in this work, the Sava River could be preliminarily divided into five distinct sectors—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava (Fig. 11). For further divisions of sectors along the Sava River, additional material is needed.

Presented sectioning of the Sava River is in accordance with the general natural characteristics of the region. The Upper Sava course (upper reach or upper geomorphologic unit—hereby referred as alpine, subalpine, Upper Sava plain) is characterised by a steep slope, torrential tributaries and domination of coarse fractions in the bottom substrate [34, 35]. The hilly mountain terrain dominates. The reach is about 260 km long (together with the Sava Dolinka, longer headwater). The region is characterised by diverse environmental conditions and consequently

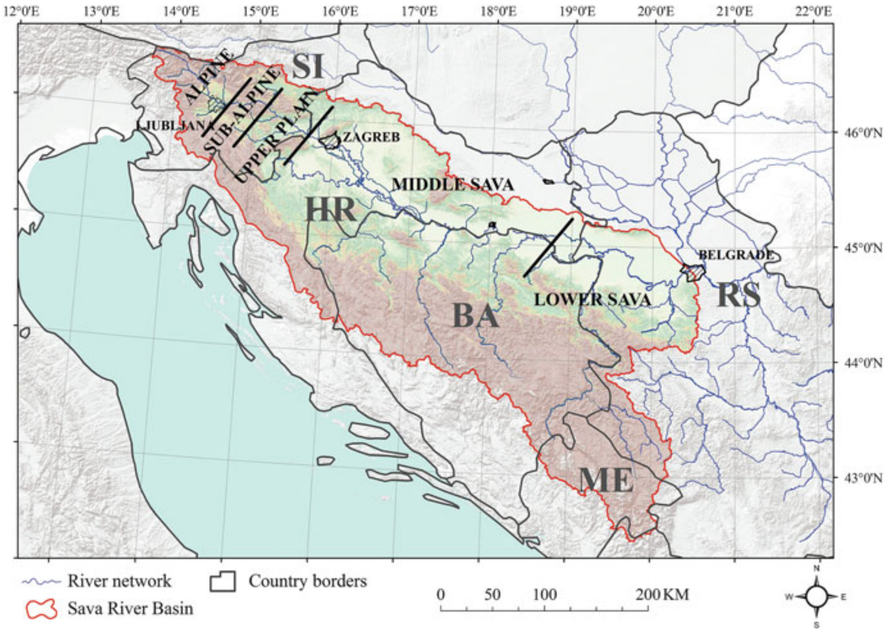


Fig. 11 Preliminary sectioning of the Sava River based on aquatic macroinvertebrates

complex biogeographical features, which are illustrated by division to ecoregions—three ecoregions are shared within a narrow area: 4 Alps, 5 Dinaric western Balkan and 11 Pannonian plain [36].

Further, general changes in bottom characteristics determine the border between the Middle and the Lower Sava River. According to available data, the gravel dominates down to the Una confluence and Sisak. In the stretch between Sisak and Slavonski Brod, the bottom is dominated by sand and gravel, while further downstream, the sand and silt dominate in bottom substrate. Since the bottom character is one of the dominant factors influencing the macroinvertebrate distribution [26], the changes in the community are expected.

5.3 Nonindigenous Macroinvertebrate Taxa

The last century has witnessed an increasing realisation of the role of humans in the dispersal of species beyond their natural range. Based on previous studies, the Sava River is also exposed to biological invasions [10–12, 37, 38]. Many of nonindigenous species recorded all over Europe are aquatic macroinvertebrates. In the following text, we provide short overview of nonindigenous aquatic macroinvertebrates recorded in the Sava River.

During our investigation, 11 nonindigenous aquatic macroinvertebrates were detected (marked with * in Table 1).

The dispersal of nonindigenous Ponto-Caspian amphipods (Crustacea: Amphipoda) in Croatian stretch of the Sava River was extensively discussed by Žganec et al. [37], and the details on the distribution of two species (*Chelicorophium curvispinum* and *Dikerogammarus haemobaphes*) were presented. Our investigation, as well as findings of Paunović et al. [12], confirmed the presence of one more amphipod invasive alien species, *D. villosus*, in the most downstream stretch of the Sava River (site 12). In addition, within the same stretch, the occurrence of spiny-cheek crayfish (*Orconectes limosus*; Crustacea: Decapoda), an invasive decapod species was confirmed during 2012, (site 12, Fig. 12). Further investigation will provide more details on the dispersal and abundance of nonindigenous crustaceans within the Sava River Basin. In that regard, the occurrence of the signal crayfish, *Pacifastacus leniusculus* (Dana 1852) (fast spreading nonindigenous invasive North American crayfish) could be expected in the Sava River, since the species was recently discovered in Korana River (Sava Basin) in Croatia [39]. Signal crayfish already successfully colonised many European freshwaters [39–42].

Besides crustaceans, several mollusc species were found to be successful invaders of the Sava River [1, 10–12]. Based on our study, as well as previous research [1, 10–12, 20, 21, 43], *C. fluminea*, *Dreissena polymorpha* and *Sinanodonta woodiana* are the most prominent mollusc invaders recorded in the Sava River. *C. fluminalis* was also recorded in the most downstream stretch of the Sava River [20, 21].

There are still a lot of efforts needed to properly assess the pressures caused by biological invasions within the Sava River, to identify the most prominent invaders, to recognise the most effective ways of introduction and to design appropriate, achievable measures for prevention of further introduction and spreading of aquatic invaders.

The general feeling is that there is a lack of systematised data on invasive aquatic macroinvertebrates within the Sava River Basin, i.e., there is no detailed list of invasive taxa, their abundance and influence on native biota and habitats.

Fig. 12 Specimen of spiny-cheek crayfish collected at site 12 (photo by Paunović 2012)

5.4 *Basic Threats to the Biodiversity of Aquatic Macroinvertebrates of the Sava River*

Based on the review of literature data (Paunović et al. 2008, 2012) [1–10, 34, 35], as well as based on our data, the following threats to aquatic macroinvertebrate diversity could be revealed:

- Physical habitat degradation—water regulation (flood protection and navigation), damming (electricity production, water supply and flood protection), change of bottom characteristics (sedimentation due to hydrological change and gravel and sand extraction), hydrological changes (damming and other regulative works), disruption of longitudinal and lateral connectivity (damming and other regulative works), drying out of riparian ecosystems (agriculture and regulative works), etc.
- Organic and nutrient pollution (untreated wastewaters from settlements and farms) and agriculture
- Pollution by hazardous and other harmful substances (different pressures caused by industrial production, as well as thermal power plants)
- Biological invasions (presented in the previous subchapter)

The consequences of the above-mentioned activities should be further elaborated in order to provide bases for effective water management practice. Some of the threats were already quantified, but for some of them, there is still need for further elaboration [34, 35].

6 Conclusions

The investigated section of the Sava River, despite anthropogenic impacts (organic pollution, impact of agricultural activity and damming in Slovenian stretch), has considerable habitat diversity and the resulting macroinvertebrate fauna diversity.

A total of 227 macroinvertebrate taxa were recorded in the Sava River based on the result of our study. Having in mind that the upper stretch of the river, which is different in overall environmental conditions, was not studied in detail, the taxa richness is certainly higher. Based on the review of previous works on the macroinvertebrate fauna of the Sava River, as well as based on the comparison with findings in other large rivers within the Danube Basin, it could be expected that more than 300 species will be confirmed for the Sava River.

There is an obvious need for further investigation of the Sava River in order to complete the data on aquatic macroinvertebrates and to the provide basis for accurate assessment of environmental status of the river. This work represents the contribution to the basic knowledge on the aquatic fauna of this large river, as the basis for future designs of more effective water resource management within the Sava River Basin.

Based on previous discussions provided in this work, the Sava River could be preliminarily divided into five distinct sectors—alpine, subalpine, Upper Sava plain, Middle Sava and Lower Sava. For further divisions of sectors along the Sava River, additional material is needed.

Different forms of physical habitat degradation; organic, nutrient and chemical pollution; as well as biological invasions were underlined as the major threats to the biological diversity of aquatic macroinvertebrates.

There is an obvious need for further work on aquatic macroinvertebrates of the Sava River that primarily includes research on diversity and distribution, identification of relation of distribution of taxa and environmental factors, study on nonindigenous aquatic macroinvertebrate distribution patterns, functional community and ecosystem analyses and the work on better involvement of know-how on aquatic macroinvertebrates in water management practice.

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Ichthyofauna of the River Sava System

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Abstract On the survey of the recent records, the fish and lamprey fauna of the River Sava catchment consists of 74 species, 15 of which being considered alien. The indigenous species diversity, explained using the relation $N = 0.546\,A^{0.232}$, fits well into the range common for large catchments in Europe. Both taxonomic and ecological diversity, as well as the character of fish communities in streams and rivers, are strongly correlated with the stream order. On the relative abundance of species in fish communities, the upper rhithron fish communities cluster distinctly from those belonging to the middle rhithron, within which several subgroups of fish communities were distinguishable. Fish communities of the middle rhithron character in streams and small rivers stand distinctly apart from those belonging to particular sections of large rivers (e.g., the Rivers Sava, Drina, Vrbas, and Bosna), with the transitional type of middle rhithron fish community in larger rivers (e.g., those in the Rivers Una and Sana) that resemble more to the fish communities

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common in middle rhithron streams. Fish communities in the middle section of the River Sava in Croatia and in the bordering area with Bosnia and Herzegovina mainly belong to the lower rhithron, attaining the character of potamon in the most downstream, Serbian section. River Sava's fish communities strongly interact with the ones occurring in the most downstream sections of their largest tributaries, e.g., the Rivers Una, Vrbas, Bosna, Drina, and Kolubara, which makes them very similar in structure in the areas of river mouths. Classification of fish communities based solely on the presence and absence of species revealed similar general pattern of fish community classification, though with the more sharp delimitation between those belonging to the upper and middle rhithron on one and to the lower rhithron and potamon on the other side. That was supported by the determination of fish communities belonging to the upper rhithron with brown trout *Salmo* cf. *trutta*, European bullhead *Cottus gobio*, and minnow *Phoxinus phoxinus* as the most common fish species. Fish communities belonging to the middle rhithron were determined mainly with chub *Squalius cephalus* and spirlin *Alburnoides bipunctatus*, whereas brook barbel *Barbus balcanicus* and stone loach *Barbatula barbatula* occurred in both upper rhithron and middle rhithron. Nase *Chondrostoma nasus* were associated with both middle and lower rhithron fish communities. The most common fish species that determine the lower rhithron fish communities were common bream *Abramis brama*, ide *Idus idus*, and bleak *Alburnus alburnus*, with the northern pike *Esox lucius*, Balon's ruffe *Gymnocephalus baloni*, and racer goby *Neogobius gymnotrachelus* as significant species explaining fish communities of both lower rhithron and potamon. The level of production of fish in the River Sava varies remarkably within the sections with the similar ecological features, as well as between the sections that differ for the type of fish community. The greatest biomass and annual natural production were recorded in the sections homing the potamon and lower rhithron fish communities, especially in the flooding areas of side arms and oxbows which serve as spawning areas and nurseries. A total of 15 alien fish species was recorded in the River Sava catchment, the Prussian carp *Carassius gibelio* and brown bullhead *Ameiurus nebulosus* being assessed the most invasive in the areas with the potamon fish community. A strong impact from both long-term and recent stocking with alien hatchery-reared brown trout strains and rainbow trout in the upper rhithron fish communities was recently recognized. Mudminnow *Umbra krameri* and huchen (or Danube salmon) *Hucho hucho* are considered the two most threatened fish species of the River Sava catchment, where various types of riverbed modifications, especially the damming, were seen the most prominent threatening factors for fish diversity.

Keywords Fish fauna • Lamprey fauna • Diversity • Community structure • The River Sava Basin

1 Introduction

First records about fishes in the River Sava drainage area date far back, in the seventeenth century [1]. In the nineteenth and early twentieth centuries, fish were much more investigated there. Reports of investigations from the River Sava section [2–13] resulted in a list of 54 fish species from 10 families, including particular introduced fish species, e.g., rainbow trout *Oncorhynchus mykiss*, brook trout *Salvelinus fontinalis*, and brown bullhead *Ameiurus nebulosus*. The most recent records of fish from the upper part of the River Sava drainage area were given by Vovk and Budihna [14], Povž [15], Povž and Sket [16], and Šumer et al. [17]. During that period, an introduction of largemouth bass *Micropterus salmoides* and translocation of marble trout *Salmo marmoratus* into the River Sava catchment, as well as a disappearance of sterlet *Acipenser ruthenus*, the only resident sturgeon species in the middle and lower section of the River Sava in Slovenia [18], were reported by Povž [19, 20].

The first investigation of the lower part of the River Sava ichthyofauna downstream of the town of Sisak was given by Plančić [21], where 25 species were then recorded. The most recent records for this part were given by Veljović [22], Suić [23], Zanella et al. [24], Mrakovčić et al. [25], Mikavica et al. [26], Ćaleta [27], and Sofradžija [28].

Mrakovčić et al. [29] stated that 42 native European lamprey and fish species from 13 families occur in the River Sava catchment area, majority of whom (27 species) are from the f. Cyprinidae. Mikavica et al. [26] recorded 29 fish species from seven families in the River Sava section from the confluence with the River Una to the confluence with the River Vrbas, whereas Sofradžija [28] stated 52 fish species for the whole River Sava middle section.

There are a lot of papers related to the fish fauna of tributaries and backwaters of the River Sava, some of the more recent ones being those of Aganović et al. [30], Mehmedagić [31], Mikavica et al. [32], Mikavica and Savić [33], Sofradžija et al. [34], Korjenić [35], Bakrač-Bećiraj and Mujić [36], Skenderović et al. [37], Adrović et al. [38], and Bećiraj and Šahinović [39].

Seven fish species (huchen *Hucho hucho*, mudminnow *Umbra krameri*, Danubian roach *Rutilus pigus*, Kessler's gudgeon *Gobio kessleri*, Danubian gudgeon *Gobio uranoscopus*, striped ruffe *Gymnocephalus schraetser*, zingel *Zingel zingel* and streber *Zingel streber*) that occur in the River Sava catchment are endemics or subendemics of the River Danube catchment. In addition to that, the River Sava catchment holds the specific, Balkan lineage of grayling *Thymallus thymallus*, with the variety of haplotypes, i.e., high level of diversity in the southernmost part of the dispersal area of this widely dispersed species [40]. There is also a notification about the differentiation of the huchen in the River Sava catchment into two distinct stocks: the western one occurring in the upper and middle course in Slovenia and the eastern one that comprises huchen from streams and rivers in eastern Bosnia, Serbia, and northern Montenegro [41, 42]. In contrast to that, the indigenous diversity assessed in alien hatchery-reared brown trout *Salmo* cf. *trutta* strain was very limited at the mtDNA level in the River Sava drainage area [43, 44].

In contrast to tributaries, where only recreative fishing is allowed, the River Sava itself is both recreative fishery and commercial fishery, except in Slovenia, where only recreative fishery is on board. Both recreative fishing as a modern leisure activity and commercial fishing as an occupation have arisen from the small traditional fishing of the people living near streams and rivers that have provided fish flesh as a food through centuries, using hook-, trap-, and net-based fishing gears. Fishing is legally regulated in all countries in the River Sava catchment, but that legislative frame differs, depending on tradition, fishery settings, state capacity, and opportunities for fishing as an economic category. Each of the states in the River Sava catchment has inland waters' fishery system based on midterm and annual management plans that asses the status of fish stocks and project the rate of fishery utilization, as well as fishery measures, activities (e.g., hatching, rearing, and stocking), and regulations, whose implementation greatly varies from state to state. The gross income from inland water fishery is the greatest in Slovenia, where the River Sava catchment holds many internationally renowned trout and grayling fly-fishing streams (e.g., the Rivers Unec, Sava Bohinjka, and Radovna) with high price of fishing licenses. Certain formerly famous fly-fishing destinations for international fishermen were recently reaffirmed at streams and rivers of the River Sava catchment in Croatia (e.g., the Rivers Kupa, or Kolpa, and Dobra) and Bosnia and Herzegovina (e.g., the Rivers Una, Sana, Klokot, Krušnica, Ribnik, Pliva, Janj), and a new one started to appear in Montenegro (e.g., upper River Lim) and Serbia (the River Gradac). Angling for other fish species is also popular throughout the River Sava watershed. Chub *Squalius cephalus*, nase *Chondrostoma nasus*, common barbel *Barbus barbus*, and Danubian roach are favorite angling species in streams and rivers in highland areas and carp *Cyprinus carpio*, wels *Silurus glanis*, zander *Sander lucioperca*, and northern pike *Esox lucius* in lowland rivers and reservoirs. Other common fish species favored by anglers are clustered in "white fish" comprising breams (*Abramis brama*, *A. sapa*, *A. ballerus*, *Vimba vimba*, *Blicca bjoerkna*) and Prussian carp (*Carassius gibelio*) and introduced bigheads (gray *Hypophthalmichthys molitrix* and white *Hypophthalmichthys nobilis*) and white grass carp (*Ctenopharyngodon idella*). Commercial fishermen use to target economically more valuable fish, like wels, starlet, and zander, though in certain parts of fishing season and on catching "value fish" they also trade with other fish, which is considered second and third grade for their quality and price. Fishery market for the trading with the commercial catches of fish mainly relies on fishermen as individual entrepreneurs in selling, both on shore and at open markets, which slowly changes toward the setting of properly equipped fish markets. Limits and constraints set by fishery legislation in the River Sava catchment vary, e.g., for the minimal landing size and closed season for fishing of huchen in Bosnia and Herzegovina and Serbia, but there is an obvious intent to harmonize national regulations with the international conventions and initiatives, which adds to the harmonization between the states in the River Sava catchment much more and quicker than through their direct negotiations. It seems that despite of variety in opportunities for the development of fishery, it will share the destiny of the gross development of economies in the states of the River Sava catchment.

The overall diversity of fish (including lamprey) species in the River Sava catchment, including tributaries, was never surveyed hitherto, although it was well known from the investigations of both academic and applied characters. The main aim of this chapter is to reveal that diversity and its main determinants, with the amount of data that could serve as a starting point for prospect investigations and inferring about the status of fish over the River Sava catchment. In addition, the fishery in the area was reviewed after the available records.

2 Materials and Methods

Data set for analysis of fish community structure was created from the lists of samples taken in each of the countries using various electrofishing and netting gears and consisted of the number of each fish species in the sample caught at each locality representing the absolute abundance, which was transformed in the set of relative abundances for each species at each locality. The only exception is data set obtained from Slovenia that consisted of records denoting the presence and absence of particular fish species at each locality.

Estimation of taxonomic richness of lamprey and fish species in streams and rivers of the River Sava system was estimated following Welcomme [45], after expression:

$$N = f\,A^b,$$

where N is the number of species and A is the surface of catchment (in square kilometers). Records for surfaces for particular streams' and rivers' catchments were taken from Marković [46].

Overall taxonomic diversity, as well as that of fish community at each of sampling locality, was considered using the Shannon–Weaver Information Index H', with the additional measure that complements the ecological component of diversity esteemed using the Evenness Index (J) [47] for the fish community at each of sampling localities.

Characterization of fish communities was worked out by calculating the Ecological Index E_i that Šorić [48] introduced for fish species in inland waters of the River Danube system in Serbia and adjacent regions. That index uses the rank f (i.e., weight) of each fish species in the sample according to its relative abundance ($f_{(<1\ \%)} = 1$; $f_{(1–3\ \%)} = 2$; $f_{(3–10\ \%)} = 3$; $f_{(10–20\ \%)} = 4$; $f_{(20–40\ \%)} = 7$; $f_{(>40\ \%)} = 9$) and K indicator values for each type of aquatic habitats (1 for upper rhithron, 2 for middle rhithron, 3 for lower rhithron, and 4 for potamon) that is common for particular fish species. It is calculated using the expression:

$$E_i = \sum (K_i f_i) / \sum f_i.$$

Fish communities with the value of E_i lower than 1.5 are upper rhithronic, those with the E_i up to 2.5 are middle rhithronic, those with the E_i up to 3.5 are lower rhithronic, and those over 3.5 belong to the potamon fish community type.

Relationships between fish community structure, stream order, components of diversity, biomass, and annual natural production were checked by Pearson Correlation Coefficient r [49].

Analysis of similarity between fish community samples for their structure was accomplished using cluster analysis of samples on relative abundance of fish species in them, accomplished by Ward's method of clustering on the Chebyshev distance metrics. Ward's method of clustering is a hierarchical (i.e., agglomerative) clustering tool that minimizes the total variance within the cluster [50], whereas the Chebyshev distance metric favors the maximum of distance between two vectors or objects in any of their dimensions, i.e., $D_{\text{Chebyshev}}(x,y) = \max(|x_i - y_i|)$. In addition to that, another method of analysis was applied, in order to investigate the structure of fish communities in the part of the River Sava catchment in Slovenia, where only qualitative data were available. That data set consisting of the presence/absence data for particular fish species in particular streams and rivers was clustered on Euclidean distances [51] between their fish communities using the Ward's clustering method.

To understand correlation between type of fish communities and river zonation, constrained Redundancy Analysis (RDA) [52] with dummy variables (explanatory variables) was used to relate fish species (response variables) with particular locality (samples). RDA is a constrained form of the linear ordination method of principal component analysis (PCA). The output of this analysis is displayed in an ordination diagram with the loadings of response variables represented by arrows and multivariate scores of sampling localities represented by points. RDA was performed for the 74 fish species as response variables studied. To evaluate significance of particular species, the Monte Carlo permutation test ($P > 0.05$) with manual selection was used. The software for this statistical analysis was performed using CANOCO for Windows 4.5 software package [52].

Fish productivity was evaluated from the records of average biomass and annual rate of survival for each age class of fish species in samples taken during an accomplishment of Fishery Management Plans available for streams and rivers in the River Sava catchment.

3 Results

Fish (including lamprey) fauna of the River Sava catchment consists of 74 species belonging to 14 families. Fifteen species are considered alien (Tables 1–8). Their taxonomic diversity assessed for 23 river catchments in the River Sava system is

Table 1 Occurrence of lamprey and fish species in the River Sava from its source downstream to the mouth with the River Danube

Fish species	Sava Dolinka	Sava Bohinjka	Sava Slo (upstream section)	Sava Slo (middle section)	Sava Slo (downstream section)	Sava Medsave	Sava Zagreb	Sava L Dubrovcak	Sava Trebež	Sava Jasenovac	Sava Gradiška	Sava Davor	Sava Slavonski Brod	Sava Babina Greda	Sava Račinovci	Sava Jarak	Sava Mišar	Sava Obrenovac	Sava (R. Kolubara junction)	Sava Makiš
Ukrainian lamprey *Eudontomyzon mariae*	+	+			+															
Sterlet *Acipenser ruthenus*							+											+		+
Brown trout *Salmo trutta*	+	+	+	+	+															
Rainbow trout *Oncorhyncus mykiss*	+	+	+	+	+															
Arctic char *Salvelinus alpinus*		+																		
Brook trout *Salvelinus fontinalis*	+	+	+																	
Huchen *Hucho hucho*	+	+	+	+	+															
European grayling *Thymallus thymallus*	+	+	+	+	+															
Northern pike *Esox lucius*			+	+	+				+	+	+	+	+	+	+		+	+	+	+
Bream *Abramis brama*			+	+	+	+	+		+	+	+		+	+	+	+	+	+		+
Whiteye bream *Abramis sapa*											+		+	+		+		+	+	
White bream *Blicca bjoerkna*				+	+				+	+	+	+	+	+	+	+		+	+	+
Blue bream *Abramis ballerus*									+											
Vimba *Vimba vimba*			+	+	+	+	+	+	+	+	+	+	+	+	+			+		+
Tench *Tinva tinca*			+	+	+										+					
Common carp *Cyprinus carpio*			+	+	+				+	+	+	+	+	+			+			+
Crucian carp *Carassius carassius*				+	+	+														
Giebel carp *Carassius gibelio*			+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
White grasscarp *Ctenopharyngodon idella*				+	+															
Rudd *Sacrdinius erythrophthalmus*			+	+	+						+									
Asp *Aspius aspius*				+	+	+	+	+	+	+	+	+	+	+	+		+			+

(continued)

Table 1 (continued)

Fish species	Sava Dolinka	Sava Bohinjka	Sava Slo (upstream section)	Sava Slo (middle section)	Sava Slo (downstream section)	Sava Medsave	Sava Zagreb	Sava L Dubrovcak	Sava Trebež	Sava Jasenovac	Sava Gradiška	Sava Davor	Sava Slavonski Brod	Sava Babina Greda	Sava Račinovci	Sava Jarak	Sava Mišar	Sava Obrenovac	Sava (R. Kolubara junction)	Sava Makiš
Danubian roach *Rutilus pigus*			+	+	+	+	+	+	+	+	+	+	+	+	+					
Roach *Rutilus rutilus*			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bitterling *Rhodeus sericeus*				+	+	+	+	+	+	+	+	+	+	+	+					
Bleak *Alburnus alburnus*			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+
Spirlin *Alburnoides bipunctatus*			+	+	+	+	+			+										
Minnow *Phoxinus phoxinus*	+	+	+	+	+															
Bladgeon *Leuciscus souffia*	+	+	+	+	+															
Dace *Leuciscus leuciscus*				+		+	+	+	+											
Chub *Squalius cephalus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Nase *Chondrostoma nasus*		+	+	+	+	+	+		+	+	+	+	+	+	+	+			+	
Orfe *Idus idus*					+				+	+	+	+	+	+	+		+			
Common barbel *Barbus barbus*		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Brook barbel *Barbus balcanicus*		+	+	+	+	+	+		+											
Gudgeon *Gobio gobio*		+	+	+	+	+	+	+	+											
Danubian gudgeon *Gobio uranocopus*		+	+	+	+		+			+	+									
Whitefin gudgeon *Gobio albipinnatus*				+	+				+	+	+	+	+	+	+					
Kessler's gudgeon *Gobio kessleri*			+	+	+															
Topmouth gudgeon *Pseudorasbora parva*				+	+	+	+	+	+	+	+	+	+	+	+					
Gray bighead *Hypophthalmichthys nobilis*																	+		+	+
Stone loach *Barbatula barbatula*	+		+	+	+	+	+	+												

Weather loach *Misgurnus fossilis*			+	+							+									
Balkan loach *Cobitis elongata*					+	+	+	+	+	+	+	+	+	+	+					
Riffle loach *Cobitis elongatoides*	+		+		+	+	+	+	+	+	+	+	+	+	+					
Golden loach *Sabanejewia aurata*					+							+		+	+					
Wells *Silurus glanis*		+			+	+	+		+	+	+	+	+	+	+	+		+	+	+
Brown bullhead *Ameiurus nebulosus*										+	+	+	+	+	+					+
Burbot *Lota lota*		+		+	+				+	+		+	+	+						
Eurasian perch *Perca fluviatilis*		+		+	+			+	+	+	+	+	+	+	+	+				
Common ruffe *Gymnocephalus cernuus*				+	+				+	+		+	+	+	+					
Striped ruffe *Gymnocephalus schraetseri*					+				+							+		+		
Zander *Sandra lucioperca*				+	+					+		+	+	+		+	+	+	+	+
Zingel *Zingel zingel*														+				+		
Streber *Zingel streber*				+	+								+		+					
Pumpkinseed *Lepomis gibbosus*					+	+		+	+	+	+	+	+	+	+		+			+
Miller's thumb *Cottus gobio*					+											+	+	+	+	+
Monkey goby *Neogobius fluviatilis*			+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Round goby *Neogobius melanostomus*													+	+	+	+	+	+	+	+
Racer goby *Neogobius gymnotrachelus*																+	+	+	+	+
Bighead goby *Neogobius kessleri*												+	+		+			+	+	+
Tubenose goby *Proterorhinus marmoratus*																+	+	+	+	+
Fish species number	11	18	29	39	47	22	23	17	30	29	28	28	31	31	29	18	16	19	17	22

Table 2 Occurrence of lamprey and fish species in the tributaries at the Slovenian section of the River Sava catchment listed in order by their position from the upper section downstream, eastward, as well as by stream order (with numbers, in rising order from headwater section downstream) where applicable and locality of sampling

Fish species	Sora	Ljubljanica	Mirna	Krka	Kolpa	Savinja	Sotla
Ukrainian lamprey *Eudontomyzon mariae*		+	+	+	+		+
Brown trout *Salmo trutta*	+	+	+	+	+	+	+
Rainbow trout *Oncorhyncus mykiss*	+	+	+	+	+	+	+
Brook trout *Salvelinus fontinalis*	+	+		+	+	+	
Huchen *Hucho hucho*	+	+	+	+	+	+	
European grayling *Thymallus thymallus*	+	+	+	+	+	+	
Northern pike *Esox lucius*		+	+	+	+	+	+
Bream *Abramis brama*		+		+		+	+
White bream *Blicca bjoerkna*		+		+			+
Vimba *Vimba vimba*		+		+	+	+	+
Tench *Tinca tinca*		+		+	+	+	+
Common carp *Cyprinus carpio*		+	+	+	+	+	+
Crucian carp *Carassius carassius*		+		+	+	+	+
Giebel carp *Carassius gibelio*				+		+	+
White grasscarp *Ctenopharyngodon idella*					+	+	
Rudd *Sacrdinius erythrophthalmus*		+		+		+	+
Asp *Aspius aspius*			+	+		+	
Danubian roach *Rutilus pigus*	+	+	+	+	+	+	+
Roach *Rutilus rutilus*		+	+	+	+	+	+
Bitterling *Rhodeus sericeus*		+		+	+	+	+
Bleak *Alburnus alburnus*		+	+	+	+	+	+
Spirlin *Alburnoides bipunctatus*	+	+	+	+	+	+	+
Minnow *Phoxinus phoxinus*	+	+	+	+	+	+	
Bladgeon *Leuciscus souffia*	+	+	+		+	+	+
Dace *Leuciscus leuciscus*			+				
Chub *Squalius cephalus*	+	+	+	+	+	+	+
Nase *Chondrostoma nasus*	+	+	+	+	+	+	+
Orfe *Idus idus*				+			+
Common barbel *Barbus barbus*	+	+	+	+	+	+	+
Brook barbel *Barbus balcanicus*	+	+	+	+	+	+	+
Gudgeon *Gobio gobio*	+	+	+	+	+	+	+

(continued)

Table 2 (continued)

Fish species	Sora	Ljubljanica	Mirna	Krka	Kolpa	Savinja	Sotla
Danubian gudgeon *Gobio uranocopus*	+			+	+	+	+
Whitefin gudgeon *Gobio albipinnatus*			+	+		+	
Kessler's gudgeon *Gobio kessleri*				+	+		+
Topmouth gudgeon *Pseudorasbora parva*				+		+	+
Stone loach *Barbatula barbatula*	+	+	+	+	+	+	+
Weather loach *Misgurnus fossilis*		+		+		+	+
Balkan loach *Cobitis elongata*			+	+	+	+	+
Riffle loach *Cobitis elongatoides*	+	+	+	+	+	+	+
Golden loach *Sabanejewia aurata*	+	+	+	+	+	+	
Wells *Silurus glanis*		+		+	+		
Brown bullhead *Ameiurus nebulosus*							+
Burbot *Lota lota*		+	+	+			+
Eurasian perch *Perca fluviatilis*		+	+	+	+	+	+
Common ruffe *Gymnocephalus cernuus*				+	+	+	
Balon's ruffe *Gymnocephalus baloni*	+						
Striped ruffe *Gymnocephalus schraetseri*					+		
Zander *Sandra lucioperca*		+		+		+	+
Streber *Zingel streber*			+	+	+	+	+
Pumpkinseed *Lepomis gibbosus*		+		+	+	+	+
Monkey goby *Neogobius fluviatilis*	+	+	+	+	+	+	
Fish species number	20	36	29	45	37	41	37

Table 3 Occurrence of lamprey and fish species in the River Una catchment, listed in order by their position from the upper section downstream by stream order (with numbers, in rising order from headwater section downstream) where applicable, by tributaries and by locality of sampling

Fish species	Una 1 Unac	Una 2 Martinbrod	Una 3 Bihać	Una 4 B Krupa	Una 5 B Krupa	Una 6 Otoka	Krušnica	Klokot	Mlječanica	Sana 1 Ključ	Sana 2 Sanica	Sana 3 Sanski Most	Pakra Banova Jaruga
Brown trout *Salmo trutta*	+	+	+	+	+	+	+	+		+	+	+	
Rainbow trout *Oncorhyncus mykiss*							+	+					
European grayling *Thymallus thymallus*		+					+	+			+		
Northern pike *Esox lucius*					+	+	+	+				+	
Bream *Abramis brama*													+
Vimba *Vimba vimba*												+	
Giebel carp *Carassius gibelio*													+
Danubian roach *Rutilus pigus*			+	+	+	+	+	+					
Roach *Rutilus rutilus*	+		+		+	+		+					+
Bleak *Alburnus alburnus*						+		+				+	+
Spirlin *Alburnoides bipunctatus*	+		+	+	+	+				+	+	+	
Minnow *Phoxinus phoxinus*	+		+	+			+	+	+	+	+		
Chub *Squalius cephalus*	+		+	+	+	+	+	+	+			+	+
Nase *Chondrostoma nasus*												+	

Common barbel *Barbus barbus*	+		+	+									
Brook barbel *Barbus balcanicus*	+		+	+	+	+			+	+	+	+	
Gudgeon *Gobio gobio*					+	+	+		+			+	+
Stone loach *Barbatula barbatula*			+	+		+			+	+			
Riffle loach *Cobitis elongatoides*									+				
Spined loach *Cobitis taenia*			+	+	+	+				+			
Burbot *Lota lota*									+				
Eurasian perch *Perca fluviatilis*												+	+
Common ruffe *Gymnocephalus cernuus*													+
European bullhead *Cottus gobio*	+	+	+	+				+	+		+	+	
Fish species number	8	3	11	10	9	11	8	10	8	7	6	11	8

Table 4 Occurrence of lamprey and fish species in the River Vrbas catchment and Pakra reservoir, listed in order by position of localities from the upper section downstream (with numbers, in rising order from headwater section downstream) with the name of the locality of sampling

Fish species	Vrbas 1 Jelić	Vrbas 2 Bugojno	Vrbas 3 Jajce	Vrbas 4 Jajce	Vrbas 5 HE Jajce	Pakra reservoir
Brown trout *Salmo trutta*	+	+	+	+	+	
European grayling *Thymallus thymallus*				+	+	
Northern pike *Esox Lucius*						+
Common carp *Cyprinus carpio*						+
White grasscarp *Ctenopharyngodon idella*						+
Roach *Rutilus rutilus*						+
Bleak *Alburnus alburnus*						+
Spirlin *Alburnoides bipunctatus*		+	+	+	+	
Minnow *Phoxinus phoxinus*					+	
Chub *Squalius cephalus*			+	+	+	+
Nase *Chondrostoma nasus*		+	+		+	
Common barbel *Barbus barbus*				+	+	
Brook barbel *Barbus balcanicus*		+	+	+	+	
Brown bullhead *Ameiurus nebulosus*						+
Eurasian perch *Perca fluviatilis*						+
Zander *Sandra lucioperca*						+
Pumpkinseed *Lepomis gibbosus*						+
European bullhead *Cottus gobio*		+				
Fish species number	1	5	5	6	8	10

explained with the expression $N = 0.546\,A^{0.232}$ ($r = 0.59$; $F_{(1,21)} = 11.092$; $p < 0.05$). Increase in stream order is significantly correlated with the increase in number of fish species ($r^2 = 0.717$; $p < 0.001$) (Fig. 1), being for the River Sava even stronger ($r^2 = 0.884$; $p < 0.001$). Increase in stream order is also significantly correlated with the values of Shannon–Weaver Index H' ($r^2 = 0.664$; $p < 0.001$) representing the taxonomic diversity (Fig. 2) and Ecological Index E_i ($r^2 = 0.786$; $p < 0.001$) that

Table 5 Occurrence of lamprey and fish species in the River Bosna and its tributaries, listed in order of localities by their position from the upper section downstream and by stream order (with numbers, in rising order from headwater section downstream) where applicable

Fish species	Bosna 1 izvor	Bosna 2 Zenica	Bosna 3 Zavidovići	Bosna 4 Maglaj	Krivaja 4 ušće	Krivaja 3 Maoča	Krivaja 2 Solun	Krivaja 1 Olovo	Lašva 5 ušće	Lašva 4 Donja Rovnja	Lašva 3 Mali Mošunj	Lašva 2 crkva	Lašva 1 izvor
Brown trout *Salmo trutta*	+						+			+	+	+	+
European grayling *Thymallus thymallus*											+		
Vimba *Vimba vimba*				+									
Giebel carp *Carassius gibelio*		+											
Bleak *Alburnus alburnus*		+		+									
Spirlin *Alburnoides bipunctatus*		+	+	+	+	+	+	+					
Minnow *Phoxinus phoxinus*	+												
Chub *Squalius cephalus*		+	+	+	+	+	+		+				
Nase *Chondrostoma nasus*					+	+							
Common barbel *Barbus barbus*				+									

(continued)

Table 5 (continued)

Fish species	Bosna 1 izvor	Bosna 2 Zenica	Bosna 3 Zavidovići	Bosna 4 Maglaj	Krivaja 4 ušće	Krivaja 3 Maoča	Krivaja 2 Solun	Krivaja 1 Olovo	Lašva 5 ušće	Lašva 4 Donja Rovnja	Lašva 3 Mali Mošunj	Lašva 2 crkva	Lašva 1 izvor
Brook barbel *Barbus balcanicus*		+	+	+	+	+	+	+	+	+			
Gudgeon *Gobio gobio*		+	+		+								+
Spined loach *Cobitis taenia*		+			+								
European bull-head *Cottus gobio*	+												+
Fish species number	3	7	4	6	6	4	4	2	2	2	2	1	3

Table 6 Occurrence of lamprey and fish species in tributaries of the River Bosna, listed in order of localities by their position from the upper section downstream and by stream order (with numbers, in rising order from headwater section downstream) where applicable

Fish species	Željeznica	Zujevina	Fojnica	Zlaća	Krabanja	Oskova	Gostelja	Spreča 1	Modrac reservoir	Spreča 2	Tinja	Brka	Prača	Sniježnica
Brown trout *Salmo trutta*	+	+	+	+	+	+	+						+	
Rainbow trout *Oncorhyncus mykiss*			+				+							
European grayling *Thymallus thymallus*			+										+	
Northern pike *Esox lucius*								+	+					
Bream *Abramis brama*								+	+	+				+
Whiteye bream *Abramis sapa*							+			+				
Vimba *Vimba vimba*										+	+			
Tench *Tinca tinca*							+		+					+
Common carp *Cyprinus carpio*									+					+
Crucian carp *Carassius carassius*									+					
Giebel carp *Carassius gibelio*						+	+		+					+
Rudd *Sacrdinius erythrophthalmus*								+	+					+
Asp *Aspius aspius*									+					
Danubian roach *Rutilus pigus*														
Roach *Rutilus rutilus*						+	+	+	+					+
Bitterling *Rhodeus sericeus*	+						+	+	+			+		+
Sichel *Pelecus cultratus*														

(continued)

Table 6 (continued)

Fish species	Željeznica	Zujevina	Fojnica	Zlaća	Krabanja	Oskova	Gostelja	Spreča 1	Modrac reservoir	Spreča 2	Tinja	Brka	Prača	Sniježnica
Bleak *Alburnus alburnus*			+				+	+	+	+				+
Shemaya *Chalcalburnus chalcoides*														
Spirlin *Alburnoides bipunctatus*	+		+				+	+		+	+	+		
Minnow *Phoxinus phoxinus*	+	+	+	+	+	+	+					+	+	
Chub *Squalius cephalus*	+	+	+			+	+	+	+	+	+	+		
Nase *Chondrostoma nasus*	+							+						+
Common barbel *Barbus barbus*		+								+				
Brook barbel *Barbus balcanicus*	+		+			+	+	+	+	+	+	+		
Gudgeon *Gobio gobio*		+	+			+	+	+	+	+	+	+		
Topmouth gudgeon *Pseudorasbora parva*												+		
Stone loach *Barbatula barbatula*						+	+					+		
Balkan loach *Cobitis elongata*		+							+					
Riffle loach *Cobitis elongatoides*	+		+				+	+	+			+		
Golden loach *Sabanejewia aurata*						+	+					+		
Wells *Silurus glanis*									+	+				
Black bullhead *Ameiurus melas*									+					

Eurasian perch *Perca fluviatilis*							+	+	+	+				
Common ruffe *Gymnocephalus cernuus*							+	+	+					
Balon's ruffe *Gymnocephalus baloni*														+
Zander *Sandra lucioperca*								+	+					
Streber *Zingel streber*			+											
Pumpkinseed *Lepomis gibbosus*							+		+					+
European bullhead *Cottus gobio*	+	+	+			+							+	
Fish species number	9	7	12	2	2	10	19	15	22	11	5	10	4	11

Table 7 Occurrence of and fish species in the River Drina and its tributaries, listed in order of localities by their position from the upper section downstream and by stream order (with numbers, in rising order from headwater section downstream) where applicable

Fish species	Drina 1 Šćepan polje	Drina 2 Goražde	Drina 3 Perućac	Drina 4 Ljubovija	Drina 5 Zvornik	Drina 6 Loznica	Drina 7 Ušće	Ljuboviđa 1	Ljuboviđa 2	Drinjača 1	Drinjača 2
Sterlet *Acipenser ruthenus*						+					
Brown trout *Salmo trutta*	+	+						+	+	+	
Huchen *Hucho hucho*	+	+	+	+		+	+				
European grayling *Thymallus thymallus*	+	+		+							
Northern pike *Esox lucius*						+	+				
Bream *Abramis brama*			+		+	+	+				
White bream *Blicca bjoerkna*							+				
Vimba *Vimba vimba*			+	+	+	+	+				
Tench *Tinca tinca*			+								
Common carp *Cyprinus carpio*					+		+				
Crucian carp *Carassius carassius*							+				
Giebel carp *Carassius gibelio*				+	+	+					
Rudd *Sacrdinius erythrophthalmus*						+	+				
Asp *Aspius aspius*				+			+				
Danubian roach *Rutilus pigus*	+	+	+	+		+	+				
Roach *Rutilus rutilus*					+	+					
Bitterling *Rhodeus sericeus*					+	+	+				
Sichel *Pelecus cultratus*							+				
Bleak *Alburnus alburnus*	+	+	+	+	+	+	+				

Shemaya *Chalcalburnus chalcoides*				+		+	+				
Spirlin *Alburnoides bipunctatus*	+	+		+		+			+	+	+
Minnow *Phoxinus phoxinus*	+			+				+	+	+	
Bladgeon *Leuciscus souffia*	+										
Chub *Squalius cephalus*	+	+	+	+	+	+	+		+		+
Nase *Chondrostoma nasus*	+	+	+	+	+	+	+				+
Orfe *Idus idus*							+				
Common barbel *Barbus barbus*	+	+	+	+	+	+	+				
Brook barbel *Barbus balcanicus*	+	+	+	+	+			+	+	+	+
Gudgeon *Gobio gobio*	+	+	+	+		+					+
Danubian gudgeon *Gobio uranocopus*											
Whitefin gudgeon *Gobio albipinnatus*				+	+						
Kessler's gudgeon *Gobio kessleri*											
Topmouth gudgeon *Pseudorasbora parva*					+						
Stone loach *Barbatula barbatula*							+				+
Weather loach *Misgurnus fossilis*							+				
Balkan loach *Cobitis elongata*				+							
Spined loach *Cobitis taenia*			+	+	+	+					
Wells *Silurus glanis*			+			+	+				

(continued)

Table 7 (continued)

Fish species	Drina 1 Šćepan polje	Drina 2 Goražde	Drina 3 Perućac	Drina 4 Ljubovija	Drina 5 Zvornik	Drina 6 Loznica	Drina 7 Ušće	Ljuboviđa 1	Ljuboviđa 2	Drinjača 1	Drinjača 2
Burbot *Lota lota*						+	+				
Eurasian perch *Perca fluviatilis*				+		+	+				
Common ruffe *Gymnocephalus cernuus*			+		+						
Striped ruffe *Gymnocephalus schraetseri*			+	+	+	+	+				
Zander *Sandra lucioperca*							+				
Zingel *Zingel zingel*				+			+				
Streber *Zingel streber*			+		+		+				
Pumpkinseed *Lepomis gibbosus*					+	+					
European bullhead *Cottus gobio*	+	+		+							
Fish species number	14	12	16	22	18	23	27	3	5	4	6

Table 8 Occurrence of lamprey and fish species in two localities (Krstonošić oxbow and Vok canal) of the Obedska swamp, in the River Bosut as well as in the River Kolubara and its tributaries, listed in order by their position from the upper section downstream and by stream order (with numbers, in rising order from headwater section downstream) where applicable

Fish species	Krstonošić	Vok	Bosut	Kolubara 1	Kolubara 2	Jablanica	Obnica	Gradac 1	Gradac 2
Ukrainian lamprey *Eudontomyzon mariae*				+					
Brown trout *Salmo trutta*								+	+
European grayling *Thymallus thymallus*								+	
Northern pike *Esox lucius*	+	+	+						
Bream *Abramis brama*	+	+	+		+				
White bream *Blicca bjoerkna*			+						
Blue bream *Abramis ballerus*			+						
Tench *Tinca tinca*	+		+						
Common carp *Cyprinus carpio*	+		+						
Crucian carp *Carassius carassius*	+								
Giebel carp *Carassius gibelio*	+		+						
Rudd *Sacrdinius erythrophthalmus*	+		+						
Asp *Aspius aspius*			+						
Roach *Rutilus rutilus*	+	+	+						
Bleak *Alburnus alburnus*	+	+	+		+				
Spirlin *Alburnoides bipunctatus*						+	+	+	+
Minnow *Phoxinus phoxinus*								+	+
Chub *Squalius cephalus*				+		+		+	+
Nase *Chondrostoma nasus*				+					
Orfe *Idus idus*			+		+				
Common barbel *Barbus barbus*				+					
Brook barbel *Barbus balcanicus*				+		+	+	+	+
Gudgeon *Gobio gobio*						+	+		
White bighead *Hypophthalmichthys molitrix*			+						

(continued)

Table 8 (continued)

Fish species	Krstonošić	Vok	Bosut	Kolubara 1	Kolubara 2	Jablanica	Obnica	Gradac 1	Gradac 2
Stone loach *Barbatula barbatula*				+		+	+		+
Spined loach *Cobitis taenia*	+								
Wells *Silurus glanis*	+	+	+						
Brown bullhead *Ameiurus nebulosus*			+						
Burbot *Lota lota*		+							
Eurasian perch *Perca fluviatilis*		+	+						
Common ruffe *Gymnocephalus cernuus*			+						
Zander *Sandra lucioperca*			+						
Volga zander *Sandra volgense*			+						
Pumpkinseed *Lepomis gibbosus*	+	+							
European bullhead *Cottus gobio*								+	+
Fish species number	12	8	19	6	3	5	4	7	7

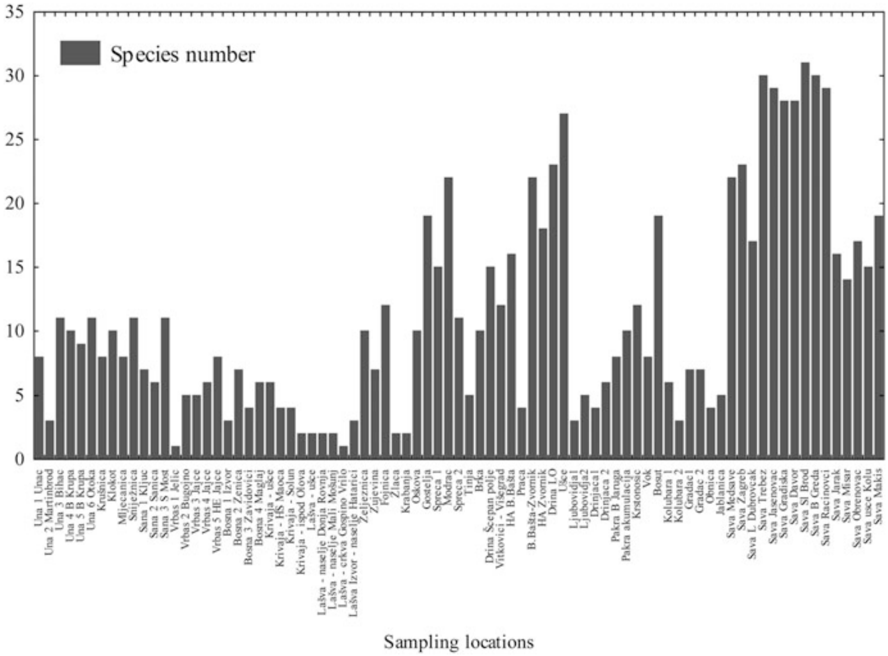

Fig. 1 Number of lamprey and fish species for streams and rivers at localities in the River Sava catchment

assigns the type of fish communities in streams of the River Sava catchment (Fig. 3). In contrast to that, there is no correlation ($r^2 = 0.147$; $p > 0.1$) with the Evenness Index J (Fig. 2). Likewise, considering only the River Sava, the increase in order downstream is not significantly correlated either to the fish biomass ($r^2 = -0.208$; $p > 0.1$) or their annual natural production ($r^2 = 0.308$; $p > 0.1$).

Streams and rivers in the River Sava system with the similar E_i values usually clustered together, but some of them deviated from that general pattern at the first glance (Fig. 4). The most distinct main cluster standing apart from all others was that of upper rhithron streams Ljuboviđa 1, Krabanja, Zlaća, Vrbas 1 Jelić, and Lašva 2 crkva, holding either exclusively or predominantly brown trout with associated minnow and brook barbel in much smaller abundance. All other upper rhithron fish communities (e.g., Una 2 Martinbrod, Sana 2 Sanica, Vrbas 2 Bugojno, Prača, Lašva 2, 3, and 4, Bosna 1 izvor, Fojnica, Krivaja 1 Olovo and 2 Solun, and Gradac 1 and 2) homed also other fish species of the upper rhithron fish community (e.g., European bullhead and stone loach) in greater abundance but also some of fish species (e.g., grayling, spirlin, and common gudgeon *Gobio gobio*) that belong to the next, middle rhithron type of fish community, which clustered them with the streams of that type that were the greatest cluster comprising the majority of fish communities. River Sava was regularly divided for its fish community character: middle rhithron fish communities from the section Zagreb–Babina Greda clustered

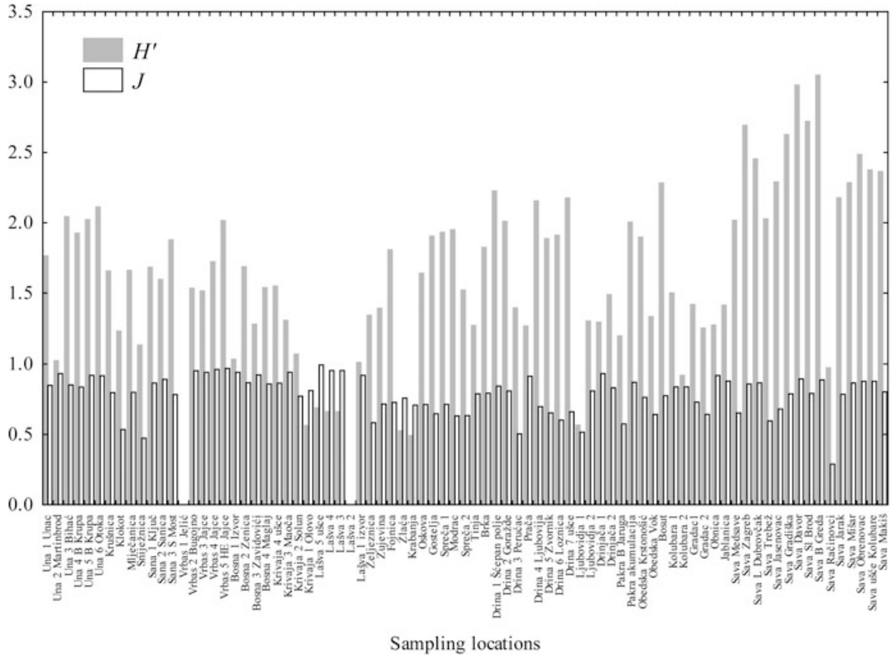

Fig. 2 Shannon Diversity (H') and Evenness (J) Indices for generated from records for structure and abundance of lamprey and fish species in streams and rivers at localities in the River Sava catchment

distinctly, as well as those of the character of potamon from the section Mišar–Obrenovac–Makiš. Only the section in Jarak was more similar to the lowest, lower rhithron sections (6 Loznica and 7 ušće) of the River Drina. Potamon fish communities in lentic habitats (e.g., Modrac, Pakra reservoir, Drina 5 Zvornik, Obedska Vok, and Obedska Krstonošić) clustered irregularly in various clusters with the lotic habitats.

Patterns revealed for the similarity in structure of fish community were even more pronounced using the data set with the only presence and absence of particular fish species in fish communities (Fig. 5). Fish communities in lower and middle sections of the River Sava and of streams Ljubljanica, Kolpa, Mirna, Krka, Sotla, and Savinja were more similar to those in the sections of the River Sava from Jasenovac and Gradiška to Babina Greda. However, fish communities from the Rivers Sava Bohinjka, Sava Dolinka, and Sora clustered with those from the streams that have both upper rhithron fish community, e.g., Klokot and Krušnica in the River Una drainage area, and the fish community that is transitional to the middle rhithron, e.g., the Rivers Una, Sana, and Drina in their most lotic sections at Bihać, Ključ, and Šćepan Polje, respectively.

In RDA with 74 fish species as response variables, first four axes were retained in the analysis, accounting for 80 % of the total variability explained by fish abundance (Table 9). The Monte Carlo permutation test showed that 11 fish species were

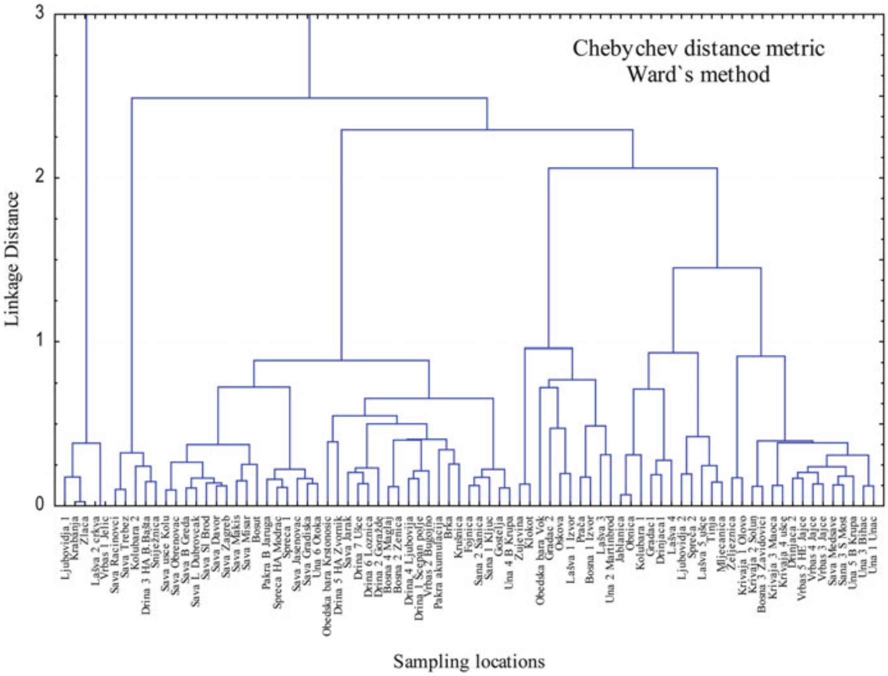

Fig. 3 Relationships between fish communities ascertained using the Ward's clustering of Chebyshev distances between them, as revealed from abundance data recorded in streams and rivers at sampling localities in the River Sava catchment

statistically significant at the levels $p < 0.05$ and $p < 0.01$ as representatives of particular river zones, i.e., fish communities (Fig. 5). Localities with the upper rhithron fish communities (e.g., the spring section of the Rivers Bosna, Vrbas, Una, Sana, Drinjača, and Lašva, as well as the Rivers Gradac, Ljuboviđa, Zlaća, Krabanja, Prača, Krušnica, and Žujevina) were explained with characteristic fish species for that type of fish community (e.g., brown trout, minnow, and European bullhead). Spirlin and brook barbel, which according to the E_i values characterize the upper rhithron fish community, determined fish communities at several localities in the streams (e.g., Obnica, Jablanica Brka, Tinja, Oskova and Gostelja, upper Rivers Drina and Kolubara, as well as lower Rivers Una, Lašva, Krivaja, and Drinjača) that were transitional to the middle rhithron type of fish community. Likewise, they were closely associated with chub and common gudgeon (e.g., in the middle course of Rivers Una, Sana, Drina, Bosna, Spreča, and Sava at several localities). Though being considered common members of the middle rhithron fish community, nase appeared slightly transitional toward the lower rhithron fish community (e.g., at particular localities in middle section of the Rivers Sava, Drina, and Spreča). Fish typical for the lower rhithron, e.g., bleak, were interconnected with the typical potamon fish representatives, such as common bream, northern pike, ide, Balon's ruffe, and racer goby. Those species were

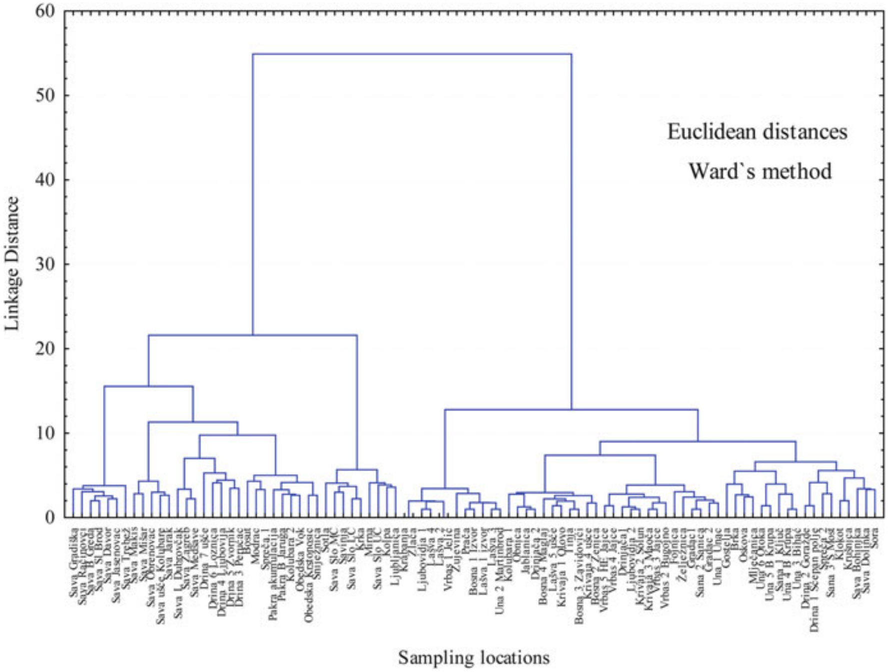

Fig. 4 Relationships between fish communities ascertained using the Ward's clustering of Euclidean distances between them, as revealed from occurrence of particular lamprey and fish species in streams and rivers at particular sampling localities in the River Sava catchment

more closely related to river sections homing the potamon fish community (e.g., Vok and Krstonošić at the Obedska swamp and River Pakra reservoir) than to the lower rhithron fish community (e.g., in the River Sava at localities Obrenovac and join of the River Kolubara, as well as in the River Drina at the Zvornik reservoir).

Survey of Fishery Management Plans available for the Croatian, bordering Croatia/Bosnia and Herzegovina and Serbian sections of the River Sava, revealed in general that there is no clear gradient in the level of productivity that follows the change of the fish community structure (Fig. 6). The greatest biomass record was for the fish community sampled at the locality Mišar (near Šabac, Serbia) characterized as potamon (Table 9). The second greatest one was that at the locality Medsave, the most upstream one in Croatia, whose fish community was characterized as transitional between the middle rhithron and lower rhithron. Annual natural production also did not reveal regular gradient. The greatest absolute natural production followed the greatest biomass record at the locality Mišar in Serbian section. However, the ratio of 16.26 % between them was less than that at the localities Jarak and Makiš, where that ratio was 38.59 % and 22.25 %, respectively. Despite the potamon character (Fig. 3) that fish communities at particular localities in the most downstream sections (e.g., Obrenovac and ušće Kolubare) of the River Sava in Serbia had, their values for biomass and natural production were not that

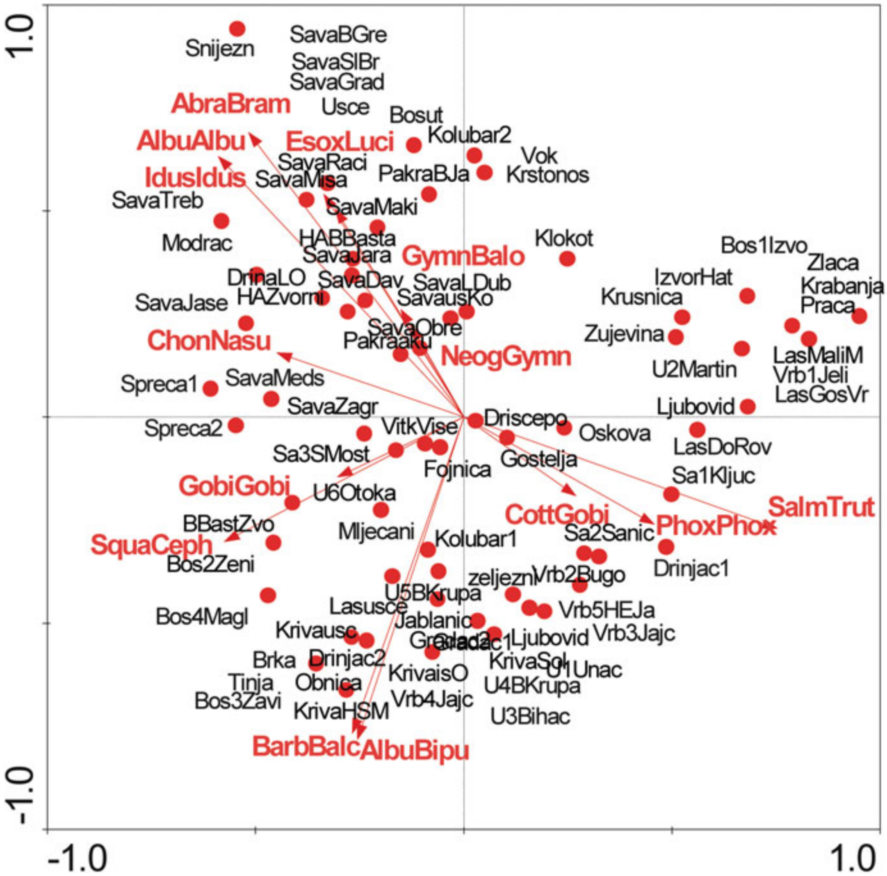

Fig. 5 RDA ordination of fish communities and river sections (explanatory variables: SalmTrut = *Salmo trutta*; PhoxPhox = *Phoxinus phoxinus*; CottGobi = *Cottus gobio*; BarbBalc = *Barbus balcanicus*; AlbuBipu = *Alburnoides bipunctatus*; SquaCeph = *Squalius cephalus*; GobiGobi = *Gobius gobius*; ChonNasu = *Chondrostoma nasus*; IdusIdus = *Idus idus*; AlbuAlbu = *Alburnus alburnus*; AbraBram = *Abramis brama*; EsoxLuci = *Esox lucius*; GymnBalo = *Gymnocephalus baloni*; NeogGymn = *Neogobius gymnotrachelus*)

different from those at particular localities in Croatian and bordering sections with fish communities of lower rhithron type, e.g., at Gradiška and Zagreb (Fig. 6). Both biomass and annual natural production of 13 fish species in the Krstonošić oxbow of the Obedska swamp out of the spawning season in the late summer 2011 were extremely high, in difference to the biomass and annual natural production in the Vok canal that connects River Sava to the Krstonošić oxbow.

The fish productivity recorded in the main tributaries of the River Sava was less (Table 9). For the Rivers Bosna, Vrbas, and Drina, biomass varied at particular localities in similar ranges, with the proportion of huchen of 1–2 % in that biomass at particular localities. Its tributary Krivaja was also very rich in fish, whereas the

Table 9 RDA output results on four axes, with their eigenvalues (λ), response–explanatory correlations (R.E. corr), cumulative percentage variance of response data (CPVRD), cumulative percentage variance of response–explanatory relation (CPVR-ER), sum of all eigenvalues ($\sum\lambda_i$), and sum of all canonical eigenvalues ($\sum\lambda_{ci}$)

Axes	1	2	3	4	Total variance
λ_i	0.223	0.146	0.051	0.036	1.000
R.E. corr	0.887	0.892	0.785	0.773	
CPVRD	22.3	36.9	42.0	45.6	
CPVR-ER	39.3	64.9	73.9	80.3	
$\sum\lambda_i$					1.000
$\sum\lambda_{ci}$					0.568

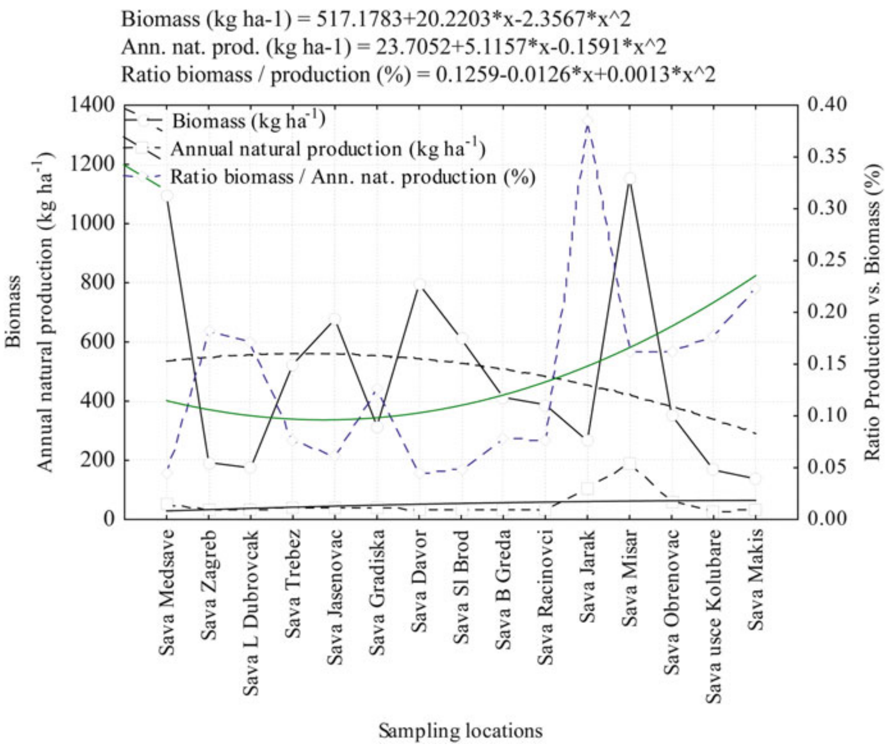

Fig. 6 Biomass, annual natural production, and ratio between them, as revealed from the records for samples from the River Sava in Croatian and Serbian sections at particular localities

most productive fishery was that of the River Spreča in the vicinity of the city of Doboj in northeastern Bosnia, majority of which (72.7 %) consisted of chub, nase, and common bream [53]. The most productive section of the River Drina was the Drina 3 Perućac section. In other sections, both biomass and annual natural production were less. The very big values for biomass and annual natural production were recorded for the lower section of the River Jadar, a tributary of the River Drinjača in the eastern Bosnia and Herzegovina in the River Drina drainage area. Although both biomass and annual natural production in the brown trout streams (e.g., River Rača, River Rogačica, Gornja Trešnjica stream, all three being tributaries of the Drina River in the Drina 4 section) were commonly much less in comparison to those in streams given above, there are streams (e.g., Gradac stream, a tributary of the River Kolubara) where great biomass and annual natural production of brown trout add mostly to their great overall productivity.

4 Discussion

Survey of the lamprey and fish fauna in the catchment of the River Sava was accomplished using the valid nomenclature that provides continuity with the previous records containing species listed for various parts of the River Sava catchment. The variety in capability of contemporary researchers to identify particular de novo promoted fish species (e.g., *Alburnus sarmaticus*, *Carassius auratus*, and *Cottus metae*) closely related to the common and widespread ones (Danube bleak *Chalcalburnus chalcoides*, Prussian carp, and European bullhead, respectively) in various regions of the catchment and to report them is to be considered another important reason. Neglecting any of those reasons might result in either lacking of valid records or excessive heterogeneity in occurrence of fish and lamprey species in reports published so far, which decreases the opportunity to make competent comparisons and reliable inferences about differences and changes that explain faunistic and community structure in the River Sava catchment.

4.1 Overall Taxonomic Diversity

In comparison to other European catchments, that of the River Sava seems similar in taxonomic diversity of lamprey and fish species to that of Europe in general ($b = 0.236$ for seven catchments), being slightly less than taxonomic diversity of Greece ($b = 0.240$ for 12 catchments), but slightly greater than that of Portugal ($b = 0.190$ for 12 catchments) [45]. It seems that the size of its catchment is large enough to comprise the diversity of lamprey and fish fauna representative in European scale, holding species common to the River Danube drainage area that belong to two great zoogeographic subregions (Mid-European and Ponto-Caspian) of the Palearctic [54].

4.2 Fish Community Structure

Very complex data set revealed several patterns of fish community structure for different kinds of inland waters in the River Sava drainage areas. The most distinct cluster of headwaters of stream orders 1 and 2 comprising the source section of streams Ljubovađa, Zlaća, and Krabanja, as well as of the upper section of the River Lašva and source section of the River Vrbas, featured the purest upper rhithron fish community (Fig. 3) consisting exclusively of brown trout *Salmo trutta* (Fig. 4). Other upper rhithron fish communities in headwaters of other streams and rivers comprising other fish species common for that type of fish community (e.g., minnow, brook barbel, European bullhead, and stone loach) were characterized as more or less transitional toward the next, the middle rhithronic type of fish community occurring downstream. That type of fish community was associated with particular fish species (e.g., spirlin, chub, nase, and/or common barbel) featuring it. The position of those streams and rivers in the series of clusters was either determined by occurrence and abundance of particular species characteristic to the downstream middle rhithronic fish community of the same river system (e.g., two most upstream sections of the River Sana in the areas of Ključ and Sanica, stream Željeznica that joins the River Krivaja) or by similarity in that kind of association across the same kind of distant waters belonging to different river systems (e.g., the spring sections of streams Gradac in the River Kolubara drainage, Drinjača in the River Drina system, and Lašva in the River Bosna system; headwater sections of Rivers Una and Bosna, stream Lašva in the River Bosna system; and downstream section of the stream Gradac in the River Kolubara system). The second prominent pattern of fish community determination features also transitional middle rhithron fish communities of distant large rivers, e.g., downstream section of the River Drinjača, River Vrbas at Jajce, River Sana at Sanski Most, River Una at Bosanska Krupa, and River Sava at Medsave (Fig. 3).

Although fish community in the section of the River Sava at Medsave resembles to other middle rhithron fish communities, in the rest of its course, it shows two main community types: the ones being lower rhithron, situated more upstream from Zagreb to Babina Greda, and those situated more downstream from Mišar (near Šabac) to Obrenovac and Makiš, which have the character of potamon (Fig. 3). It is evident that fish in the River Sava and in the most downstream sections of its main tributaries impact each other's fish communities. The lower rhithron fish community of the River Sava at Jarak resembles more to those of the closely situated most downstream sections of the River Drina (at Drina 6 Loznica and Drina 7 ušće at the junction to the River Sava). Likewise, the lower rhithron fish community of the River Sava at the sections at Jasenovac and Gradiška resembles more to that in the most downstream section of the nearby situated River Una at Otoka. Fish communities in certain upstream, i.e., middle sections of the River Sava (e.g., at Račinovci and Trebež), reveal almost the potamon character, making them more similar to the fish community of the lowermost section of the River Kolubara in the most downstream section of the River Sava, as well as to fish communities of the Perućac reservoir (Drina 3 Perućac) of the River Drina and Snježnica reservoir.

Another prominent feature is the distinctness of middle rhithron fish communities in the large rivers that flow to the River Sava, e.g., the River Drina, which resembles to particular sections of the River Bosna, as well as of the River Vrbas for the structure of its fish communities along its course (Fig. 4). That distinctness clearly delimits them from smaller rivers and streams that hold also fish communities whose structure assigns them middle rhithron character, e.g., upper River Kolubara with the streams Obnica and Jablanica, lower section of the River Drinjača, as well as Rivers Lašva and Krivaja in their middle and lower sections. That difference in middle rhithron fish community structure between large and smaller rivers results in grouping together almost all (five of seven) sections of River Drina, with only the first, the most upstream section at Šćepan Polje, and third, the reservoir Perućac section standing aside from the rest of them. The series of sections reveals the gradual change of the structure of fish communities along the River Drina, retaining sufficiently similar abundance of the most common fish species in the neighboring, successive sections to maintain the resemblance and retain the character of middle rhithron fish community. That succession along the river course features also Rivers Bosna and Vrbas, though in much shorter sections (Fig. 3). For their fish community structure in general, all those large tributaries (Rivers Vrbas, Bosna, and Drina) are more similar to the section of River Sava corresponding them for the fish community structure and geographic position than to their lower-order smaller tributaries. In addition to those two types, there is a group of middle rhithron fish communities in large Rivers Una and Sana, which clearly stand apart from those in both large and small rivers, resembling more to those in the group of streams and smaller rivers than to large rivers (Fig. 4). That supports in general the significant correlation between the increase in stream and gradual increase in the number of fish species (Fig. 1), which adds to the complexity of fish communities and their diversity.

Break in succession of fish community structure of the River Drina (Fig. 4) is probably caused by damming and pollution, respectively. Fish communities of the River Drina in sections 1 Šćepan Polje and 4 Ljubovija were more similar to each other than to the adjacent sections of 2 Goražde, 3 Perućac, and 5 Zvornik, due to the change in the fish community structure from middle to the lower rhithron and even to the potamon that occurs in reservoirs constructed there. The "tailwater" effect of dams on the restoration of middle rhithron fish community in sections downstream of reservoirs is evident in the Drina 4 Ljubovija section downstream of the Perućac reservoir. Similar effect is also evident in the section Spreča 2 downstream of the Modrac reservoir. That effect in general adds to the fishery value by increasing the variety of fish species for angling.

In addition to the riverbed regulation activities for the flood control and water transportation purposes on the River Sava and its tributaries that commenced already in nineteenth century, damming is the next most widespread activity, with the six high dams occurring in the Slovenian section, as well as eight, two, and one high dams in drainage areas of the Rivers Drina, Vrbas, and Bosna, respectively. Only two of those 17 high dams have the operational fish passes. Apart from the obstruction of migration in potamodromous fish, the alteration of habitat in reservoirs resulted in the strong shift of their fish communities. That shift was usually from middle rhithron community featuring the nearby lotic river sections toward the potamon (e.g., in the Pakra, Zvornik, and Modrac reservoirs). Less frequently, that shift was toward the lower rhithron (e.g., in the Perućac reservoir) (Fig. 3), which was in addition to damming strongly aided by stocking activities that followed it, allegedly aiming to increase the fishery value of reservoirs. That forced the disappearance of native fish species in the altered lentic environment, resulting in even lower diversity than in adjacent lotic river sections (Fig. 2).

4.3 Productivity of Fishery

The lack of correlation between downstream increase in order of the River Sava at the localities Trebež, Jasenovac, Davor, Slavonski Brod, Babina Greda, and Račinovci and fish biomass and increase in order and annual natural production comes from the occurrence of strong and irregular fluctuation in biomass, annual natural production, and ratio between those two parameters. That suggests the harvesting of yield in a very strong intensity there. It is also likely that the productivity level is related to the availability and/or size of the floodplain zone area necessary for the spawning of majority of fish species. The most productive sites in the River Sava valley (the area of Posavina) that serve as spawning grounds are those of Lonjsko Polje in Croatia, Bardača in Bosnia and Herzegovina, and Obedska swamp (here represented with two localities, Krstonošić and Vok) in Serbia, which remained connected to the main riverbed after its regulation as backwaters affected by seasonal flooding. High values for annual natural production in relation to those of biomass at localities Jarak and Makiš are likely a consequence of sufficient spawning areas in the floodplain zone occurring there, with the dikes set sufficiently far apart from the main riverbed and several large wetlands, where high biomass and annual natural production add to that of the main riverbed.

There is also a prominent variability in biomass and natural production in tributaries of different order. Explanation of that variability still lacks, due to scarcity of data about the productivity at other trophic levels in them. In addition to that, it is difficult to judge about the similarity between rivers of different sizes for the relative fish biomass and annual natural production without the data about the fishing pressure, i.e., fishing rate occurring there, which usually do not exist. For example, the extremely high values for the biomass of fish occur for the River Gradac (in the headwater section of the River Kolubara in Serbia), whose greatest part consisted of brown trout and where the catch-and-release fishing regime was enforced in the first decade of the twenty-first century. Those values greatly overcome the values for the biomass of brown trout in streams of similar size holding the upper rhithron fish community, where the fishing control is scarce and brown trout was used to be landed on catching and taken out by poaching. However, the annual natural production in the River Gradac was only slightly greater in comparison to those streams, implying the similar level of productivity for fish in them. That implies the questioning of justification of the unconditional catch and release as a measure of fishery management. On the other hand, there might be some other reasons that influence the productivity of trout streams. The vast majority of trout streams are typical stone creeks, with the low level of productivity in them in comparison to the stone creeks that hold fish farms rearing rainbow trout. Those farms add the nutrients into the feeding stream and increase their productivity to some higher level. A relative new circumstance is occurrence of tailwaters and their effect on fishery, especially that on the fly-fishing for trout and grayling but also on the coarse fishing to other fish species (e.g., nase, chub, Danubian rudd, and common barbel) that are traditionally target of recreational anglers in the area of the Balkans. It is not still clear if tailwaters, in addition to the restoration of native fish communities, also raise the productivity level. Considering the relative scarcity of records about the productivity of fish communities in Fishery Management Plans and a common lack of fishery statistics, that effect will be hard to infer. It seems that the most productive type of stream is chalk streams, which are much more rare than stone creeks in the River Sava catchment, especially those that feed fish farms with water and receive additional nutrients from them (e.g., the River Ribnik, a tributary of the River Sana in the River Una drainage area in Western Bosnia). Their very rich and diverse fish communities are especially convenient for the setting of the highest grade of fishery. However, the management with those fisheries whose ecosystem is strongly pushed to its mere limits should be accomplished very carefully from both environmental and conservational point of view. For the more reliable inferences about the productivity of fish communities and its various implications for the fishery, however, more complete and accurate data are necessary.

4.4 Alien and Invasive Fish Species

Nonnative fish species in the River Sava catchment and their status were recently and partially assessed in the study of Simonović et al. [55], where for waters of the most downstream, Serbian section, the Prussian carp was assigned the most invasive alien fish species, followed by brown bullhead. That assessment revealed the very high risk they pose to the recipient ecosystems they enter into, due to their environmental versatility, adaptability, and reproductive traits. Those traits are favored by both features of environment (i.e., habitat) and structure of lower rhithron and potamon fish communities common for the lower section of the River Sava and tributaries that join it, with the oxbows, side arms, and marshes connected with them.

There are certain records about the introduction of alien trout species (e.g., rainbow trout, brook trout, Arctic charr *Salvelinus alpinus*) and of hatchery-reared brown trout of the Atlantic strain into the appropriate environment of mountain streams throughout the River Sava catchment [19, 20, 43, 56, 57]. Nevertheless, the reports about their impact on the native trout species and strains in the recipient ecosystems are still scarce and arbitrary. The main vectors for their entrance into the waters were aquaculturists and fishery managers, as revealed clearly in Slovenia by Marić et al. [56]. There are reliable records about the introgression of the stocked brown trout of Atlantic and marble trout *Salmo marmoratus* strains into the gene pool of the native brown trout of Danubian lineage [20, 43, 57, 58]. In addition, there are also yet unconfirmed hints about the naturalization of the feral rainbow trout in the streams of Slovenia. That must be thoroughly investigated, since that poses additionally high risk and shed different light on the currently low invasive potential of this alien fish species widely spread in aquaculture.

4.5 Conservation of Indigenous Diversity

Considering the great size of the River Sava drainage area in the northwestern Balkans and great habitat and ecological diversity of aquatic ecosystems in it, it is to expect that more diversity, especially that on the level of genetics similar to the diversity found for grayling [40], is to be assessed using the molecular techniques. Preliminary results on the genotyping of huchen stocks [41, 42] from Slovenia, Serbia and Montenegro in the River Sava drainage area revealed monomorphism at the mtDNA level. That was confirmed by Weiss et al. [59] and supported by both the low level and large geographic scale of variability in two microsatellites occurring in stocks from Serbia, Bosnia and Herzegovina, and Montenegro. The low variability level was explained by relatively late immigration of taimen from Siberia during the last Quaternary glaciation [60–62] and specific life-history characteristics such as long life span, small population size, and low metabolism level [63]. The discovery of the three unique alleles at the HLJZ003 microsatellite locus in huchen from the territory of Serbia (in the River Drina and upper section of the River Ibar) warns for caution in the application of fishery measures and activities for the sake of the conservation of native stocks in the River Sava catchment.

The recent advance in genotyping contributed to the assessment of alien strains and lineages of particular native salmonid species in streams of the River Sava catchment. The introduction of the hatchery-reared, i.e., domesticated brown trout of Atlantic mtDNA (At) lineage (sensu [64, 65]) into the River Sava catchment started in Slovenia far back in 1920 [66], where almost all streams in the River Sava drainage area were widely stocked [56]. However, the first record of brown trout of At lineage in Serbia was in the River Gradac, the River Kolubara headwater [44], where it established so far, showing invasive character [57]. Likewise, the Da25 mtDNA haplotype of grayling native to streams and rivers in the River Sava catchment in Slovenia was found as introduced into the River Drina in frequency of 40 % [40]. Advance in knowledge about the indigenous character of brown trout and grayling stocks throughout the River Sava catchment area will lead to the more effective conservation measures in the fishery management with them.

In addition to fish species listed and explained in the chapter dealing with the threatened species in the River Sava catchment [67], there are two especially important threatened fish species. The first one is the mudminnow *Umbra krameri*, of the IUCN status V (vulnerable) A2c, whose historical occurrence in the River Sava catchment was recorded for the River Lonja at Lupoglav in Croatia, in 1899 and 1908, as well as for the floodplain area in Surčin, upstream of Belgrade in 1950s

[68]. IUCN [69] stated that the main threatening factors causing the decrease of mudminnow are river regulation for water transport that reduces the oxbows and drainage of wetlands to arable land. The contemporary findings in the Zasavica swamp area in Serbia, downstream of the junction of the River Drina with the River Sava [70], and in the Gromiželj wetland in Bosnia and Herzegovina, upstream of the junction with the River Drina [71], lead to declaring protected areas for both of those recent habitats of mudminnow. The other important fish species is huchen, which inhabits the southernmost part of its dispersal area in the River Sava catchment. Its southernmost place of occurrence is the Lake Plav and its tributary Ljuča in the northeastern Montenegro, with the River Lim, which outflows from the Lake Plav and joins the River Drina, where huchen attains the greatest age and size. Giving already the recent discoveries for particular features important for the conservation of this endemics and having in mind the prospect intentions to dam large mountain rivers and to construct myriad of hydropower plants, it is necessary to warn about the importance of this already threatened fish species and to undertake activities for its conservation in situ, from the proper and efficient methods of sampling and data assessment to the implementation of knowledge in the management practices of all activities within the integrative management with the River Sava catchment.

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Fauna of the Riparian Ecosystems: Amphibians, Reptiles, Birds, and Mammals

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Abstract In pristine environments, riparian ecosystems are continuously distributed along large river flows. As ecotones, they harbor more species diversity than ecosystems bordering them from both sides. Along the Sava River flow, riparian ecosystems are discontinuously distributed, being preserved mainly in protected areas of Slovenia, Croatia, Bosnia and Herzegovina, and Serbia. Nine riparian ecosystem types could be listed, harboring in total 17 amphibian, 13 reptile, more than 280 bird, and 80 mammal species. Looking at global species conservation status (global IUCN status: 2009, amphibians and reptiles; 2012, birds; 2008,

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mammals), the highest concerns should be focused on *Triturus dobrogicus* (NT), *Emys orbicularis* (NT), *Falco cherrug* (EN), *Aythya nyroca* (NT), *Rhinolophus euryale* (VU), *R. ferrumequinum* (NT), *R. hipposideros* (NT), *Barbastella barbastellus* (VU), *Miniopterus schreibersii* (NT), *Myotis bechsteinii* (VU), *M. blythii* (NT), *M. dasycneme* (NT), *Plecotus macrobullaris* (NT), *Lutra lutra* (NT), and *Eliomys quercinus* (NT). Most of the vertebrate species occurring along the Sava River are also protected by national legislations. However, it seems that both their populations and native habitats need more appropriate treatment at place.

Keywords The Sava River • Riparian ecosystems • Amphibians • Reptiles • Birds • Mammals

1 Riparian Ecosystems Along the Sava River

Riparian ecosystems are basically ecotonal [1].They are functionally characterized as mosaic open fragmented with forest vegetation, as they are stretched between (and thus exposed to strong interchanges with) aquatic ecosystems on the one side and upland terrestrial ecosystems on the other [1–3]. They are defined as specific assemblages of plant, animal, and aquatic communities which are more or less under the influence of stream-induced factors.

The edge effect [1] could be easily recognized in riparian ecosystems: these places are rich in wildlife because they provide living conditions for a greater number of species than surrounding ecosystems (elaborated in Kauffman and Krueger [4]). In the United States, for example, the riparian zones are more productive in both plant and animal biomass than the adjacent managed rangelands [5]. It was recognized decades ago that riparian zones harbor apparent biological diversity and productivity and thus they are of essential importance to the management of land and wildlife resources [4]. Therefore, in the conservation programs related to big river systems, riparian ecosystems are assigned as necessary to maintain in a sustainable way.

Permanent and seasonal watercourses or “river-floodplain” systems (see in Bayley [6]) have been the most frequently recognized as riparian zones. However, recent analyses point on small headwaters and ephemeral tributaries [7] as equally important constituents of riparian zones.

Despite the fact that European riparian zones are predominantly forest ones [8], the main land cover classes occurring there include (1) broad-lived forests, (2) coniferous forests (as natural forests in the upper part of Sava River flow in Slovenia), (3) mixed forests, (4) artificial poplar plantations, (5) natural grasslands, (6) moors and heathlands, (7) sclerophyllous vegetation, (8) transitional woodland shrub, and (9) sands, beaches, and dunes. The right bank of the Sava River mainly delineates the northern edge of the Balkan Peninsula, while the left one enters the southern edge of the Pannonian Plain. On both river sides, the natural landscapes belong to the group of European predominantly deciduous forests [9].

1.1 Riparian Habitats of Special Conservation Concern

There are 49 localities along the Sava River watercourse which are identified as important for biodiversity conservation, being named as "sites of biological importance along the Sava River" [10]. There is 8 of them in Slovenia, Croatia and Bosnia and Herzegovina have 16 each, and 9 localities occur in Serbia [10, 11].

Additionally, 18 habitat types along the Sava River have been recognized as focal for biodiversity conservation. Among them, 8 represent important riparian habitats. Their occurrence at representative localities varies from site to site (% of the total number of important sites which harbor each riparian habitat type is presented in brackets). These are Pannonian salt marshes (2 %), muddy river banks with *Chenopodion rubri* p.p. and *Bidention* p.p. vegetation (38 %), alluvial meadows of river valleys of the *Cnidion dubii* (34.7 %), northern boreal meadows of river valleys (22.4 %), transition mires and quaking bogs (8.2 %), alkaline fens (28.6 %), alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (71.4 %), and riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *U. minor*, *Fraxinus excelsior* or *F. angustifolia* (40.8 %).

According to the official report [10], the most endangered riparian habitats in the area were **Pannonian salt steppes and salt marshes**, as they are the rarest ones (occurring in just 1 of 49 sites) and with unfavorable conservation status. Also rare, but of relative good conservation status, are **transition mires and quaking bogs** (occurring in 8.2 % of the sites and in 25 % of them, the conservation status is favorable). All the other riparian habitat sites are present at more than 30 % of important localities from the list. They are evaluated as having relative good and favorable conservation status at 65–79 % and 20–25 % of these localities, respectively. **Northern boreal meadows of river valleys** could be endangered in the future if appropriate management is lacking, due to the unfavorable conservation status at even 36.4 % of important sites they inhabit. Finally, the **alluvial forests** are the most frequently occurring at important sites (71.4 %). Their conservation status is relatively good in general. However, indicative is that it falls to unfavorable category at even 20 % of the sites where they occur. **Riparian mixed forests** are in somewhat better position, as their conservation status is favorable in 20 % of the sites where they occur and unfavorable in 15 % of them. In general, rarity and conservation status are just one aspect of threat assessment. Therefore, future update of this report should include information on surface area per fragment per habitat type, as well as total surface area of habitat type within riparian zone.

Gravel banks and islands are particularly important for some bird species (*Sterna albifrons*, *Sterna hirundo*, *Charadrius dubius*). Those habitats are highly threatened by gravel extraction and river regulation for traffic and flood control. Following localities along the Sava floodplain, the lower part and mouth of the Drina River, Sava River by Zagreb, and the upper part of the river also should be considered as important for local biodiversity. Additionally, some valuable riparian habitats are fragile by their nature. Moreover, open meadows-grasslands, pastures, as well as shallows cannot be preserved for a long time without maintaining measures such as traditional use of grazing or mowing.

2 The Fauna

2.1 Amphibian Fauna

Amphibian species which occur in riparian habitats along the Sava River are mostly listed as typical faunal elements of the subprovince of Balkan-Middle European forests—fire salamander, green toad, tree frog, agile frog, and some of water frogs, e.g., edible frog [12]. There are also few amphibians typical of the subprovince of Pannonian-Dakian steppes—fire-bellied toad and Danube crested newt. Finally, a common toad—a typical herpetofaunal element of the subprovince of Boreal forests of taiga type and one of the most widespread European amphibians—also inhabits in a riparian zone of the Sava River.

General distributional data could be extracted from Radovanović [13], Đurović et al. [14], Gasc et al. [15], Arnold and Ovenden [16], Redžić et al. [17, 18], Tanović and Adrović [19], and Jelić et al. [20]. Some amphibian species are widespread in the whole area, as fire salamander, smooth newt, common and green toads, and tree, edible, marsh, and agile frogs (Table 1). Some others are typical only for the upper part of a watercourse, as Italian crested newt. There is also a group of species with more "western" local distribution (e.g., alpine newt, yellow-bellied toad, moor frog, and common frog). The common spadefoot—the species which distribution on the territory of Balkan peninsula is relatively well known [21]—was recorded also in riparian habitats of the Sava River in Bosnia and Herzegovina ([22]; Jelić unpublished). Kitnaes et al. [10] confirmed presence of the yellow-bellied toad in Velika and Mala Tišina near Bosanski Šamac. Amphibian distribution maps in Bosnia and Herzegovina presented by Lelo and Vesnić [23] suggested that the alpine newt and common frog could also occur in the Sava River wetlands in this country. In Serbia, the common frog occurs in species lists of some protected areas along the Sava River. However, there are no records published in scientific journals. In Croatia, the alpine newt was only recorded in two localities (Turopolje and inflow of Una River into the Sava).

2.2 Reptile Fauna

In comparison to amphibians, reptile fauna related to surroundings of the Sava River shows less qualitative diversity [13–18, 20, 24]. Most of reptile species there belong to typical faunistic elements of the subprovince of Balkan-Middle European forests—slow worm, sand lizard, smooth snake, grass snake, and Aesculapian

Table 1 Amphibian species occurring in riparian ecosystems along the Sava River

Species	Common name	SLO $N = 14$	CRO $N = 17$	BIH $N = 13$ (15)	SER $N = 12$ (13)
Urodela					
Salamandridae					
Salamandra salamandra	Fire salamander	+	+	+	+
Ichthyosaura alpestris	Alpine newt	+	+	–	–
Lissotriton vulgaris	Smooth newt	+	+	+	+
Triturus dobrogicus	Danube crested newt	–	+	+	+
Triturus carnifex	Italian crested newt	+	+	–	–
Anura					
Bombinatoridae					
Bombina bombina	Fire-bellied toad	–	+	+	+
Bombina variegata	Yellow-bellied toad	+	+	+	–
Bufonidae					
Bufo bufo	Common toad	+	+	+	+
Pseudepidalea viridis	Green toad	+	+	+	+
Hylidae					
Hyla arborea	Tree frog	+	+	+	+
Pelobatidae					
Pelobates fuscus	Common spadefoot	–	+	+	+
Ranidae					
Pelophylax kl. esculentus	Edible frog	+	+	+	+
Pelophylax lessonae	Pool frog	+	+	+	+
Pelophylax ridibundus	Marsh frog	+	+	+	+
Rana arvalis	Moor frog	+	+	?	–
Rana dalmatina	Agile frog	+	+	+	+
Rana temporaria	European common frog	+	+	?	?

$N =$ number of species on the list
? = single record from the literature or not confirmed recently

snake [12]. Moreover, some elements of the subprovince of sub-Mediterranean Balkan forests are also common there, such as green lizard. Almost 70 % of reptile species from the list (Table 2) are common for the whole watercourse of Sava River. The species with predominantly "western" or "eastern" distribution are few in comparison with amphibians: nose-horned viper and viviparous lizard versus large whip snake, on western and eastern part of the area, respectively (Table 2).

Wetlands of the lower part of river flow harbor populations of adder [25], which is, together with the viviparous lizard, a typical element of the subprovince of European forests of taiga type. According to the old literature [26], adder was an inhabitant of riparian habitats along the Sava River in Slovenia; however, recent

Table 2 Reptile species occurring in riparian ecosystems along the Sava River

Species	Common name	SLO $N = 11(12)$	CRO $N = 11$	BIH $N = 10$ (11)	SER $N = 11$
Chelonia					
Emydidae					
Emys orbicularis	European pond terrapin	+	+	+	+
Sauria					
Anguidae					
Anguis fragilis	Slow worm	+	+	+	+
Lacertidae					
Lacerta agilis	Sand lizard	+	+	+	+
Lacerta viridis	Green lizard	+	+	+	+
Podarcis muralis	Common wall lizard	+	+	+	+
Zootoca vivipara	Viviparous lizard	+	+	–	–
Ophidia					
Colubridae					
Coronella austriaca	Smooth snake	+	+	+	+
Dolichophis caspius	Large whip snake	–	–	?	+
Natrix natrix	Grass snake	+	+	+	+
Natrix tessellata	Dice snake	+	+	+	+
Zamenis longissimus	Aesculapian snake	+	+	+	+
Viperidae					
Vipera berus	Adder	?	+	+	+
Vipera ammodytes	Nose-horned viper	+	–	–	–

N = number of species on the list
? = single record from the literature or not confirmed recently

records are limited on the territory outside the area in question. In Croatia, adders inhabit a continuous area of wetlands along the Sava River, from Zaprešiće to Spačva (border with Serbia). Some of these populations are very abundant. On the contrary, in Serbian part they are considered rare, due to heavily degraded wetlands [27]. A few characteristic elements of the subprovince of Pannonian-Dakian steppes reach the lowlands of the Sava River flow—large whip snake and dice snake [12]. While the first one was recorded sporadically (as its typical habitats are not riparian ones), and only in the eastern part of the watercourse, the second one is generally common and widespread throughout the region [28]. Oviparous populations of the viviparous lizard are recorded only in the western part of the river flow [29].

The upper flow of the Sava River harbor some reptile species not common for the wetlands, such as the nose-horned viper, a typical element of sub-Mediterranean rocky grounds and rock deserts [12].

2.3 Bird Fauna

The Sava River floodplains have high diversity of avifauna. More than 280 species (17 orders and 58 families) occur in the area, while 150 regularly breed. Most of the species are linked to wetlands—marshes, reedbeds, and riparian forests—as well as to open meadows and shallows (see Table 3). Among the most significant groups are herons and storks. Of them, 12 species breed along the Sava River. They include the Eurasian spoonbill, glossy ibis, purple heron, and black stork. Large heron colonies are located in Obedska bara in Serbia and Lonjsko Polje and Jelas Polje in Croatia. Although the region is rich with different types of wetlands, not many wader species (Charadriiformes) breed there (related to deforestation by foresters, to natural overgrow (succession of vegetation), and to generally low number or absence of artificial open water bodies). Most of them occur only during migration or during wintering. However, the little ringed plover, northern lapwing, Eurasian woodcock, and common sandpiper are common breeders along the Sava River floodplain. The similar situation is with ducks and geese (Anseriformes) where only 5–6 species regularly breed in the area. Some man-made habitats such as fishponds and sewage ponds offer good conditions for breeding of wetland birds. The order of diurnal raptors is also well represented with more than 20 species occurring in the region. Large and relatively preserved broad-leaved forests along the Sava River (Obedska bara, Bosutske šume, Spačva, Lonjsko Polje) are of particular importance for raptors such as the white-tailed eagle, lesser spotted eagle, and black kite. Woodpeckers (Piciformes) are common in forested and semi-forested habitats. Middle spotted woodpecker is one of the characteristic species of old oak forests. Songbirds (Passeriformes) are well represented in all habitat types. Typical forest species are the collared flycatcher and short-toed treecreeper. The reed warbler, great reed warbler, sedge warbler, and Savi's warbler are typical for marshes and reedbeds. Natural and seminatural open habitats (grasslands, pastures) are nowadays reduced and mainly deteriorated or abandoned (in relation to traditional use for grazing and mowing). Still, some characteristic species such as the red-backed shrike or whinchat have significant populations in the region. Other typical grassland species like the Eurasian roller and corncrake are nowadays rare and have very restricted distribution in the region.

2.4 Mammalian Fauna

Mammals related to the surroundings of the Sava River number 80 recently confirmed species [30–45]. There are 9 insectivores, 27 bat species (including co-occurrence species), 12 carnivores (including co-occurrence species), 4 hoofed mammals, 24 rodents, and one hare species (Table 4). Aquatic habitats are preferred

Table 3 A shortened list of bird species occurring in riparian ecosystems along the Sava River

Species	Common name	SLO $N = 43$ (44)	CRO $N = 48$	BIH $N = 47$ (48)	SER $N = 48$
Pelecaniformes					
Family Phalacrocoracidae					
Phalacrocorax pygmeus	Pygmy cormorant	+	+	+	+
Phalacrocorax carbo	Great cormorant	+	+	+	+
Ciconiiformes					
Family Ardeidae					
Botaurus stellaris	Great bittern	+	+	+	+
Ixobrychus minutus	Little bittern	+	+	+	+
Nycticorax nycticorax	Black-crowned night heron	+	+	+	+
Ardeola ralloides	Squacco heron	–	+	+	+
Egretta garzetta	Little egret	+	+	+	+
Casmerodius albus	Great egret	+	+	+	+
Ardea cinerea	Gray heron	+	+	+	+
Ardea purpurea	Purple heron	+	+	+	+
Family Ciconiidae					
Ciconia nigra	Black stork	+	+	+	+
Ciconia ciconia	White stork	+	+	+	+
Family Threskiornithidae					
Plegadis falcinellus	Glossy ibis	–	+	+	+
Platalea leucorodia	Eurasian spoonbill	+	+	+	+
Anseriformes					
Family Anatidae					
Aythya nyroca	Ferruginous duck	+	+	+	+
Falconiformes					
Family Accipitridae					
Milvus migrans	Black kite	+	+	+	+
Haliaeetus albicilla	White-tailed eagle	+	+	+	+
Aquila pomarina	Lesser spotted eagle	+	+	+	+
Falco cherrug	Saker falcon	–	+	–	+
Gruiformes					
Family Rallidae					
Rallus aquaticus	Water rail	+	+	+	+
Porzana porzana	Spotted crake	+	+	+	+
Porzana parva	Little crake	+	+	+	+
Crex crex	Corncrake	+	+	+	+
Charadriiformes					
Family Charadriidae					
Charadrius dubius	Little ringed plover	+	+	+	+

(continued)

Table 3 (continued)

Species	Common name	SLO $N = 43$ (44)	CRO $N = 48$	BIH $N = 47$ (48)	SER $N = 48$
Family Scolopacidae					
Gallinago gallinago	Common snipe	+	+	+	+
Scolopax rusticola	Eurasian woodcock	+	+	+	+
Actitis hypoleucos	Common sandpiper	+	+	+	+
Family Laridae					
Larus ridibundus	Common black-headed gull	+	+	+	–
Family Sternidae					
Sterna hirundo	Common tern	+	+	+	+
Sterna albifrons	Little tern	?	+	+	+
Chlidonias hybrida	Whiskered tern	+	+	+	+
Strigiformes					
Family Tytonidae					
Tyto alba	Barn owl	+	+	+	+
Family Strigidae					
Strix aluco	Tawny owl	+	+	+	+
Coraciiformes					
Family Alcedinidae					
Alcedo atthis	Common kingfisher	+	+	+	+
Piciformes					
Family Picidae					
Dryocopus martius	Black woodpecker	+	+	?	+
Dendrocopos medius	Middle spotted woodpecker	+	+	+	+
Passeriformes					
Family Motacillidae					
Anthus campestris	Tawny Pipit	–	+	+	+
Motacilla flava	Yellow wagtail	+	+	+	+
Saxicola rubetra	Whinchat	+	+	+	+
Family Locustellidae					
Locustella fluviatilis	Eurasian river warbler	+	+	+	+
Locustella luscinioides	Savi's warbler	+	+	+	+
Family Acrocephalidae					
Acrocephalus scirpaceus	Common reed warbler	+	+	+	+
Acrocephalus arundinaceus	Great reed warbler	+	+	+	+
Family Muscicapidae					
Muscicapa striata	Spotted flycatcher	+	+	+	+
Ficedula albicollis	Collared flycatcher	+	+	+	+

(continued)

Table 3 (continued)

Species	Common name	SLO $N = 43$ (44)	CRO $N = 48$	BIH $N = 47$ (48)	SER $N = 48$
Family Certhiidae					
Certhia brachydactyla	Short-toed treecreeper	+	+	+	+
Family Remizidae					
Remiz pendulinus	Eurasian penduline tit	+	+	+	+
Family Laniidae					
Lanius collurio	Red-backed shrike	+	+	+	+
Family Emberizidae					
Emberiza hortulana	Ortolan Bunting	–	–	+	+

N = number of species on the list
? = single record from the literature or not confirmed recently

foraging zone for Eurasian and Mediterranean water shrew. Flooded forests along the Sava River are inhabited by populations of typical Middle European mammal species like the yellow-necked and wood mice, bank vole, hazel dormouse, wild boar, and wildcat. Over 20 bat species were recorded to forage over the water (e.g., Daubenton's bat) or banks of the river (e.g., pipistrelle bats) and surrounding fields (e.g., greater mouse-eared bat) and forests (e.g., barbastelle and Bechstein's bats), and forests are also a roosting habitat for many tree-dwelling bat species (e.g., *Noctules*). Several cave bat species exploit riparian habitats of the Sava River in Croatia and Slovenia as a hunting area (e.g., the greater, Mediterranean, and lesser horseshoe bats and Schreiber's bent-wing bat). The northern bat was recorded on the spring of Sava Dolinka and in the upper parts of the Sava River valley. Flooded meadows and planes are habitat to vole species—field and European field voles—as well as to striped field mouse. Reed plantations and other tall vegetation along marshes and waterways are home to tiny harvest mouse. The golden jackal inhabits this area, and there were some findings of wandering individuals of gray wolf (Croatia, Bosnia and Herzegovina), in the flooded plain of the Sava River. Though supposed in the past, sporadic occurrence of the brown bear in Bosnia and Herzegovina's part of the Sava River plain is almost impossible [46, 47]. Two allochthonous semiaquatic species—coypu and muskrat—live near the water, together with the reintroduced Eurasian beaver (reintroduced in Croatia in 1996 and in Serbia in 2004—Zasavica and Obedska bara) and native population of Eurasian otter. The fallow deer was introduced by hunters and in some parts forms stable populations. The raccoon dog is allochthonous invasive species in expansion.

Table 4 Mammalian species occurring in riparian ecosystems along the Sava River

Species	Common name	SLO $N = 69$	CRO $N = 64$ (73)	BIH $N = 63$ (72)	SER $N = 60$ (67)
Eulipotyphla					
Family Erinaceidae					
Erinaceus roumanicus	Northern white-breasted hedgehog	+	+	+	+
Family Soricidae					
Sorex araneus	Common (Eurasian) shrew	+	+	+	+
Sorex minutus	Eurasian pygmy shrew	+	+	+	+
Sorex alpinus	Alpine shrew	+	–	–	–
Neomys anomalus	Mediterranean water shrew	+	+	?	+
Neomys fodiens	Eurasian water shrew	+	+	+	–
Crocidura leucodon	Bicolored (white-toothed) shrew	+	+	+	+
Crocidura suaveolens	Lesser white-toothed shrew	+	+	+	+
Family Talpidae					
Talpa europaea	European mole	+	+	+	+
Chiroptera					
Family Rhinolophidae					
Rhinolophus euryale	Mediterranean horseshoe bat	+	+	+	–
Rhinolophus ferrumequinum	Greater horseshoe bat	+	+	+	+
Rhinolophus hipposideros	Lesser horseshoe bat	+	+	+	–
Family Vespertilionidae					
Miniopterus schreibersii	Schreiber's bent-wing bat	+	+	+	+
Barbastella barbastellus	Western barbastelle	+	+	+	+
Eptesicus nilssonii	Northern bat	+	–	–	–
Eptesicus serotinus	Serotine	+	+	?	+
Myotis bechsteinii	Bechstein's bat	+	+	+	+
Myotis blythii	Lesser mouse-eared bat	+	+	+	+
Myotis brandtii	Brandt's bat	+	?	?	a
Myotis dasycneme	Pond bat	–	?	?	+
Myotis daubentonii	Daubenton's bat	+	+	+	+
Myotis emarginatus	Geoffroy's bat	+	+	+	a
Myotis myotis	Greater mouse-eared bat	+	+	+	a
Myotis mystacinus	Whiskered bat	+	+	+	a
Myotis nattereri	Natterer's bat	+	+	+	a
Nyctalus leisleri	Leisler's bat	+	?	–	+

(continued)

Table 4 (continued)

Species	Common name	SLO N = 69	CRO N = 64 (73)	BIH N = 63 (72)	SER N = 60 (67)
Nyctalus noctula	Noctule	+	+	+	+
Pipistrellus kuhlii	Kuhl's pipistrelle	+	+	+	+
Pipistrellus nathusii	Nathusius's pipistrelle	+	+	+	+
Pipistrellus pipistrellus	Common pipistrelle	+	+	+	+
Pipistrellus pygmaeus	Soprano pipistrelle	+	+	?	+
Hypsugo savii	Savi's pipistrelle	+	?	+	a
Plecotus auritus	Brown long-eared bat	+	+	+	+
Plecotus austriacus	Gray long-eared bat	+	+	+	+
Plecotus macrobullaris	Mountain long-eared bat	+	?	?	–
Vespertilio murinus	Parti-colored bat	+	?	–	a
Carnivora					
Family Canidae					
Nyctereutes procyonoides[b]	Raccoon dog	–	?	?	+
Canis aureus	Golden (common) jackal	+	+	+	+
Canis lupus	Gray wolf	–	+	+	–
Vulpes vulpes	Red fox	+	+	+	+
Family Mustelidae					
Lutra lutra	Eurasian otter	+	+	+	+
Martes foina	Stone (beech) marten	+	+	+	+
Martes martes	Pine marten	+	+	+	+
Meles meles	Eurasian badger	+	+	+	+
Mustela erminea	Ermine (stoat)	+	+	+	+
Mustela nivalis	Least weasel	+	+	+	+
Mustela putorius	Western polecat	+	+	+	+
Family Felidae					
Felis silvestris	Wildcat	–	+	+	+
Cetartiodactyla					
Family Suidae					
Sus scrofa	Wild boar	+	+	+	+
Family Cervidae					
Cervus elaphus	Red deer	+	+	+	+
Dama dama[b]	Fallow deer	+	?	?	+
Capreolus capreolus	European roe deer	+	+	+	+
Family Bovidae					
Ovis orientalis[b]	Wild sheep (mouflon)	–	?	–	+

(continued)

Table 4 (continued)

Species	Common name	SLO N = 69	CRO N = 64 (73)	BIH N = 63 (72)	SER N = 60 (67)
Rodentia					
Family Sciuridae					
Sciurus vulgaris	Eurasian red squirrel	+	+	+	+
Family Gliridae					
Glis glis	Fat dormouse (edible dormouse)	+	+	+	+
Muscardinus avellanarius	Hazel dormouse	+	+	+	+
Dryomys nitedula	Forest dormouse	+	–	+	–
Eliomys quercinus	Garden dormouse	–	–	+	–
Family Castoridae					
Castor fiber	Eurasian beaver	+	+	?	+
Family Arvicolidae					
Clethrionomys glareolus	Bank vole	+	+	+	+
Arvicola amphibius	European (or northern) water vole	+	+	+	+
Arvicola scherman	Montane water vole	–	+	+	–
Ondatra zibethicus[b]	Muskrat	+	+	+	+
Microtus agrestis	Field vole	+	+	+	+
Microtus arvalis	Common vole	+	+	+	+
Microtus subterraneus	European pine vole	+	+	+	+
Microtus liechtensteini	Liechtenstein's pine vole	+	+	+	–
Family Muridae					
Micromys minutus	Harvest mouse	+	+	+	+
Apodemus agrarius	Striped field mouse	+	+	+	+
Apodemus flavicollis	Yellow-necked mouse	+	+	+	+
Apodemus sylvaticus	Wood mouse	+	+	+	+
Apodemus uralensis	Ural field mouse	–	–	–	+
Rattus norvegicus	Brown rat	+	+	+	+
Rattus rattus	Black rat	+	+	+	+
Mus musculus	House mouse	+	+	+	+
Mus spicilegus	Mound-building (steppe) mouse	–	+	+	+
Family Myocastoridae					
Myocastor coypus[b]	Coypu	+	+	+	+
Lagomorpha					
Family Leporidae					
Lepus europaeus	European (brown) hare	+	+	+	+

N = number of species on the list
[a]Bat species that are probably present in Serbian part, but there are not published data yet
[b]Introduced or invasive–nonnative species
? = Single record from the literature and/or not confirmed recently

3 Species Conservation Status

3.1 IUCN Global

The International Union for Conservation of Nature (IUCN) is the oldest and largest global environmental organization, with a central mission to conserve biodiversity worldwide. Through a large network of international experts, IUCN is setting and maintaining international standards for species extinction risks. IUCN regularly updates its Red List of Threatened Species and produces publications related to the status of endangered species (for European amphibians and reptiles, see Anthony et al. [48] and Cox and Temple [49]). Only 2 % of species listed in this study are globally threatened, while 7 % could be threatened in the future (Table 5).

3.2 CITES

The Convention on International Trade in Endangered Species of Wild Fauna and Flora establishes and regulates conditions that govern the transfer of wild species or their parts or derivatives across the administrative borders of countries. These rules inevitably should be followed for all wild species appearing on the CITES list, but unfortunately wild species are still being transported for commercial or noncommercial (including purely scientific) purposes. Not so many species listed here are covered by CITES annexes—around 8 % (Table 5).

3.3 Bern Convention

The Bern Convention has a main goal to conserve wild flora and fauna and their natural habitats and to promote European cooperation in that field. It covers most of the natural heritage of the European continent. Species and habitats of conservation concern are listed under several appendices. However, it is obvious that some species were not properly evaluated by the Bern Convention, despite having a very restricted distribution range. These are the species occurring exclusively in the Balkans and/or in Eastern Europe. All amphibian, reptile, and bird species listed here are included into annexes of the Bern Convention, while almost 28 % of mammals in riparian habitats of the Sava River are not (Table 5).

Table 5 Global conservation status and international/national levels of legal protection of amphibians, reptiles, birds, and mammals occurring in riparian ecosystems of the Sava River and their degree of legal protections on international and local level

Species	IUCN[1]	CITES[2]	Bern[3]	Habitats[4]/Birds[5]	Slovenia[6a,b]	Croatia[7]	Bosnia and Herzegovina[8]	Serbia[9]
Amphibia								
Urodela								
Salamandra salamandra	LC	–	III	–	1A	–	+	1
Ichthyosaura alpestris	LC	–	III	–	1A,2A	–	+	1
Lissotriton vulgaris	LC	–	III	–	1A,2A	–	+	1
Triturus dobrogicus	NT	–	II	II	1A,2A	1	+	1
Triturus carnifex	LC	–	II	II,IV	1A,2A	1	+	/
Anura								
Bombina bombina	LC	–	II	II,IV	1A,2A	1	+	1
Bombina variegata	LC	–	II	II, IV	1A,2A	1	+	1
Bufo bufo	LC	–	III	–	1A,2A	–	+	1
Pseudepidalea viridis	LC	–	II	IV	1A,2A	1	+	1
Pelobates fuscus	LC	–	II	IV	1A,2A	1	+	1
Hyla arborea	LC	–	II	IV	1A,2A	1	+	1
Rana arvalis	LC	–	II	IV	1A,2A	1	?	/
Rana dalmatina	LC	–	II	IV	1A,2A	1	+	1
Rana temporaria	LC	–	III	V	1A	–	+	1
Pelophylax lessonae	LC	–	III	IV	1A,2A	1	+	2
Pelophylax ridibundus	LC	–	III	V	1A,2A	–	+	2
Pelophylax kl. esculentus	LC	–	III	V	1A,2A	–	+	2
Reptilia								
Chelonia								
Emys orbicularis	NT	–	II	II,IV	1A,2A	1	+	1
Sauria								

(continued)

Table 5 (continued)

Species	IUCN[1]	CITES[2]	Bern[3]	Habitats[4]/Birds[5]	Slovenia[6a,b]	Croatia[7]	Bosnia and Herzegovina[8]	Serbia[9]
Anguis fragilis	LC	–	III	–	1A	–	+	–
Lacerta viridis	LC	–	II	IV	1A	1	+	–
Lacerta agilis	LC	–	II	IV	1A,2A	1	+	–
Podarcis muralis	LC	–	II	IV	1A	1	+	–
Zootoca vivipara	LC	–	III	–, *IV	1A	1	+	1
Ophidia								
Coronella austriaca	LC	–	II	IV	1A	1	+	1
Dolichophis caspius	LC	–	II	IV	/	1	+	1
Zamenis longissimus	LC	–	II	IV	1A	1	+	1
Natrix natrix	LC	–	III	–	1A	–	+	1
Natrix tessellata	LC	–	II	IV	1A	1	+	1
Vipera berus	LC	–	III	–	1A	–	+	1
Vipera ammodytes	LC	–	II, III	IV	1A	1	+	2
Aves								
Pelecaniformes								
Phalacrocorax pygmeus	LC	–	II	BD–I	1A,2B	1*	+	1
Phalacrocorax carbo	LC	–	II	–	1A	–	+	1,L
Ciconiiformes								
Botaurus stellaris	LC	–	II	BD–I	1A,2B	1*	+	1
Ixobrychus minutus	LC	–	II	BD–I	1A,2A	1*	+	1
Nycticorax nycticorax	LC	–	II	BD–I	1A,2A	1*	+	1
Ardeola ralloides	LC	–	II	BD–I	1A,2B	1*	+	1
Egretta garzetta	LC	–	II	BD–I	1A,2B	1*	+	1
Casmerodius albus	LC	–	II	BD–I	1A	1*	+	1
Ardea cinerea	LC	–	III	–	1A	–	+	2

Ardea purpurea	LC	–	II	BD–I	1A,2B	1*	+	1
Ciconia nigra	LC	II	II	BD–I	1A,2A	1	+	1
Ciconia ciconia	LC	–	II	BD–I	1A,2A	1*	+	1
Plegadis falcinellus	LC	–	II	BD–I	1A,2B	1**	+	1
Platalea leucorodia	LC	–	II	BD–I	1A,2B	1*	+	1
Anseriformes								
Aythya nyroca	NT	–	III	BD–I	1A,2A	1*	+	1
Accipitriformes								
Milvus migrans	LC	II	II	BD–I	1A,2A	1*	+	1
Haliaeetus albicilla	LC	I	II	BD–I	1A,2A	1*	+	1
Aquila pomarina	LC	II	II	BD–I	1A,2A	1*	+	1
Falco cherrug	EN	II	II	BD–I	1A,2B	1*	+	1
Gruiformes								
Rallus aquaticus	LC	–	III	–	1A,2A	–	+	1
Porzana porzana	LC	–	II	BD–I	1A,2A	1*	+	1
Porzana parva	LC	–	II	BD–I	1A,2A	1*	+	1
Crex crex	LC	–	II	BD–I	1A,2A	1*	+	1
Charadriiformes								
Charadrius dubius	LC	–	II	–	1A,2A	1*	+	1
Gallinago gallinago	LC	–	III	–	1A,2A	1*	+	1
Scolopax rusticola	LC	–	III	–	1A,2A	1*	+	2
Actitis hypoleucos	LC	–	III	–	1A,2A	1*	+	1
Larus ridibundus	LC	–	III	–	1A	***	+	2
Sterna hirundo	LC	–	II	BD–I	1A,2A	1*	+	1
Sterna albifrons	LC	–	II	BD–I	1A,2A	1*	+	1
Chlidonias hybrida	LC	–	II	BD–I	1A,2B	1*	+	1
Strigiformes								

(continued)

Table 5 (continued)

Species	IUCN[1]	CITES[2]	Bern[3]	Habitats[4]/Birds[5]	Slovenia[6a,b]	Croatia[7]	Bosnia and Herzegovina[8]	Serbia[9]
Tyto alba	LC	II	II	–	1A,2A	1*	+	1
Strix aluco	LC	II	II	–	1A	1*	+	1
Coraciiformes								
Alcedo atthis	LC	–	II	BD–I	1A,2A	1*	+	1
Piciformes								
Dryocopus martius	LC	–	II	BD–I	1A,2A	1*	+	1
Dendrocopos medius	LC	–	II	BD–I	1A,2A	1*	+	1
Passeriformes								
Anthus campestris	LC	–	II	BD–I	1A,2A	1*	+	1
Motacilla flava	LC	–	II	–	1A	1*	+	1
Saxicola rubetra	LC	–	II	–	1A,2A	1*	+	1
Locustella fluviatilis	LC	–	II	–	1A	1*	+	1
Locustella luscinioides	LC	–	II	–	1A,2A	1*	+	1
Acrocephalus scirpaceus	LC	–	II	–	1A,2A	1*	+	1
Acrocephalus arundinaceus	LC	–	II	–	1A,2A	1*	+	1
Muscicapa striata	LC	–	II	–	1A	1*	+	1
Ficedula albicollis	LC	–	II	BD–I	1A,2A	1*	+	1
Certhia brachydactyla	LC	–	II	–	1A,2B	1*	+	1
Remiz pendulinus	LC	–	III	–	1A	1*	+	1
Lanius collurio	LC	–	II	BD–I	1A,2A	–	+	1
Emberiza hortulana	LC	–	III	BD–I	1A,2A	–	+	1
Mammalia								
Eulipotyphla								
Erinaceus roumanicus	LC	–	III	–	1A	–	–	2
Sorex araneus	LC	–	III	–	2A	–	+	2

Sorex minutus	LC	–	III	–	2A	–	?	2
Sorex alpinus	LC	–	III	–	2A	–	+	1
Neomys anomalus	LC	–	III	–	2A	–	+	2
Neomys fodiens	LC	–	III	–	2A	–	+	1
Crocidura leucodon	LC	–	III	–	2A	–	+	2
Crocidura suaveolens	LC	–	III	–	2A	–	+	2
Talpa europaea	LC	–	–	–	–	1	–	2
Chiroptera								
Rhinolophus euryale	VU	–	II	II/IV	1A,2A	1	+	1
Rhinolophus ferrumequinum	NT	–	II	II/IV	1A,2A	1	+	1
Rhinolophus hipposideros	NT	–	II	II/IV	1A,2A	1	+	1
Miniopterus schreibersii	NT	–	II	II/IV	1A,2A	1	+	1
Barbastella barbastellus	VU	–	II	II/IV	1A,2A	1	+	1
Eptesicus nilssonii	LC	–	II	IV	1A,2A	1	/	/
Eptesicus serotinus	LC	–	II	IV	1A	1	–	1
Myotis bechsteinii	VU	–	II	II/IV	1A,2A	1	+	1
Myotis blythii	NT	–	II	II/IV	1A,2A	1	+	1
Myotis brandtii	LC	–	II	IV	1A,2A	1	/	1
Myotis dasycneme	NT	–	II	II/IV	/	1	+	1
Myotis daubentonii	LC	–	II	IV	1A	1	+	1
Myotis emarginatus	LC	–	II	II/IV	1A,2A	1	+	1
Myotis myotis	LC	–	II	II/IV	1A,2A	1	+	1
Myotis mystacinus	LC	–	II	IV	1A	1	+	1
Myotis nattereri	LC	–	II	IV	1A,2A	1	+	1
Nyctalus leisleri	LC	–	II	IV	1A,2A	1	+	1
Nyctalus noctula	LC	–	II	IV	1A	1	+	1
Pipistrellus kuhlii	LC	–	II	IV	1A	1	+	1
Pipistrellus nathusii	LC	–	II	IV	1A,2A	1	–	1
Pipistrellus pipistrellus	LC	–	III	IV	1A,2A	1	+	1

(continued)

Table 5 (continued)

Species	IUCN[1]	CITES[2]	Bern[3]	Habitats[4]/Birds[5]	Slovenia[6a,b]	Croatia[7]	Bosnia and Herzegovina[8]	Serbia[9]
Pipistrellus pygmaeus	LC	–	II	IV	1A	1	–	1
Hypsugo savii	LC	–	II	IV	1A	1	+	1
Plecotus auritus	LC	–	II	IV	1A,2A	1	+	1
Plecotus austriacus	LC	–	II	IV	1A,2A	1	+	1
Plecotus macrobullaris	NT	–	II	IV	(1A,2A)	1	+	/
Vespertilio murinus	LC	–	II	IV	1A,2A	1	+	1
Carnivora								
Nyctereutes procyonoides	LC	–	–	–	L	–	–	–
Canis aureus	LC	–	–	V	1A	–	–	L
Canis lupus	LC	II	II	IV	1A,2*A	1	–	1,L
Vulpes vulpes	LC	–	–	–	L	–	–	L
Lutra lutra	NT	I	II	II/IV	1A,2A	1	+	1
Martes foina	LC	–	III	–	L	–	–	L
Martes martes	LC	–	III	V	L	–	–	L
Meles meles	LC	–	III	–	L	–	–	L
Mustela erminea	LC	–	III	–	1A,2A	–	+	1
Mustela nivalis	LC	–	III	–	1A,2A	–	+	2
Mustela putorius	LC	–	III	V	1A	–	–	2
Felis silvestris	LC	II	II	IV	1A,2A, L	1	+	1,L
Cetartiodactyla								
Sus scrofa	LC	–	–	–	L	–	–	L
Cervus elaphus	LC	–	III	–	L	–	+	L
Dama dama	LC	I	III	–	L	–	–	–
Capreolus capreolus	LC	–	III	–	L	–	–	L
Ovis orientalis	LC	II	III	–	L	–	–	–

Rodentia								
Sciurus vulgaris	LC	–	III	–	1A	–	–	L
Glis glis	LC	–	III	–	L	–	+	L
Muscardinus avellanarius	LC	–	III	–	1A,2A	1	+	1
Dryomys nitedula	LC	–	III	–	1A,2A	1	+	1
Eliomys quercinus	NT	–	III	–	1A	–	+	/
Castor fiber	LC	–	III	II/IV	1A,2A	1	–	1
Clethrionomys glareolus	LC	–	–	–	–	–	–	–
Arvicola amphibious	LC	–	–	–	–	–	+	2
Arvicola scherman	LC	–	–	–	–	–	/	/
Ondatra zibethicus	LC	–	–	–	L	–	–	–
Microtus agrestis	LC	–	–	–	–	–	+	2
Microtus arvalis	LC	–	–	–	–	–	–	–
Microtus subterraneus	LC	–	–	–	–	–	–	–
Microtus liechtensteini	LC	–	–	–	–	–	+	1
Micromys minutus	LC	–	–	–	–	–	+	1
Apodemus agrarius	LC	–	–	–	–	–	+	–
Apodemus flavicollis	LC	–	–	–	–	–	–	–
Apodemus sylvaticus	LC	–	–	–	–	–	+	–
Apodemus uralensis	LC	–	–	–	/	–	/	2
Rattus norvegicus	LC	–	–	–	–	–	–	–
Rattus rattus	LC	–	–	–	–	–	–	–
Mus musculus	LC	–	–	–	–	–	–	–
Mus spicilegus	LC	–	–	–	/	–	–	–
Myocastor coypus	LC	–	–	–	L	–	–	–

(continued)

Table 5 (continued)

Species	IUCN[1]	CITES[2]	Bern[3]	Habitats[4]/Birds[5]	Slovenia[6a,b]	Croatia[7]	Bosnia and Herzegovina[8]	Serbia[9]
Lagomorpha								
Lepus europaeus	LC	–	III	–	L	–	–	2,L

/ = species not present in country, – = legal conservation act does not apply to this species, ? = no information about occurrence and status

[1]**IUCN** (*International Union for Conservation of Nature*)—listed categories of threat according to IUCN categorization: LC, species is not threatened (*least concern*); NT, species is almost threatened (*near threatened*); VU, EN-species is considered to be facing a high risk of extinction in the wild (*vulnerable, endangered*)

[2]**CITES** (*Convention on International Trade in Endangered Species*) (Appendix I, species that face extinction; Appendix II, species that should be under the control of trade to avoid the threat of extinction; Appendix III, species that are protected on the territory of at least one country)

[3]**Bern—***Bern Convention on conservation of European wild flora*, *fauna*, *and natural habitats* (Appendix II, strictly protected animal species; Appendix III, protected animal species)

[4]**Habitats Directive—***European directive on conservation of natural habitats and of wild fauna and flora* (Annex II, vulnerable/sensitive species which could become endangered in the near future if the factors of threat continue to act; Annex IV, species that require strict protection; Annex V, species which breeding in the wild and exploitation could be a matter of management); *subspecies *pannonica*, e.g., lowland oviparous populations are under Annex IV

[5]**Birds Directive—***European directive on conservation of bird species* (BD-1 or Annex 1, species in danger of extinction, rare, vulnerable to specific changes in their habitat, or requiring particular attention for reasons of the specific nature of their habitat)

[6a]*Decree on protected wild animal species*. Official Gazette of the Republic of Slovenia No. 46/04, subsequent corrections. 1A, Annex 1 (section A) protected native animals; 2A, Annex 2 (section A) protected native animals with habitat management protection; 2*A, priority species for conservation where the EU has a particular responsibility in relation to the proportion of their natural range, which lies within the European Union; 2B, Annex 2 (section B) protected nonnative animals with habitat management protection

[6b]Wild Game and Hunting Act. Official Gazette of the Republic of Slovenia No. 16/04, subsequent corrections, and its consequent *Decree specifying the wild game and hunting periods*. Official Gazette of the Republic of Slovenia No. 101/04, subsequent corrections. L, species listed as game animals; however, conservations requirement must be followed

[7]*Nature Protection Act* (National Gazzete No. 80/13); *Ordinance on Proclamation of Wild Taxa as Protected and Strictly Protected* (Official Gazette 144/13); *1* strictly protected species, *nesting populations, **non-nesting populations, ***lowest protection status; any kind of human interference strictly regulated

[8]Species lists are not available yet for the Federation of Bosnia and Herzegovina, while *Decree on Red List of Protected Species of Flora and Fauna* has been established recently in Republika Srpska (Official Gazette of Republika Srpska no. 124/12). Consequently, at the moment we can present here only protected (+) vs. non-protected (−) species in Republika Srpska

[9]*Book of rules on declaring and protection of strictly protected and protected wild species of plants*, *animals*, *and fungi* (Official Gazette RS no. 5/2010); 1, strictly protected species; 2, protected species; L, species on the list of *Law on Game and Hunting* (Official Gazette RS no. 18/2010)

3.4 Habitats Directive

Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, known as the Habitats Directive, combines the idea of maintaining a network of protected sites with a strict system of species protection on a European level.

The main aim of the Habitats Directive is to promote the maintenance of biodiversity by requiring Member States to take measures to maintain or restore natural habitats and wild species listed on the annexes to the Directive at a favorable conservation status, introducing robust protection for those habitats and species of European importance (Annex IV). The provisions of the Directive require Member States also to contribute to a coherent European ecological network of protected sites by designating Special Areas of Conservation (SAC) for habitats listed on Annex I and for species listed on Annex II. These measures are also to be applied to Special Protection Areas (SPAs) classified under Article 4 of the Birds Directive. Together SAC and SPAs make up the Natura 2000 network. Member States must also establish systems of strict protection for those animal and plant species which are particularly threatened (Annex IV) and prohibit the use of nonselective methods of taking, capturing, or killing certain animal and plant species (Annex V). The species which are considered as widely distributed on the territory recognized today as the European Union are not included into Habitats Directive. Of 17 amphibian species listed in our study, 24 % are not covered by the Habitats Directive, as well as 31 % of reptile and 56 % of mammal species (Table 5).

3.5 Birds Directive

The Birds Directive is another EU directive in relation to wildlife and nature conservation. It was adopted first in 1979 and then replaced by the new version in 2009. The main goal is protection of all European wild birds and the habitats of listed species. Species covered by this directive are listed in Annexes I–III. Species listed in Annex I require specific conservation measures concerning their habitats; the species listed in Annex II may be hunted under national legislation. Around 36 % of bird species listed here are not included in the Birds Directive (Table 5).

3.6 National Legislatives

3.6.1 Slovenia

Nature Conservation Act (ZON-UPB2) (Ur .l. RS 96/2004) defines measures necessary for the preservation of biodiversity and the system of protection of natural features with the intent to contribute towards preservation of nature (ZON, Article 1). Protected animal species are defined by the Decree on protected wild animal species (46/2004, 109/2004, 84/2005, 115/2007, 96/2008, 36/2009, 102/2011, respectively).

3.6.2 Croatia

The basic legislation governing the nature protection in Croatia is the Nature Protection Act (National Gazette No. 80/13). According to the Act, existing conservation measures include strict protection of wildlife species. The law stipulates that a strictly protected wild species should not be disturbed or harassed (catching, keeping, killing, etc.), except for certain purposes specified in the Act (research, education, repopulation, reintroduction, etc.). The latter can only be done with special permission issued by the Croatian Ministry of Environment and Nature Protection.

3.6.3 Bosnia and Herzegovina

The legal framework governing the issue of nature protection in Bosnia and Herzegovina is the Nature Protection Act (Official Gazette of FBiH no. 33/03) and the Law on Nature Protection (Official Gazette of FBiH no. 53/02) in the Federation of Bosnia and Herzegovina, as well as Law on Nature Protection in Republika Srpska (Official Gazette of Republika Srpska no. 50/02, no. 34/08, and no. 59/08). The Law on Nature Protection in Federation BiH defines strictly protected species/subspecies and protected species/subspecies. Strictly protected animals, fungi, and plants may not be exported or imported. Notwithstanding, some strictly protected species may be exported and imported for scientific research purposes, for exchange, exposure, etc., on the basis of the Federal Ministry permission. The use of protected wildlife species/subspecies is allowed in the manner and amount which do not cause any threat to their populations. The Law on Nature Protection of Republika Srpska also defines strictly protected and protected wild species and is accompanied with the Decree on Red List of Protected Species of Flora and Fauna of Republika Srpska (Official Gazette of Republika Srpska no.124/12).

3.6.4 Serbia

According to the Book of Rules on declaring and protection of strictly protected and protected wild species of plants, animals, and fungi in the Republic of Serbia (Official Gazette RS no. 5/2010 from 5.2.2010), with the exception of species which are assigned as “protected” by control of their collection, exploitation, and trade, other wild species which inhabit the territory of Serbia are either assigned as "strictly protected" or are not protected at all. The difference between the statuses of "protected" and "strictly protected" wild species is reflected in the permanent ban on any collection, killing, or keeping in captivity of "strictly protected wild species." The only exceptions are for the purpose of scientific experiments, when the collection of a small number of specimens is allowed. Even then, it is necessary for the competent institution in the competent ministry to consider the experimental proposal first and consequently to allow or prohibit the requested collection. "Protected" wild species could be collected for commercial purposes, but only in a way and in quantities allowed by the competent ministry. Their collection for noncommercial purposes also requires a permit issued by the competent ministry.

4 Main Threats

Modern society has had numerous and adverse effects on the local populations of wild animal species. These effects can be classified into four major causes of contemporary mass extinction: habitat fragmentation and degradation, over-exploitation, colonization of allochthonous and invasive species, and chain effects of species extinctions [50].

In the report on the sites important for biodiversity along the Sava River, Kitnaes et al. [10] analyzed the threats and impacts, also for riparian habitat types. The open ones, belonging to meadows in a broad sense, are moderately suffering from modern agricultural practices, which quickly deteriorate living space for focal vertebrate species. Abandonment of traditional exploitation by grazing and mowing, which results in succession, and overgrowing is even more dangerous for open meadows and grassland pastures. The riparian forests are at the moment still at the moderate equilibrium between favorable and unfavorable conservation status but continuously in danger of intensive forestry, deforestation, and replanting of nonnative tree species (predominantly by poplar plantations).

4.1 Amphibians

Today the world's amphibians are threatened by a series of direct negative impacts to their long-term survival [48]. As a group, amphibians are rightly considered more endangered and faster declining than, for example, birds and mammals, and urgent conservation measures are needed at the global level [51].

Riparian habitats along the Sava River are of great economic importance to humans, e.g., for agriculture, fisheries, exploitation of minerals, hydropower operations, etc. They are also pertinent in the processes of construction of reservoirs, dams, deepening of river beds, and artificial waterways regulations, digging channels to prevent flooding and thus lowering the groundwater level. By habitat alterations, natural floodplains and swamps lose their effect of nature services as natural sponges which absorb and harbor extra water during floods. Additionally, they also lose the role of prime habitats for whole variety of amphibian species living there. Unfenced roads which pass directly through the natural habitats cause big problems to spring and autumn amphibian migrations as many of these roads have intensive traffic. Chemicals, such as pesticides, which end up in the water, reduce viability of the amphibians, especially at the larval stage. A serious problem for amphibians' eggs and larvae in their stagnant hatcheries causes introductions of invasive fish species, which prey on them. One of these very dangerous and highly resistant opportunistic species is Rotan (*Perccottus glenii*), currently recorded moving upwards the Sava River 'till Slavonski Brod. This species is estimated as one of the most dangerous invasive fish species having strong effect on amphibian populations [52, 53].

4.2 Reptiles

Decline in the numbers of the world populations of reptiles was never so thoroughly explored, as is the case for amphibians, but there are indications that their number also decreased significantly ([54, 55]). Many of these declines can be attributed to a number of threats such as pollution, loss or degradation of habitat, spread of diseases, overexploitation, or climate change, but some causes of decline are either only partly defined or completely unknown [56]. Lowland valleys of large rivers such as the Sava constitute the prime areas for human development and are in rapid change. Reptiles of riparian habitats are threatened primarily by disappearance of suitable habitats, by increasing level of habitat fragmentation and break of continuous corridors. Prime drivers of these changes are construction of transport infrastructure ([57] for Obedska bara in Serbia) and residential areas, and also the shift to intensive agriculture, which cause not only destruction of the remaining suitable habitats but also create insurmountable barriers between populations. Moreover, intensive population of humans is often followed by deliberate introduction of exotic species, such as red-eared slider [20]. Environmental stress has visible effects on reptile populations, and some of the species present in this area could be valuable indicators of extent of human impact on wildlife [58].

4.3 Birds

Habitat loss is by far the most significant threat for bird populations. For more than 70 % of the threatened species of birds, habitat loss was cited as the main source of risk [59]. When we compare historical data on birds and general landscape characteristic along the Sava River [60–62] with the current situation [63–67], it is obvious that natural wetlands have been significantly deteriorated and reduced in their coverage (direct human impact or natural succession). That consequently caused the drop of population numbers and restriction of distribution of many wetland birds. Large areas along the Sava River previously covered by alluvial forests have been cleared and turned into arable land or settlements. However, relatively preserved and large forest patches still exist along the Sava River, but forest management which is practiced there is often too intensive and not compatible with conservation needs. Other human activities such as extraction of river sediments, development of infrastructure, or intensive agriculture also pose significant threat to natural habitats of birds.

Hunting activities are common in the region. Those activities can have significant impacts on bird populations, especially in case of rare and endangered species (not only through illegal killing but also by disturbance, especially near the nests and important feeding habitats). Some large bird species like eagles or colonial species like terns or herons are very sensitive to human disturbance, especially during nesting period. Too intensive disturbance by human presence (hunters, fisherman, tourists, farmers, traffic) can often cause reproduction failure (leaving of nest).

4.4 Mammals

The European Red List [68] accounts 15 % of all listed 231 European mammals to endangered species, and almost the same percentage of mammal species living along the Sava River are classified as vulnerable or near threatened. Common regional red list of mammals does not exist; however, general conservation status is more or less similar all over the Sava River area.

Habitat loss and degradation have by far the largest impact on both threatened and non-threatened species of mammals along the Sava River. Forestry and drainage activities have altered pristine forests, while agricultural practices have been reducing forests and changing flooded meadows. Marshes and reedbeds have been reduced, and trenches have replaced natural creeks and ponds. For the majority of mammals, it is hard to quantify population decrease or even population trends, since the research on the subject is poor. Their level of threat mostly can be assumed by the state of preservation or destruction of their habitats.

Destruction of flooded meadows and ponds due to drainage activities and possible effect of pesticides perform a negative impact on amphibious mammalian species as Mediterranean or Eurasian water shrews. Also other insect-eating animals (shrews and bats on general) may be under increasing pressure of diminishing wet forests and riparian habitats which harbor proportionally higher numbers of insects than other areas [69]. In recent years also effect of light pollution is increasing in some areas, and again, on a long run, some population of insects could be directly negatively affected. Additionally, insects are lured away from the foraging habitats of several bat species. Forest management reduces the number of old trees with cracks, crevices, rot holes, and woodpecker holes as well as those with loose bark (all very important roost of several bat species, as a place for hibernation, maternity colonies, and mating). Removal of these old trees directly destroys key roosting habitats for bats and is likely reducing their populations as well. The European beaver or gray wolf was affected by human presence and hunting and had in the past disappeared from a region. However, today's beaver populations, originating from reintroduced animals, are increasing, and wolf is slowly gaining back its area along the Sava River. Nevertheless, both species and also otter, because of their small population sizes, are vulnerable to poaching and even to road kills, but the main threat is deterioration or total destruction of their habitats. Additionally, big game animals are sometimes exposed to poaching and in some areas are also victims of land mines. However, this seems not to endanger their populations on the whole. The increase of their populations is also the cause of increased damage to crops which leads to conflict with farmers, but in general hunters' organizations are managing populations well enough.

Nevertheless, some species benefited from human influence in this area, e.g., deforestation favored populations of field and meadow vole species. Some species, like the golden jackal, are attracted to the new form of such human-altered habitat, where also the nonnative species, like the muskrat, coypu, and raccoon dog, find their place. Real effects of introduced species to native fauna have yet to be revealed.

5 Species of Special Conservation Concern

5.1 Amphibians

The majority of amphibian species listed here are generally common European species. The most typical example is the common toad, which is very widespread and also genetically not divergent in this area [70]. However, information from the Tables 1 and 5 point that some amphibian species require specific conservation actions. They have higher global conservation status in comparison with the rest of the list as well as distinguished regional or national priority status. Among tailed amphibians, populations of the widespread smooth newt belong to the same haplotype group as those from Western Europe and Western Balkans, including most of Serbia [71], and thus are not considered fragile. On the contrary, the crested newt taxa deserve special treatment, due to their particular evolutionary history [72]: Danube crested newt has already been recognized as globally potentially endangered, and it is also among the Natura 2000 target amphibian species, while Italian crested newt populations, occurring only in the upper part of the Sava River watercourse, should have specific conservation priority in the area. The other generally vulnerable amphibian species in the Sava River region are red-bellied and yellow-bellied toads, also Natura 2000 target species.

In Croatian Red Book of threatened amphibians and reptiles, Jelić et al. [20] listed Danube crested newt and red-bellied toad as near threatened (NT) and common spadefoot as data-deficient species (DD). Wolterstorff's moor frog (*Rana arvalis wolterstorffi*) is also mentioned as species of high conservation concern because their populations are thought to be in decline, though there is not sufficient evidence. This species should be subjected to more field research in Croatia, Bosnia and Herzegovina, and Serbia. Authors of the Red Book propose a number of specific conservation actions in order to reduce the threat level for this species. Large river plains of Croatia (including the Sava River plain) are suggested as important herpetofauna area.

Crnobrnja-Isailović and Paunović [73] listed Danube crested newt, yellow-bellied toad, common spadefoot, as well as water frogs as the most threatened amphibian species in Serbia. Either because of their sensitivity to habitat alteration or being a subject of long-term overexploitation, these species were recognized as potentially locally endangered due to rapid anthropogenic impact and thus are in need of active support by implementation of specific legislations and protection in situ.

5.2 Reptiles

There is no globally endangered reptile species occurring along the Sava River flow. However, some of them could be treated as disturbed as their favorable habitat types are getting lost in the process of anthropogenic changes.

In Croatian Red Book of threatened amphibians and reptiles, Jelić et al. [20] listed the European pond terrapin and adder as near threatened (NT) and viviparous lizard as data-deficient species (DD). Pannonian subspecies of viviparous lizard, *Z. vivipara pannonica*, known only from the Spačva forest (the Sava River plain in Croatia) was listed as near threatened (NT). Authors of the Red Book proposed a number of specific conservation actions in order to reduce the threat level for this species. Large river plains of Croatia (including the Sava River plain) are suggested as important herpetofauna area.

European pond terrapin is near threatened globally, and it is already recognized as Natura 2000 focal species. Its survival depends on the well preservation of both aquatic and riparian habitats, and recent studies point on general problems this species faces in Slovenia [74], Croatia [75], and Serbia [76].

In the lower part of the Sava River watercourse, in Serbia, riparian habitats are apparently altered. Therefore, some of the most widespread European species, the adder, is very rare in Pannonian part of Serbia—Vojvodina [27]. Intensive colonization of Vojvodina in the last few centuries induced habitat alteration in combination with overexploitation of timber. As a result, most of suitable places for adder—marshy areas and autochthonous riparian mostly oak forests—were converted into arable land and poplar forests.

5.3 Birds

Several globally threatened species of birds are present in the Sava River region. Saker falcon is a globally endangered species (EN) which regularly breeds in Srem region in Serbia and possibly in Croatia (a few pairs breed in eastern Croatia, northern of Sava River flood plain). Other breeding species which are red listed are the ferruginous duck (NT, regularly breeds in Croatia and Serbia and in Bardača-Bosnia and Herzegovina) and whimbrel (NT, breeds in Slovenia). Red listed species which occur regularly during migration or wintering are the European roller (NT), red-footed falcon (NT), great snipe (NT), black-tailed godwit (NT), and red kite (NT). Rare visitors of the region are the Dalmatian pelican (VU), lesser white-fronted goose (VU), red-breasted goose (EN), long-tailed duck (VU), velvet scooter (EN), greater spotted eagle (VU), and pallid harrier (NT).

Additionally, five species which have breeding populations along the Sava River are also recognized as European species of global conservation concern [65]. Those are the pygmy cormorant, ferruginous duck, white-tailed eagle, saker falcon, and corncrake.

5.4 Mammals

Considering the IUCN Global [77] as well as the European Red Lists [68] and the state of mammals along the Sava River and surrounding riparian areas, we can highlight some species within the category of vulnerable (VU) species which are highly dependent to riparian and nearby habitats. Ten of all mammal species that occur along the Sava River plain have global status higher than the LC category.

Forest bat species, Bechstein's bat and western barbastelle, are only two mammal species with VU category that have roosts in the Sava River plain. They are typical representatives of old forest stand faunas. The first of the two mentioned species uses almost exclusively woodpecker holes and rot holes [78], while the second one occupies loose barks and crevices on trees [79]. Since one colony utilizes several dozens of roosts, conserving a net of suitable roost is of an utmost importance for conservation of this species [69]. For some other bat species with higher categories of protection (VU, Mediterranean horseshoe; NT, Schreiber's bent-wing bat, lesser mouse-eared bat, greater and lesser horseshoe bats), this area is an important foraging habitat, though they roost in caves of neighboring hills or utilize different kinds of buildings as their summer roosts.

The whole area is a habitat of relatively rare European otter (NT). Otter is a charismatic animal which uses littoral river areas but also nearby forests, arable land, and bigger water bodies. Optimal habitat includes foraging areas, but also critical for its presence is existence of opportunities for peaceful resting place, e.g., structured coast, diverse and dense riparian vegetation, and old trees with extensive root system. Despite great flexibility regarding habitat requirements, the choice of suitable sites for dens is much more difficult. Females choose well-protected sites. Its inland habitats are the most upper streams of rivers and streams (in the forest) or the appropriate standing water, where the risk of flooding is less. Therefore, the system tributaries of large rivers are extremely important for the existence of otter populations [80, 81].

Some other mammals may be threatened locally, although globally such species are not in higher conservation categories. An example is the European wildcat (global status LC), species that is common in flooded forests along the Sava River but is endangered by crossbreeding with local populations of feral cats.

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Genotoxicological Studies of Lower Stretch of the Sava River

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Abstract Genotoxicity monitoring of the lower stretch of the Sava River was performed by the combined approach of in situ assessment of genotoxicity and active biomonitoring of two species of mussels from the Unionidae family, *Unio pictorum* and *Unio tumidus*. Genotoxic response was studied using comet assay on hemocytes. For active biomonitoring, the mussels were acclimated to controlled laboratory conditions for 10 days and then exposed at two sites in the Sava River in the area of the city of Belgrade. Hemolymph of exposed specimens of each species was taken after 7, 14, and 30 days of exposure. For in situ assessment, the mussels were collected from five sites in the lower flow of the Sava River. The mussels were sampled immediately after the acclimation served as controls in both types of monitoring procedures. The results of our studies indicated the presence of genotoxic pollution at all studied sites at the Sava River. The level of DNA damage varied at different sites depending on the source and level of pollution. The response to genotoxic pollution was evident at the site in the urban area of Belgrade city, as well as at the sites far from the large urban settlements, suggesting that the lower flow of the Sava River is under pollution pressure.

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1 Introduction

The evaluation of the impact of pollutants by biomarkers becomes essential for assessing the condition of aquatic ecosystems due to the fact that the simple detection of pollutants failed to provide the information on the relationship between contaminant exposure and biological effects in aquatic organisms [1, 2]. The presence of pollutants in aquatic ecosystems can be detected by a range of physiological, histological, and molecular responses, including abnormal morphology, alterations of antioxidative status, and DNA integrity [3–11].

The integrity of cellular DNA is continuously attacked by various agents in the environment resulting in DNA lesions such as strand breaks, modified bases, DNA–DNA cross-links, and DNA–protein cross-links. Unrepaired DNA lesions may block replication and transcription, potentially leading to cell death, or may give miscoding information, generating mutations [12–14]. As a result, a number of biological consequences can be initiated at the cellular, organ, whole animal, and finally community and population levels. DNA damage in a variety of aquatic animals has been associated with reduced growth, abnormal development, and reduced survival of embryos, larvae, and adults [15]. Studying the origin of genotoxic pollution, as well as the effects of pollution on individuals and populations, is the main objective of ecogenotoxicology.

1.1 Comet Assay (SCGE)

The comet assay, also known as single cell gel electrophoresis (SCGE), is a sensitive and rapid technique for detection of DNA damage in individual cells based on the migration of denatured DNA during electrophoresis, in which damaged nuclei form comet-like shapes. Comet assay has been accepted as one of the major tools for assessing pollution-related genotoxicity in aquatic organisms [16]. It has been used in many ecogenotoxicological studies on freshwater mussels [3, 6, 17–22] and has shown correlation with other genotoxicity tests such as chromosomal aberration, sister chromatid exchanges, and micronucleus assay [23]. The modified alkaline version of the comet assay, described by Singh et al. [24], enables detection of both single and double DNA strand breaks, as well as alkali labile sites. Images of the comets can be analyzed manually or with the assistance of computer software. When scoring manually, nuclei are divided in classes based on different levels of DNA damage, from undamaged nuclei (class 0) to nuclei which have almost all DNA in tail (class 4), or based on the head to tail length ratio [25]. In recent studies, the comets are scored and analyzed using the Comet IV Computer

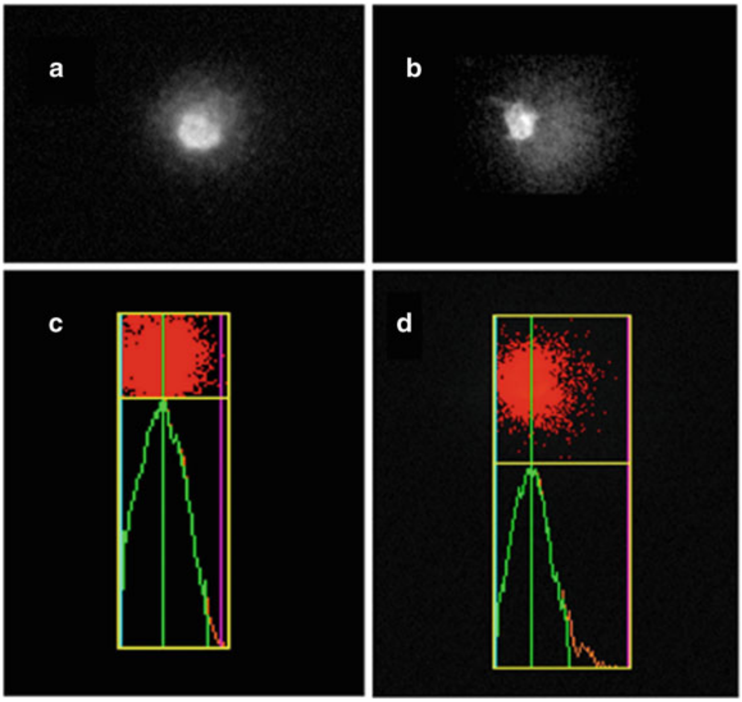


Fig. 1 (**a**) Hemocyte nucleoid from the control group of mussels with minimal DNA damage, as demonstrated by the lack of DNA fragment migration. (**b**) The hemocyte nucleoid from the mussel collected at a polluted location showing a high degree of DNA damage, with a significantly reduced nucleoid core and a large cloud of DNA fragments migrating away from the core forming the characteristic comet tail. (**c**, **d**) The comets are analyzed using the Comet IV Computer Software (Perceptive Instruments, UK)

Software (Perceptive Instruments, UK) (Fig. 1). Tail intensity, TI (the percent of DNA fluorescence in the comet tail), and Olive tail moment, OTM (calculated as a product of the TI and the distance between the means of the head and tail distributions [26]), are most often used as a measure of DNA damage.

1.2 Freshwater Mussels as Bioindicators

Mussels are commonly employed in ecogenotoxicological studies. They have several characteristics, such as wide distribution, filter feeding, a sessile life form, and an ability to accumulate pollutants, which makes them favorable organisms for estimating the environmental pollution level and the bioavailability of various types of pollutants [27–33]. In response to environmental stress they show a range of physiological, histological, and molecular responses, including abnormal

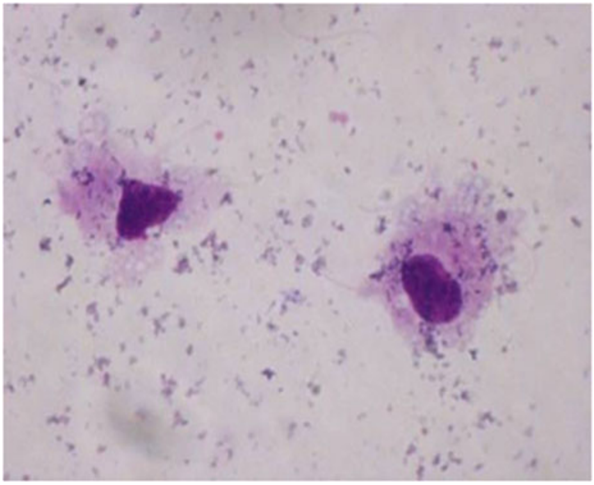


Fig. 2 Hemocytes of *U. pictorum* stained with Giemsa stain

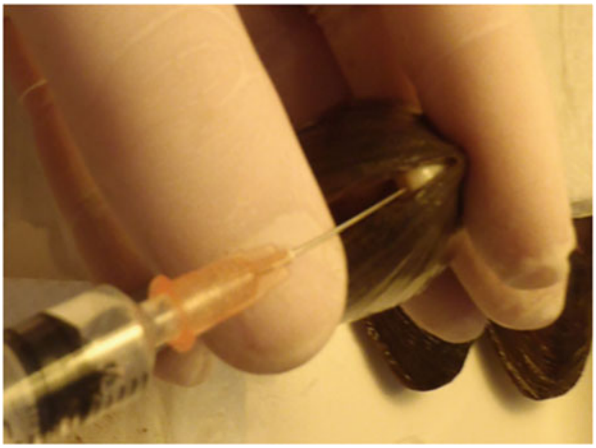
morphology, alterations of antioxidative status, induction of DNA strand breaks, etc. [3–6, 8, 9].

Selection of proper mussel tissue enables the detection of the effect that genotoxic substances have on the first site of contact. In most cases genotoxicity studies are performed on hemocytes and gill cells. Gills have a high efficiency in genotoxicity monitoring due to their large surface and constant exposure to environment. Hemocytes (Fig. 2) have a role in processes such as the transport and digestion of nutrients and elimination of toxic substances and small particles, which makes them constantly exposed to waterborne pollutants [23, 34, 35]. Hemolymph can be easily collected from the adductor muscle and, most importantly, collecting does not require sacrificing animals (Fig. 3).

1.3 Assessment of Genotoxicity: In Situ Assessment and Active Biomonitoring

In ecotoxicological studies, different approaches are used for assessing the conditions of ecosystems. In situ assessment employs the collection of the specimens from selected locations, while active biomonitoring entails the use of bioindicator organisms obtained from unstressed populations and their subsequent exposure at polluted sites [36, 37]. Active biomonitoring is increasingly used for quantifying the impact of pollutants on aquatic ecosystems because of its numerous advantages over the in situ assessment, such as avoiding the biological variability in the responses related to different age and the reproductive status of the organisms in situ. In addition, it can overcome the hydrological, hydrochemical, and other abiotic and biotic factors that can influence species distribution, contaminant bioaccumulation, and biomarker responses [19, 29, 38–41]. In the last decade, a

Fig. 3 Collection of hemolymph from the adductor muscle



range of phylogenetically separate groups of animal and plant organisms have been used in active biomonitoring [1, 2].

One of the major issues in ecogenotoxicological studies is providing data from the animals at unpolluted sites which can be used as control values of DNA damage for in situ assessment of genotoxicity. Active biomonitoring also requires the specimens from unpolluted sites to be used for translocation. However, finding an unpolluted site is not always possible. The acclimation of mussels in controlled laboratory conditions could provide an adequate solution for obtaining the control values, i.e., the baseline DNA damage, as described in different mussel species [42, 43]. In our previous study [21, 22], we have shown the ability of DNA damage recovery in mussel species from the Unionidae family.

2 Genotoxicity Monitoring of the Lower Flow of the Sava River

The Sava River Basin in Serbia covers an area of 95,719 km^2 with intensive agricultural activity [44]. In addition, situated in this section is the city of Belgrade (2,000,000 of inhabitants), the biggest settlement on the Sava River. The Sava River has great importance for water supply, irrigation, fisheries, and water-related activities and represents an important waterway. However, the lower flow of the Sava River has been influenced by numerous pollution sources from municipal and industrial facilities, and agriculture as well. The impact of untreated and improperly treated wastewaters is evident in high nutrient content, BOD values, and inorganic pollutant loads [45, 46]. There is an additional pressure by heavy boat traffic and intense exploitation of riverbed material.

Genotoxic pollution in the Sava River was detected in our previous study on the mussels *Unio pictorum* and *U. tumidus* as bioindicators [22]. We have also detected genotoxic pollution in the Danube River in studies performed on *Barbus barbus* [47] and *Sinanodonta woodiana* [48], as well as in the tributary Velika Morava by *S. woodiana* [21]. Moreover, the deterioration of water quality of the Danube River and the Danube tributaries by anthropogenic impact was shown in our previous studies [48–52].

Genotoxicity monitoring of the lower flow of the Sava River was performed by the combined approach of in situ assessment of genotoxicity and active biomonitoring with two species of mussels from the Unionidae family, *U. pictorum* and *U. tumidus*. Both mussels are native to the Danube River basin and have a wide distribution in Central, Northern, and Eastern Europe, with a relatively low genetic variability [53]. For in situ assessment, mussels were collected at the sites in the Sava River, while active biomonitoring was applied for assessment of genotoxicity at the sites which are under high pollution pressure. The level of DNA damage was assessed in hemolymph, but the results of our previous study [22] indicated that gill cells are also reliable for the assessment of genotoxic pollution in aquatic environments.

Six sites were studied at the Sava River: Štitar, Bosut, Sremska Mitrovica, Jarak, Duboko, and Sava's branch. Selected sites are under different pollution pressure, such as the impact of wastewaters from small and large urban settlements and the impact of agriculture and pollution brought by tributaries (Fig. 4).

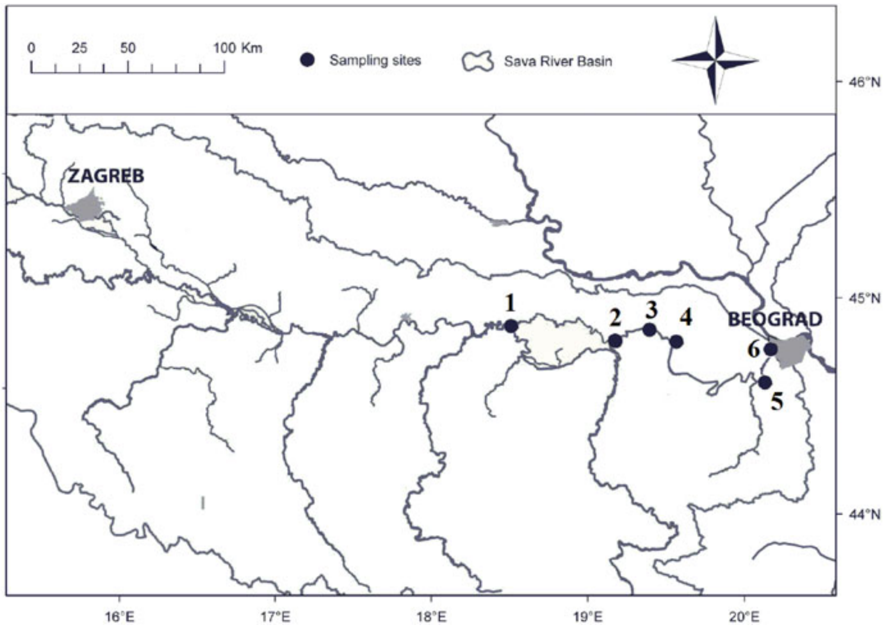


Fig. 4 Sampling sites along the lower flow of the Sava River Basin. *1* Štitar, *2* Bosut, *3* Sremska Mitrovica, *4* Jarak, *5* Duboko, *6* Sava's branch

Genotoxicity was detected by the alkaline version of the comet assay and TI was chosen as the measure of DNA damage. Statistical analysis of the results obtained in the experiment was carried out using Statistica 6.0 software (StatSoft, Inc. [54]). Kolmogorov-Smirnov test for normality of distribution was used prior to statistical analysis. Considering that the data were not compatible with the requirements for the application of parametric tests, differences between samples were tested using the Mann–Whitney *U* test.

2.1 Genotoxicity Monitoring: DNA Damage Recovery

For DNA damage recovery we have used specimens of *U. pictorum* and *U. tumidus* collected from the Orešac site (the Danube River) situated downstream of the Belgrade city. Considering that this site is under heavy pollution impact, the mussels were subjected to acclimation procedure. The mussels were kept in aquaria under controlled conditions (conc. $O_2 > 8$ mg L^{-1}, O_2 saturation >90 %, $t = 22$ °C, pH 7.2–8.1). The bottom substrate is composed of fine sand washed with clean water to eliminate debris and pretreated with heat (at 250 °C for 4 h) in order to eliminate potential disease vectors. The mussels were fed every third day with dry leaves of string nettle (*Urtica dioica*) that were macerated and minced with a mortar and pestle. After 10 days of acclimation, the hemolymph was sampled from five specimens of each species and subjected to the comet assay separately for each specimen.

Our results indicated that 10 days was sufficient for reaching a baseline level of the DNA damage (Fig. 5). Acclimated mussels were used as a control group for in situ assessment of the genotoxicity.

2.2 Genotoxicity Assessment: In Situ Assessment

The mussels were collected on September 2012 from the five sites where they were present in quantities needed for the experiments. The Štitar site (1) is mainly under the impact of agricultural runoffs. In this section the Sava River flows through the region Slavonia which is of great importance for the agriculture in Croatia. The only larger settlement close to the Štitar is located 60 km upstream of town Slavonski Brod with 60,000 inhabitants. The site is situated about 30 km downstream from the confluence of the Bosna River, the Sava River's right-bank tributary. The Bosut site (2) is located near the confluence of the small lowland Bosut River. However, about 15 km upstream of the Bosut site is the confluence of the Drina River, the largest tributary of the Sava River with a significant hydrological input. The Sremska Mitrovica site (3) is under the impact of wastewaters from the town Sremska Mitrovica. The Jarak site (4) is located 15 km downstream of the Sremska Mitrovica. Apart from the wastewaters originating from the upstream

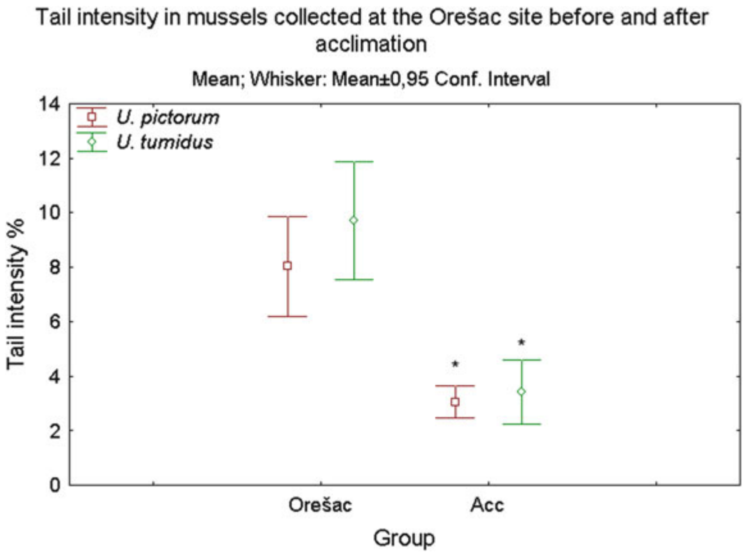


Fig. 5 Comparison of DNA damage in hemocytes of *U. pictorum* and *U. tumidus* collected at the site Orešac before and after acclimation (Acc) in controlled conditions. DNA damage was assessed by TI. For each plot, 250 nucleoids of hemocytes were scored. (*Asterisk*) Statistical significance ($p < 0.05$, Mann Whitney *U* test)

located settlement, this site is also under the impact of agricultural runoffs. The Duboko site (5) is located upstream to the city of Belgrade. This site receives only domestic wastewaters of upstream located minor settlements. The site is located 2 km downstream of the confluence of Kolubara River.

The results of the in situ assessment of genotoxicity are shown in Fig. 6. When comparing to values obtained after 10 days of acclimation, significantly higher levels of DNA damage were detected at all studied sites. The level of DNA damage in *U. pictorum* and *U. tumidus* was similar at all sites, with the exception of the Jarak site where the level of DNA damage in *U. pictorum* was significantly higher in comparison to *U. tumidus*.

The majority of investigated sites are under the impact of agricultural runoffs, which due to excessive usage of artificial fertilizers represent a potential environmental hazard. These runoffs usually contain a mixture of pollutants such as herbicides, pesticides, and PAHs which, even when present in small concentrations, can have genotoxic effect on freshwater mussels [55]. In addition, untreated wastewaters originating from the town Sremska Mitrovica resulted in high levels of DNA damage in mussels collected at this site (*U. tumidus*) and downstream located in site Jarak (*U. pictorum*). At the Bosut site, hydrological input of the tributaries Drina and Bosut was evident in the lowest level of DNA damage in hemocytes of both mussel species. When comparing the level of DNA damage at the site Štitar with the sites located downstream of the site Bosut, similar levels of genotoxicity were observed. The study of Borković-Mitić et al. [56] performed in

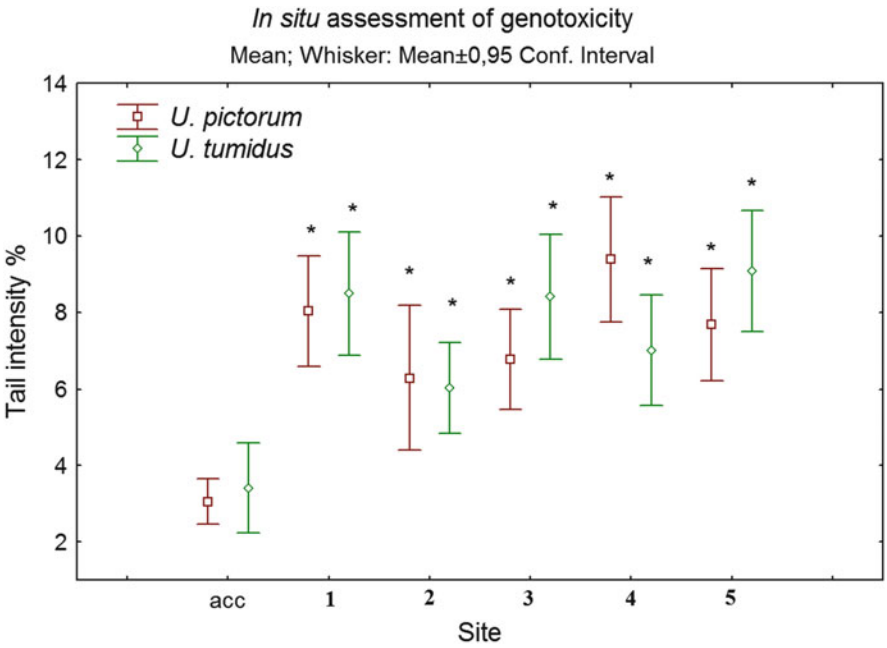


Fig. 6 The level of DNA damage expressed as TI in hemocytes of *U. pictorum* and *U. tumidus* upon sampling from the sites *1* Štitar, *2* Bosut, *3* Sremska Mitrovica, *4* Jarak, and *5* Duboko at the Sava River. Mussels held on acclimation were used as control (acc). Results of 200 comets are shown per group. (*Asterisk*) Statistical significance ($p < 0.05$)

the tissues of specimens of *U. pictorum* collected at two sites at the lower flow of the Sava River, Jamena (located about 25 km upstream of the site Bosut) and the site Šabac (located about 20 km downstream of the site Jarak) showed an insignificant variation in the level of antioxidative defense enzymes at these sites, suggesting a similar level of environmental stress at these sites.

In comparison with the acclimated mussels, we also detected significantly higher levels of DNA damage at the Duboko site which is mainly under the impact of domestic sewage. Although organic extracts of domestic wastewaters can be genotoxic, their potency is several folds below those of many industrial wastewaters [57]. In our previous study performed on *Squalius cephalus* in the Kolubara River basin, we have observed noteworthy genotoxic pollution [58, 59].

Ecogenotoxicological studies performed on *Astacus leptodactylus* [60] and *U. pictorum* [19] caged at the sites located in the upper flow of the Sava River and at the sites downstream of Zagreb (Sisak, Crnac) also indicated a presence of genotoxic pollution. Taken together, genotoxic pollution is evident in the whole stretch of the Sava River from Zagreb to Belgrade.

2.3 Genotoxicity Assessment: Active Biomonitoring

The active biomonitoring was performed in April and May 2011. In April 2011, mussels were collected from the site Orešac and subjected to an acclimation procedure as described earlier, in order to obtain baseline levels of DNA damage. For the exposure experiments, acclimated specimens were held at selected sites in plastic net bags (mesh size 2 mm) at 2 m depth for 30 days. The samples of hemolymph obtained from five mussels from each species were taken for DNA damage analysis after 7, 14, and 30 days of exposure.

The mussels were exposed at two sites in the area of the Belgrade city, one located upstream of the urban area of the Belgrade city and already used in the in situ study (Duboko) and one located in the urban area (Sava's branch site).

The Sava's branch site is located downstream of the sewage outlet of the southern area of the city. This site receives untreated urban, industrial, and hospital wastewaters. The site is situated in the branch of the Sava River which results in strongly reduced water flow. For both sites we have analyzed the microbiological and chemical quality of the water. Microbiological analyses revealed unsatisfactory water quality levels with regard to the threshold values recommended by the Bathing Water Directive [61]. Increased counts of coliform bacteria and elevated concentrations of ammonia and phosphates indicate that there is a recent fecal pollution. At the Sava's branch, the concentrations of dissolved zinc, copper, and arsenic were several folds higher than the permitted values [62]. The concentrations of herbicides, pesticides, detergent precursors, and PAHs have not exceeded the permitted values during the exposure period. All parameters, except the number of fecal coliforms and the concentrations of ammonia and phosphates, were within the permitted values at the Duboko site.

The exposure experiments revealed that the time-course of the genotoxic response and the extent of DNA damage in both analyzed species depended on the level of pollution at the investigated sites. At the site Sava's branch which was characterized by high levels of organic pollutants and several-fold increased concentrations of zinc, copper, and arsenic, the genotoxic response was induced earlier than at the Duboko site which was characterized only by a low level of organic pollution. At the Duboko site, significant increase of DNA damage appeared in *U. pictorum* only after 30 days of exposure (Fig. 7a). Increase (statistically insignificant) in DNA damage was also observed in *U. tumidus* after 30 days of exposure (Fig. 7b). It is possible that the Kolubara River, despite its low hydrological input, excretes the occasional genotoxic impact at this site.

At the highly polluted site, the Sava's branch, the genotoxic response in exposed mussels lasted throughout the entire exposure period; the induction of DNA damage was observed after 7 days and reached a maximum level after 14 days of exposure. When comparing to acclimated mussels during the whole period of exposure, the increase of TI was statistically significant in both species with the exception of *U. tumidus* after 30 days. Moreover, we observed a significant correlation between the TI values in tissues of mussels and the concentrations of zinc, copper, iron, and

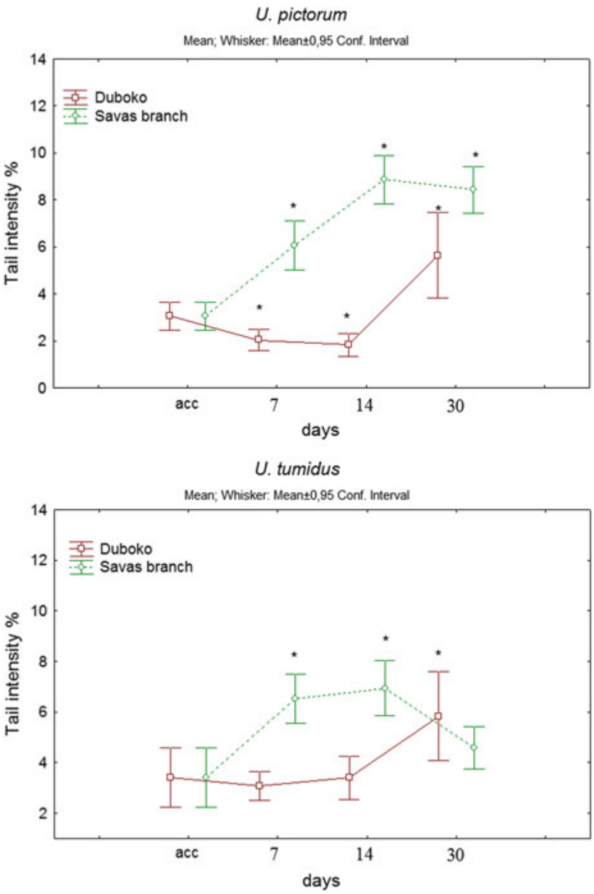


Fig. 7 DNA damage in hemocytes and of freshwater mussels *U. pictorum* (**a**) and *U. tumidus* (**b**) during the exposure period at the sites Duboko and Sava's branch. Control values (marked with acc) are from acclimated specimens. For each plot, 200–250 nucleoids of hemocytes were scored. (*Asterisk*) Statistical significance ($p < 0.05$)

arsenic at the exposure sites. Since zinc, copper, and arsenic are known to induce genotoxic effects in aquatic organisms [63–66], it is reasonable to conclude that the genotoxic responses observed in our study were mainly caused by pollution with heavy metals. However, we cannot exclude the contribution of other pollutants at the exposure sites. The genotoxic potential of industrial, hospital, and household wastewaters containing complex mixtures of chemicals has been confirmed in many studies, even if single pollutants were present at below limit concentrations [55, 57, 67–72].

It is of interest to note that the genotoxic response in both studied mussel species declined after the maximum level of DNA damage was reached at the Sava's branch site which is characterized by considerable levels of pollution. We can speculate that the physiological adjustment of the mussels to the polluted environments (increased antioxidative defense and/or enhanced DNA repair capacity) could account for the decreased genotoxic response during later exposure. Regoli et al. [73] described variations in concentrations of heavy metals and biomarkers, such as DNA damage, antioxidant parameters, and lysosomal membrane stability in the marine mussel *Mytilus galloprovincialis* during 28 days of exposure at the highly polluted Genova harbor. It is also possible that longer periods of exposure select individuals that are more resistant to environmental stress, while the sensitive ones are eliminated. In favor of this idea is the increased mortality, especially of *U. tumidus* observed after 30 days of exposure at Sava's branch.

3 Conclusions

The results of our studies performed in situ and by active monitoring indicated the presence of genotoxic pollution at all studied sites at the Sava River. The level of DNA damage varied at different sites depending on the source and level of pollution. The response to genotoxic pollution was evident at the site in the urban area of the Belgrade city as well as at the sites which are relatively far from the large urban settlements, suggesting that the lower flow of the Sava River is under pollution pressure.

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Indicative Status Assessment, Biodiversity Conservation, and Protected Areas Within the Sava River Basin

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Abstract The aim of this chapter is to provide the overview of the water status, state of the biological diversity, and protected areas along the Sava River as well as to underline the necessity of identification and implementation of effective conservation measures. The chapter is based on historical data on environment and recent investigation on macroinvertebrate communities (2011–2012). Ecological status of water bodies within the Sava River basin ranges from high to poor, while the ecological status of the majority of water bodies is assessed as moderate, which indicates the necessity of design and implementation of relevant mitigation measures. The assessment of water quality and ecological status of the river Sava based on the macroinvertebrates community, alongside with the use of several standard biological methods and regional biotic index BNBI indicates a high correlation of the obtained results. BNBI has proven to be a method reliable enough for both the assessment of water quality and the assessment of ecological status of large rivers. Based on the results of water status assessment, the Sava River could be divided into three zones. The best water quality was recorded within the Slovenian stretch

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of the river, being within the limits of betamesosaprobic zone, while the ecological status was assessed as a good one. The middle part of the Sava River, stretching mainly through Croatia and Bosnia and Herzegovina, has a somewhat worse water quality, approaching the limit of betamesosaprobic zone, while the ecological status in this part of the flow was also determined as a “good” one. The lower parts of the Sava River flow through Serbia are by all indicators more heavily polluted; the water quality is on the border between beta- and alfamesosaprobic zones, while the ecological status is between “good” and “moderate.” The biodiversity of the Sava River may be considered significant, when compared to similar watercourses of Central Europe and Balkan Peninsula. The work contains a more detailed analysis of the biodiversity of aquatic macroinvertebrates and fish of the main flow of the Sava River. Based on the condition of biodiversity of these groups, the river’s ecosystem is divided into three “macrohabitats.” The first macrohabitat includes the upper rhithron parts of the river through Slovenia, with a significant diversity of stenovalent groups of macroinvertebrates (larvae EPT) and salmonid species of fish (brown trout, grayling, and huchen trout). The second macrohabitat includes the parts of the flow through Croatia and Bosnia and Herzegovina with significant diversity of invertebrates from the groups Odonata, Mollusca, Hirudinea, and Chironomidae and fish from the families of Cyprinidae, Percidae, and Gobiidae. The highest number of protected species of fish has been registered in this section. The third “macrohabitat” includes the lower part of the potamon of the Sava River and mostly flows through Serbia wherein this part of the flow represents the most important habitat of the globally endangered and fishing-wise important sturgeon species of sterlet (*Acipenser ruthenus*) in this river. It is characterized by a decreased biodiversity of macroinvertebrates in the main flow of the river and a significant diversity in the flood zones. In the biodiversity of fish, the highest number of allochthonous species appears. In this section, the diversity of fish in flood zones especially as the habitat of endangered species such as *Umbra krameri*, *Misgurnus fossilis*, and *Carassius carassius* is also important. Research has shown that in order to perform a successful conservation of large river biodiversity, the ecosystem must be observed as a complex consisting of the main flow of the river, flood zone, and its tributaries.

Keywords Biodiversity conservation • Bioindication • Protected areas • The Sava River • Water status

1 Introduction

The objective of this chapter is to provide a review on the ecological status and biological diversity of the Sava River, to give an overview of water-related protected areas of the Sava River basin, as well as to underline the necessity of identification and implementation of effective conservation measures. As background, the general natural characteristics of the basin area are provided.

The indicative ecological status has been assessed based on the data from the Sava River Basin Management Plan and recent macroinvertebrate survey (2011–2012). The assessment of the Sava River ecosystem status based on biological indicators has not been performed comprehensively since the work of Matoničkin et al. [1] in the 1970s. The research performed within a bilateral project of Serbia and Croatia is related to the detailed research of macroinvertebrate community. Thus, this quality element has been used for the assessment of both the quality of water of the Sava River and the ecological status of this significant international aquatic ecosystem.

Aquatic macroinvertebrates are the most commonly used organisms in water quality assessment [2], according to the European Union Water Framework Directive, and in the assessment of ecological status of aquatic ecosystems [3]. This fact is primarily based on good indicator characteristics of these organisms, such as relatively low mobility, relatively long life cycle, relatively large forms, as well as good possibilities of their taxonomic evaluation [4]. Europe is the place of origin of most of the methods which, in order to assess the pollution of aquatic ecosystems, use representatives of macroinvertebrates as bioindicators, such as saprobic indices, biotic indices, and diversity indices [5].

According to many authors, such large number of methods is due to the fact that it is hard to conceive a biological method for assessment of aquatic ecosystem pollution that would be precise enough in all, more or less different geographical areas [2, 5]. For this reason, biological methods are conceived for individual smaller or larger geographical areas, certain basins, or even rivers as unique ecosystems. More or less different concepts of biological methods are the result of smaller or bigger differences in the structure of the communities of macroinvertebrates of aquatic ecosystems in different geographical areas.

The concept of using regional or local biological methods in the assessment of aquatic ecosystems pollution and the assessment of ecological status of water has been embedded into the contemporary concept of the European Union Water Framework Directive, which states that the countries who signed the directive are to decide for themselves which biological method to use on its territory.

Regarding the previous considerations, this work on the assessment of the pollution of the Sava River and its ecological status has simultaneously used a large number of globally accepted biological methods included in the European Union-funded project AQEM [6], on one side, and a regional biotic index, the so-called Balkan Biotic Index (BNBI), conceived for the Balkan Peninsula area, on the other [7].

The importance of the application of this index on the ecosystem of the Sava River lies in the fact that this was the first time it has been applied on a large river, allowing a certain amount of correction and addition to the base of indicator taxons (genus) of this index. Besides the assessment of the degree of pollution and the ecological status of the Sava River, the other important aim of this chapter is the review of species and ecosystem biodiversity of this river and a part of its basin.

Based on the structural characteristics of biodiversity of the Sava River, including total species richness and representation (by taxonomic groups, but primarily within well-studied communities, such as plankton, benthos, and nekton); indigenity; the presence of endemic, rare, and endangered species; and the status of conservation of habitat parts, the proposal of the so-called macrohabitats is given, primarily within the limits of the river flow itself, the ecosystem of the Sava River. The allocation of macrohabitats was done according to the system of "keystone" community and the ecosystems that they suggested [8]. The purpose of allocation of macrohabitats as particularly important parts of the Sava River ecosystem is to provide a more comprehensive insight into the state of biodiversity of the Sava River as well as to take appropriate measures of protection and conservation. In this case, the conservation of the allocated "macrohabitats" should ensure long-term sustainability of the river's entire ecosystem.

The proposal of particularly important "macrohabitats" of the Sava River, which is the result of our research, has been compared to previous studies which have been undertaken within the ecosystem of the Sava River and its basin, concerning the conservation of species and/or ecosystem diversity of coastal wetlands. These researches are a part of the project called Protection of Biodiversity of the Sava River Basin Floodplains (IUCN and partners). The ultimate goal of the project is to observe the mutual importance of conservation of the river's ecosystem and the protected areas of the Sava River basin within the completion of Pan-European Ecological Network (PEEN).

2 Environmental Conditions

Structural and functional characteristics of ecosystems are determined by the synergistic action of climate, geological substrate, soil, topography, different types of biotic interactions, and anthropogenic influence.

2.1 Topography

The Sava River catchment belongs to three different regions: Alpine region (the Alps and the Dinarides), mountainous region, and lowland region (peri-Pannonian).

High mountain ranges (the Alps, the Dinaric Mountains) have been formed by complex orogenic processes. Neotectonic processes (subsidence of Pannonian region and uplifting of mountainous regions) are lasting from the beginning of the Upper Miocene to the present day [9]. Besides the tectonic processes, the geomorphic evolution of the investigated area involved topography reshaping by rivers, glaciers, and karst processes. These exogenic morphostructural processes formed glacial landforms (glacial valleys, cirques, moraines), fluvial landforms (alluvial plains, loess-covered stream terraces), fluvial-denudation landforms (gorges and canyons), and karst landforms (caves, pits, erosed karst, troughs).

High mountains (the Alps and the Dinarides) dominate in the upper part of the basin which belongs to Slovenia, where the highest peak is Triglav (2,864 m a.s.l.).

The mountainous region is represented by large valleys and a karst plain. The upper drainage area of the plain (the "Notranjski Karst") collects water from the Ljubljanica River, which in the tributary region forms a large moorland ("Ljubljansko barje").

In the middle section of the Sava River, there is a remarkable distinction in landscape of the northern part (the left bank) and southern part (the right bank) of the basin. The left bank extends to the large Pannonian plain area and low hilly Slavonian regions. The right bank extends to the Dinaric Mountains in Croatia and Bosnia.

The elevation of the Sava River basin varies between approx. 71 m a.s.l. at the mouth of the Sava River in Belgrade and 2,864 m a.s.l. (Triglav, Slovenian Alps). Mean elevation of the basin is 545 m a.s.l. The dominant slope in the basin is moderately steep. Mean value of slope in the Sava River basin is 15.8 % [10].

2.2 Climate

Due to diverse orography, the climate of the Sava River catchment varies from alpine to moderate. Orography is the most significant factor that modifies climatic modifications in the Sava River catchment. Strong altitude gradient affects air temperature, precipitation, and evapotranspiration. Temperature decreases with increasing altitude. Simultaneously, precipitation and humidity increase with increasing altitude.

According to Köppen's classification of climate zones, the Sava River catchment belongs to three climate types: two microthermal climate types (i.e., the boreal "Ds" climate of high mountains and tundra "ET" climate of Alpine belt) and the moderate climate (Cfb).

Alpine (**E**) climate is characterized by average temperatures below 10 °C in all 12 months of the year. The warmest month has an average temperature between 0 and 10 °C.

Boreal (**D**) climates have an average temperature above 10 °C in their warmest months and an average temperature below −3 °C in their coldest month.

Moderate (**C**) climate of the northern hemisphere prevails within the Sava River catchment. This type of climate is characterized by an average temperature of the warmest months (April to September) which is higher than 10 °C, while the average temperature of the coldest months ranges from −3 to 10 °C. The amount of rainfall throughout the year is consistent and does not have dry season. The warmest month has a temperature lower than 22 °C, but the average temperature of the hottest 4 months is higher than 10 °C.

Köppen's climate classification system is based on annual averages of temperature and precipitation. Therefore, it is appropriate for global-scale analyses only.

A much detailed picture of climatic conditions can be observed using the thermo-pluviometric regime, which indicates the seasonal variability of climate conditions. The thermo-pluviometric regime can be described using different types of climate diagrams [11–13]. Using the "Flora" package [14, 15], we created Walter's climate diagrams for Ljubljana, Zagreb, and Belgrade. Long-term climate data, based on a 50-year period (1950–2000), were collected from WorldClim database [16].

The temperature conditions in all three regions are similar (mean annual temperatures for Ljubljana, Zagreb, and Belgrade are 10.4, 11.3, and 11.6 °C, respectively). A low temperature gradient from Ljubljana to Belgrade is caused by topography. Mean altitudes of Ljubljana, Zagreb, and Belgrade are 298, 123, and 116 m a. s. l, respectively.

The precipitation gradient from west to east is more apparent. Mean annual precipitations of Ljubljana, Zagreb, and Belgrade are 1,140, 883.3, and 656.6 l/m^2, respectively (Fig. 1).

The large-scale floodplain area involves the middle and lower sections of the Sava River and smaller waters flowing parallel to the Sava River (Fig. 2). Due to specific topography (wide lowland), the left tributaries of the Sava River (Krapina, Česma, Lonja, Pakra, Orljava, Bosut) are prone to floods. Except the Kupa River, the right tributaries of the Sava River flow through much smaller floodplains.

Other floodplains are located in the estuaries of Una, Vrbas, Bosna, and Drina Rivers. The main causes of reduction of wetland areas have been the expansion of agriculture uses and river engineering works mainly for flood control. In the large plains of the lower-middle and lower Sava, extensive flood protection systems and drainage networks were built up and have caused the loss of wetlands.

2.3 Soil

Due to diverse geological substrate, high variability of climate conditions, different vegetation cover, topography, and human influence, the soil along the Sava River is complex and very heterogeneous [17–28].

Lithological substratum within the Sava River catchment involves diverse magmatic (igneous), sedimentary, and metamorphic rocks. The most important igneous rocks are granite, diabase, dacite, andesite, feldspars, and peridotites. The sedimentary formations involve limestone, dolomites, and clastic sedimentary rocks (conglomerate, breccia, sandstone, shale, marl). Pleistocene and Holocene sediments cover the floors and edges of the valleys (moraines, gravelly outwash terraces in larger basins, silty-clayey sediment in smaller valleys). The metamorphic formations are represented by slate, phyllite, schist, gneiss, marble, chert, hornfels, quartzite, and other rocks.

Such diverse climate and lithological conditions caused the development of complex soil formations [19, 21, 22, 25]. The accumulation of organic matter, the

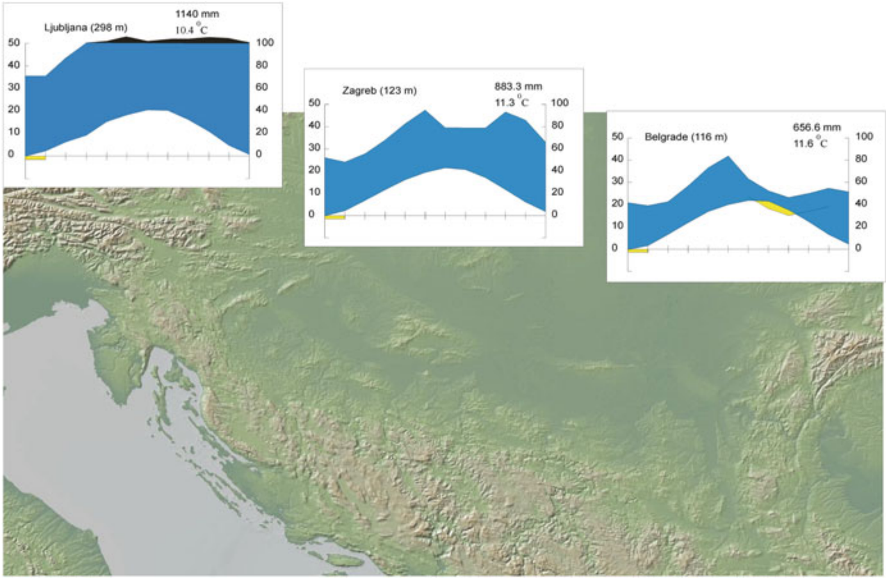

Fig. 1 Climate of Ljubljana, Zagreb, and Belgrade

Fig. 2 Floodplains along the Sava River

weathering of parent material, and leaching with clay and/or sesquioxide translocation are the main pedogenetic processes that affected the diversification of soil.

Undeveloped soils are represented by a group of **lithosols** that are frequent in canyons and gorges.

Humus-accumulative soils have the structure A-C, where A denotes the humus-accumulative horizon in which the humus is associated with mineral particles, while C indicates the parent lithological substrate that is slightly altered by pedogenetic processes. This group of soils includes **rendzina** (*rendzic leptosols*) on limestone and dolomite at Alpine and mountainous regions of the Sava River catchment. The dominating soil in the lower section of the Sava River, outside the flooding area, is chernozem on loess, a variant of *humus-accumulative* soils.

Cambic soils are characterized by A-(B)-C structure, where (B) indicates the argilogenesis horizon with the accumulation of in situ formed clay and high base saturation. This group of colluvial soils developed along the mountainous region of the Sava River, on less steep slopes, on the bottom of dolinas, or at the footslopes.

Strong weathering and eluviations of clay minerals are the main processes involved in the formation of **luvisols**. The results of these processes are soils with profile A-Ae-Bt-C, where Ae denotes a gray upper horizon of eluviations and Bt denotes the illuvial horizon of clay (and partially sesquioxides) enrichment. Luvisols developed within mountainous forest zone.

Fluvisols (hygromorphic *alluvial soils*), **gley**, and **semigley** (meadow dark soils) developed on alluvial sediments, sandbanks, and fluvial-denudation formations within the flooding region of the Sava River. These soils occur in conditions of periodical or permanent excessive wetting and flooding. When waterlogged for long periods, soils became anaerobic and rich in ferric iron. Bacterial and chemical actions reduce iron and manganese to ferrous and manganese to ferrous and manganous forms which are soluble and mobile. Seasonal drying causes reoxidation and deposition of yellow rusty spots of ferric iron and black manganese streaks. Pedogenetic processes of hygromorphic soils are not well developed because of the youth of the deposits or because sedimentation prevails over pedogenesis.

Other types of soil are sporadically developed (*peat soil* in Alpine region and halomorphic soils in the Pannonian plain).

Halomorphic soils are found in (semi)arid, continental zones, in conditions when evapotranspiration significantly exceeds precipitation. In such conditions, the dissolved salts migrate with transpirated water upwards. Salt-enriched horizons are characterized by the high Na to Ca ratio. Although the dry season along the Sava River is missing, the semiarid period occurs during the summer months (July to October). Such climate, with extreme temperatures and aridity in summer, determined the development of specific steppe and halophytic vegetation. High evaporation of groundwater during summer causes the enrichment of salt in the soil and development of specific halomorphic soil types.

3 Material and Methods

The indicative ecological status has been assessed based on the data provided in the Sava River Basin Management Plan [29] and recent investigation in the period 2011–2012. All together 189 water bodies were taken into consideration (national registers of water bodies up to 2009). The confidence level of the assessment was provided in the SRBMP [29].

In respect to the recent investigation, the degree of pollution and the ecological status of the Sava River have been estimated according to the structure of the macroinvertebrate communities. The choice of macroinvertebrates for the assessment of the status is justified, primarily because macroinvertebrate communities have been most thoroughly studied in the course of the bilateral project between Serbia and Croatia during 2011–2012. For the analysis of macroinvertebrate community, AQEM database and software package "Asterix 3.1.1" have been used [30].

Out of numerous biological methods, the package that contains the following has been used in the assessment of the ecological status of the Sava River: saprobic index according to Zelinka and Marvan [31], BMWP and ASPT [32], BBI [33], and diversity index according to Washington [34].

Besides the aforementioned methods, which are widely used throughout most of Europe, for the assessment of water quality and ecological status, the so-called Balkan Biotic Index (BNBI) [7], conceived for the running water ecosystems of the Balkan Peninsula area, was also used. Even though the index has been routinely used for a while now, this was the first time it was used on such a large river like the Sava. The basic matrix of the BNBI is shown in Table 1.

Based on a comparative analysis of the obtained results of water pollution, the ecological status of the Sava River has been defined by the researched profiles, according to the European Union Water Framework Directive scale, as excellent, good, moderate, low, and bad [3].

The degree of conservation of biodiversity of the Sava River and its basin has been reviewed by parameters whose values are used for the global assessment of the state of biodiversity of the researched habitats and/or ecosystems of tributaries within the Sava River basin. In that respect, priority has been given to the assessment of biodiversity conservation of the main watercourses of the Sava River basin.

The following parameters have been used:

1. Species richness—the total number of species (taxa) within the researched area of the Sava River ecosystem
2. Representation of taxa—representation index by relative scale: 0, absent; 1, rare; 2, common; 3, very common; and 4, heavily represented
3. Rareness of taxa—index of conservation importance $= \sum 100 n_i / N_i$ (n_i, numbers of taxa units within a part of the habitat ecosystem; N_i, number of units of that taxa on a wider area (ecosystem, basin)). Index of habitat (locality) importance $= \sum k_i / a_i$ (k_i, total number of localities in the area (ecosystem, basin); a_i, total number of localities inhabited by the taxon)
4. Habitat preservation status—index of conservation status $= \sum S_i$ (S_i, species conservation score, according to IUCN or some other categorization)

Table 1 Matrix of Balkan Biotic Index (BNBI)

H' genus	H' group	Animal group	Taxa: genus (g), families (f), subfamilies (sub.f), dominant ($d > 10$ %), and/or subdominant ($d = 5$–10 %)	Score, Class	Pollution category
>3	>2	Plecoptera Ephemeroptera Trichoptera f. Chironomidae Platyhelminthes Diptera (Ostale)	g. *Protonemura* g. *Baetis*; *B. alpinus*—group sub.f. Drusinae; g. *Drusus*; sub.f. Hyporhyacophila sub.f. Orthocladiinae; g. *Diamesa* *Crenobia alpina* and *Planaria montenegrina* g. *Liponeura*; sub.f. Prosimulinae g. *Prosimulium*	Point (P)5, I	Very clean waters BNBI 4.6–5 Color white
2.5–3	1.5–2	Plecoptera Ephemeroptera Trichoptera f. Chironomidae Orthocladiinae Diptera (others) Coleoptera Mollusca Oligochaeta Amphipoda	g. *Perla*, *Leuctra*, *Isoperla*, *Nemoura*, and others g. *Ecdyonurus*, g. *Epeorus*, g *Rhithrogena*, g. *Baetis*; *B. rhodani*, *B. alpinus*, *B. lutheri*—group with a case (g. *Micrasema*, *Silo*, *Sericostoma*) g. *Rhyacophila* sub. f. Orthocladiinae (g. *Orthocladius*, *Eukiefferiella*); sub. f. Corynoneurinae, g. *Tanytarsus* (*Tanytarsini*) g. *Satchelliella*, g. *Atherix*; sub.f. Simuliidae exc. g. *Similium* f. Elmidae (g. *Elmis*, *Limius*, *Riolus*); g. *Hydraena* g. *Ancylus* g. *Eiseniella*, g. *Lumbriculus*, f. Enchytraeidae Gammarus (4 points)	4, Ia	Clean waters under natural condition BNBI 3.6–4.5 Color blue
			g. *Ecdyonurus*, *Oligoneuriella*, *Oligoplectrum*, *Hydropsyche*,	3.5	

(continued)

Table 1 (continued)

H' genus	H' group	Animal group	Taxa: genus (g), families (f), subfamilies (sub.f), dominant ($d > 10$ %), and/or subdominant ($d = 5–10$ %)	Score, Class	Pollution category
			Dugesia gonocephala, *Gammarus*		
1.5–2.49	1–1.49	Ephemeroptera Trichoptera f. Chironomidae Diptera (Ostale) Heteroptera Amphipoda Mollusca Oligochaeta Odonata	g. *Ephemerella*, *Ephemera*, *Heptagenia*, *Caenis*, *Cloeon*, *Baetis*; *B. bioculatus*, *B. vernus*—group and others g. *Hydropsyche* and with a case g. *Mystacides*, *Anabolia*, *Hydroptila*, *Limnephilus* sub.f. Chironomidae exc. Tanytarsini; sub.f. Tanypodini g. *Simulium* g. *Aphelocheirus* g. *Gammarus* (3 boda), Dicerogammarus, Corophium g. *Lymnaea*, *Viviparus*, *Theodoxus*, *Bithynia*, *Lithoglyphus* g. *Stilodrilus*, *Psammoryctes*; f. Naididae exc. g. *Nais* g. *Gomphus*, *Onychogomphus*	3, II	Moderately polluted BNBI 2.6–3.5 Color green
1–1.49	0.5–1	Ephemeroptera Trichoptera f. Chironomidae Chironomidae Megaloptera Isopoda Mollusca Oligochaeta Hirudinea	g. *Cloeon*, *Caenis*—individual unit g. *Hydropsyche*—individual unit g. *Polypedilum*, *Trissocladius* (*Cricotopus*), *Psectrocladius*, *Macropelopia*, *Prodiamesa*, *Chironomus* g. *Sialis* g. *Asellus* g. *Physa*, *Planorbis* (*P. planorbis*) f. Tubificidae (g. *Tubifex*, *Limnodrilus*,	2, III	Heavily polluted BNBI 1.6–2.5 Color yellow

(continued)

Table 1 (continued)

H’ genus	H’ group	Animal group	Taxa: genus (g), families (f), subfamilies (sub.f), dominant ($d > 10$ %), and/or subdominant ($d = 5–10$ %)	Score, Class	Pollution category
			Potamotrix); g. *Nais* g. *Erpobdella*		
<1	<0.5	f. Chironomidae Oligochaeta Diptera (others) No dominant and diversity group	*Chironomus* gr. *thummi* very abundant population min. 100 ind. In the sample *Tubifex tubifex*, g. *Limnodrilus*—severe organic pollution ------------------------- g. *Eristalis*, *Psychoda* (toksično—organsko zagađenje) Present 1–2 individuals in the sample (silt-inert, acut toksik poll) No macroinvertebrates found	1, IV	Very heavily polluted BNBI 0–1.5 Color red
Biotope status			Water quality		
Column 1	Column 2	Column 3	Column 4		
P1	P2	P3	P4; P4 = Σ of points min 3 taxa (dominant and/or subdom/ΣP)		

1. Underlined taxa, with maximal diversity; bold taxa, the most dominant group; bold and underlined taxa, taxa dominant and with maximal diversity
2. Genus is counting with 4 points if following groups from Ia class are dominant
3. Taxa is counting with 3.5 points
4. Genus is counting with 3 points if following groups are dominant

The analysis of biodiversity of the Sava River and its basin by the selected parameters aimed to identify the areas of the Sava River ecosystem characterized by a biodiversity especially significant for the conservation of the entire ecosystem, which were therefore labeled as important “macrohabitats.”

The assessment of fish fauna diversity was done based on the literature review.

With the purpose of reviewing the measures for long-term conservation of the Sava River biodiversity, there was a comparison of the position and biodiversity of the allocated “macrohabitats” within the Sava River and the areas included in Pan-European Ecological Network, including the surrounding wetlands along the Sava River flow, which were defined by the project Protection of Biodiversity of the Sava River Basin Floodplains (IUCN and partners).

The review of water-related protected areas was done based on the data used for the preparation of the SRBMP [29]. The discussion includes areas larger than 100 ha, since the unified register does not comprise smaller areas [29].

Based on all the results, the conservation of the Sava River biodiversity has been discussed.

4 Ecological Status of the Sava River

For the indicative assessment of the ecological status within the Sava River basin, all together 189 water bodies identified on national level were taken into consideration. Out of 189 water bodies, the ecological status for 183 water bodies has been assessed based on the SRBMP [29] data. High ecological status has been achieved only in 10 water bodies, while good ecological status was assessed at 65 water bodies. The majority of water bodies (70) have been in moderate status. Poor status was found at 17 water bodies, while no water bodies were in bad status. Ecological potential was assessed at 20 heavily modified water bodies (HMWB) (or candidates) on the Sava, Vrbas, Bosut, Drina, Lim, and Kolubara Rivers. In 17 HMWB, a good ecological potential has been identified, while in three HMWB, a moderate ecological potential has been identified.

It should be mentioned that assessment of ecological status and ecological potential has been done with low and medium confidence [29]. Assessment of the ecological status has been provided the following shows:

- High ecological status—with low confidence (93.75 %) and with medium confidence (6.25 %)
- Good ecological status—with medium confidence (20.29 %) and with low confidence (79.71 %)
- Moderate ecological status—with medium confidence (31.25 %) and with low confidence (68.85 %)
- Poor ecological status—with low confidence (89.47 %) and with medium confidence (10.53 %)

The results of the analysis of the Sava River pollution assessment based on the community of macroinvertebrates (2011–2012 survey) are shown in Tables 2, 3, and 4. The assessment was done separately for natural and artificial habitats, serving as coastal defense. Natural substrate can be made of different fractions, starting from fine sludge and sand to large rocks. On the other hand, artificial base is always made of large broken stones or rocks. Based on the presented results of the structure of macroinvertebrate community and the results of all the biological methods used for the assessment of water quality and ecological status of the Sava River, the following are shown:

1. Belgian Biotic Index (BBI) shows the largest discrepancies with the detected degree of water pollution. Knowing that this index was conceived for a narrow geographic area and rivers of rhithron type makes the result an expected one, confirming the fact that it is impossible to conceive a universal biotic index.
2. All other biological methods, as well as the used diversity indices, show that the quality of the Sava River can be divided into three zones. The first zone is made from parts of the upper flow through Slovenia, where all indicators sow the best water quality, while the ecological status can be marked as "good." The researched localities of the Sava River through Slovenia are not in the zone of upper rhithron (component rivers Sava Bohinjka and Sava Dolinka), but in the already-formed flow of the upper stream of the Sava River. However, the Sava River is obviously under an anthropogenic influence in that part, which can be described as moderate or within the limits of betamesosaprobic class waters.
3. The middle parts of the Sava River, which flow through the flat part of Slovenia, Bosnia and Herzegovina, and Croatia, have a somewhat lower ecological status, especially in sections downstream from major cities (primarily Zagreb and Slavonski Brod). Major right tributaries, such as Kupa, Una, Vrbas, and Bosna, coming from the Dinaric massif, surely have a significant impact on the ecological status and water quality in this part of the flow. By all indicators, water quality on the upper limit of the second class or betamesosaprobic class is observed in this part of the flow.
4. The third zone is made from part of the flow through Serbia, where, by all indicators, the biggest change of ecological status and water quality is observed, ranging within the limits of the second and third classes or beta- to alfamesosaprobic waters. Such condition is a consequence of anthropogenic influences from the entire upper and middle flows of the Sava River, on one hand, and hydrological characteristic of lower flow of the Sava River, on the other. Those characteristics include reduced water speed, greater width of the riverbed, increased deposition of sludge, greater amount of nutrients, and increased trophic. All these facts affect the change of structure in the community of macroinvertebrates and greater dominance of tolerant taxa which prefer a muddy base and, in average, a smaller amount of dissolved oxygen in water. The ecological status of this part of the Sava River is characterized as a transition from good to moderate.

Table 2 Qualitative-quantitative structure of benthic fauna in the Sava River and saprobic, biotic, and diversity indices: natural substrates

Metric	JP 1–10	MVp1–10	Kp1–10	Op1–10	SŠ1–10	UB1–10	SM1–10	Š1–10	OSp1–10
Saprobic index (Zelinka and Marvan)	2.526	2.239	2.244	2.347	1.944	2.282	3.043	2.807	3.015
Class water (Zelinka and Marvan)	II	II	II	II	II	II	III	III	III
Average score per taxon	5.059	5.818	3.6	5.048	5.444	3.556	4.125	4.833	4.6
BMWP score	86	128	18	106	98	32	66	58	23
BBI	8	10	4	7	9	5	5	7	4
Balkan Biotic Index (BNBI)	3.00	2.83	2.30	2.80	3.00	1.90	2.40	2.57	2.75
Class water (BNBI)	II	II	II	II	II	III	III	II–III	II
Diversity (Shannon-Wiener index)	1.987	1.698	0.996	3.067	2.02	0.92	2.066	2.13	1.892
Diversity (Margalef Index)	6.001	7.142	1.957	8.361	5.088	3.413	5.456	5.673	3.584
Evenness index	0.522	0.424	0.388	0.765	0.559	0.273	0.556	0.563	0.621

Table 3 Qualitative-quantitative structure of benthic fauna in the Sava River and saprobic, biotic, and diversity indices: artificial substrates

Metric	Ju1–10	MVu1–10	Ku1–10	Oru1–10	SŠu1–10	UBu1–10	Smu1–10	Šu1–10	Osu1–10
Saprobic index (Zelinka and Marvan)	2.379	2.424	2.15	2.147	1.832	2.455	2.579	2.991	2.44
Class water (Zelinka and Marvan)	II	II	II	II	II	II	II–III	III	II
Average score per taxon	5.72	5.217	5.5	5.368	5.333	5	5.625	4.583	5.222
BMWP Score	143	120	99	102	96	45	45	55	47
BBI	8	8	5	5	7	5	5	5	5
Balkan Biotic Index (BNBI)	3.14	2.6	2.4	2.55	2.71	2.4	2.6	2.55	2.55
Class water(BNBI)	II	II	III	II–III	II	III	II	II–III	II–III
Diversity (Shannon-Wiener index)	2.803	2.957	1.308	1.814	1.112	1.965	2.018	2.499	1.73
Diversity (Margalef Index)	6.84	9.598	5.45	5.834	5.034	5.202	5.007	5.757	3.115
Evenness index	0.7	0.685	0.338	0.459	0.292	0.536	0.568	0.673	0.588

Table 4 Saprobic, biotic, and diversity indices: Natural and artificial substrates, total

	JP-JU	MVp-Mvu	Kp-Ku	Op-Oru	SŠ-Sšu	UB-Ubu	Sm-Smu	Š-Šu	Osp-Osu
Saprobic index (Zelinka and Marvan)	2.45	2.33	2.19	2.24	1.88	2.37	2.81	2.89	2.72
Class water (Zelinka and Marvan)	II	II	II	II	II	II	III	III	III
Average score per taxon	5.38	5.52	4.55	5.21	5.38	4.27	4.87	4.71	4.91
BMWP Score	114	124	58.5	104	97	38.5	55.5	56.5	35
BBI	8	8	5	6	8	5	5	6	5
Balkan Biotic Index (BNBI)	3.07	2.71	2.35	2.67	2.85	2.15	2.50	2.56	2.65
Class water (BNBI)	II	II	II	II	II	III	II–III	II–III	II
Diversity (Shannon–Wiener index)	2.39	2.32	1.15	2.44	1.56	1.44	2.04	2.26	1.81
Diversity (Margalef Index)	6.42	8.37	3.7	7.09	5.06	4.3	5.23	5.71	3.35
Evenness index	0.6	0.55	0.38	0.61	0.42	0.4	0.56	0.62	0.6

5. The ecological status and water quality of the Sava River obtained from the structure of the community of macroinvertebrates on natural and artificial bases show no major differences, except when it comes to indices of diversity, where significantly higher values are observed on rocky bases, as opposed to natural bases. This occurrence is probably the consequence of greater heterogeneity of rocky coastal defense habitats, which gradually acquired the characteristics of natural rocky bases with a large number of present microhabitats.

5 Biodiversity of the Sava River Ecosystem

A comparative review of the global species biodiversity of the Sava River and other European rivers is shown in Table 5. The table shows the total number of groups of organisms for which there are sufficient data, based on these studies and the data from literature [35, 36].

The results from the table indicate that the global biodiversity of the Sava River is similar to the biodiversity of other big European rivers, in terms of the number of species. Based on the presented analysis, the causes of differences in the number of species in certain rivers cannot be identified with high confidence. However, we do think that the insufficient and/or unequal examination is one of the important causes of the state shown.

For the assessment of biodiversity of the Sava River ecosystem, its main tributaries, and flood zones, Tables 6, 7, 8, 9, and 10 and Figs. 3, 4, and 5 show the characteristics of biodiversity of macroinvertebrates and fish (as the best-studied groups), according to the chosen parameters (total richness of taxa, representation of taxa, rareness of taxa, the importance and conservation of habitats compared to the presence of protected species).

The total biodiversity of indigenous fish of the Sava River according to the number of species is relatively uniform along the flow of the river (Table 9). A greater presence of allochthonous species is noticed in the part of the flow through SRB, CRO, and BIH, as opposed to the upper flow, or the part of the flow through Slovenia (Table 9, Fig. 6).

By qualitative characteristics of biodiversity, the fish community of the Sava River is different and can be divided into three zones. The upper flow is characterized by a larger presence and diversity of salmonids, such as brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), and hucho trout (*Hucho hucho*). The middle flow, which mostly flows through the territories of CRO and BIH, is characterized by diverse and dominant presence of rheophilic species, such as river barbel (*Barbus barbus*), common nase (*Chondrostoma nasus*), and European chub (*Leuciscus cephalus*), as well as the presence of potamonic species like the representatives of bream (*Abramis* spp.), carp (*Cyprinus carpio*), pike (*Esox lucius*), and catfish (*Silurus glanis*). The lower flow of the Sava River has a potamonic character dominated by bream, carp, and catfish, but also with the most common presence of sterlet (*Acipenser ruthenus*). Other than that, the lower flow is

Table 5 A comparative review of the global species biodiversity of the Sava River and other European rivers [35, 36]

Group/rivers	Sava	Dunav	Rajna	Kama	Oka	Elba	Vistula
Vascular plants		49		93			
Algae			455	458	380		
Bacillariophyta		68	194	235			162
Chlorophyta		22	72	131	188		80
Cyanophyta		10	72	65	38		41
Chrysophyta				9			8
Dinophyta				9			
Euglenophyta		2		8			
Volvocales				5			
Rhodophyta				1			
Zooplankton				186	60		128
Rotifera							85
Cladocera							22
Copepoda							21
Macrozoobenthos		268		296		≈600	600
Spongia		1		1		1	
Coelenterata				1			
Nematoda	1			67			
Turbellaria	4						
Oligochaeta	15	20	37	25			64
Hirudinea	6	4		6			
Hydracarina	1						

(continued)

Table 5 (continued)

Group/rivers	Sava	Dunav	Rajna	Kama	Oka	Elba	Vistula
Mollusca	15 Gastropoda 7 Bivalvia	30 Gastropoda 20 Bivalvia	33	20			50
Ostracoda				15			
Isopoda	1	1		1			
Amphipoda	5	5		6			
Mysidacea	1	2		1			
Crustacea: Decapoda		2	23	1			
Plecoptera				4			
Ephemeroptera	16	27	49	28			50
Planipennia	1						
Trichoptera	27	42	79	17			57
Heteroptera	2			2			
Odonata	9	7		1			
Coleoptera	13	22					
Hydracarina				10			
Bryozoa	1	1		1			
Diptera	50 including Chironomidae		105	89			
Diptera: Chironomidae		27	95	84			152
Fish	74		High Rhine, 36, Upper Rhine, 56, Middle Rhine, 40, Lower Rhine, 37	32	39	94	55
Amphibians		27					
Reptiles		37					
Birds		330					320

Table 6 Total number of macroinvertebrate species per location

Sites	Jarun (CRO)	Martinska Ves (CRO)	Krapje (CRO)	Orubica (CRO)	Slavonski Šamac (CRO)	Ušće Bosuta (SRB)	Sremska Mitrovica (SRB)	Šabac (SRB)	Ostružnica (SRB)	Total
Turbellaria	2	1	2	2	1	–	–	–	–	4
Gastropoda	6	6	7	10	12	6	11	11	3	19
Bivalvia	2	5	3	2	3	2	4	3	6	7
Oligochaeta	3	7	9	10	6	11	10	8	5	18
Hirudinea	1	3	3	1	1	2	–	–	–	6
Isopoda	1	–	–	1	–	–	1	–	–	1
Amphipoda	1	3	3	3	1	1	3	–	–	3
Ephemeroptera	2	11	11	4	4	–	1	–	–	11
Odonata	3	2	2	5	5	3	2	7	2	9
Heteroptera	2	1	1	–	–	–	–	–	–	2
Trichoptera	16	15	15	6	7	1	1	1	2	21
Coleoptera	6	8	8	3	5	1	1	1	–	11
Diptera	16	31	31	26	11	26	23	25	16	38

Table 7 Characteristics of biodiversity of macroinvertebrates of the Sava River

Sites/group		Turbellaria	Gastropoda	Bivalvia	Oligochaeta	Hirudinea	Isopoda	Amphipoda	Ephemeroptera	Odonata	Heteroptera	Trichoptera	Coleoptera	Diptera
Jarun (CRO)	Natural	0	3	2	2	0	1	1	1	2	1	11	2	9
	Artificial	2	6	1	2	1	0	0	2	2	1	14	5	10
	Total	2	6	2	3	1	1	1	2	3	2	16	6	16
	Rarity	0.25	0.08	0.06	0.04	0.11	0.33	0.07	0.09	0.09	0.5	0.3	0.24	0.08
Martinska Ves (CRO)	Natural	0	3	4	3	2	–	3	10	1	1	7	2	13
	Artificial	1	4	4	8	3	–	0	3	2	1	10	6	26
	Total	1	6	5	7	3	–	3	11	2	1	15	8	31
	Rarity	0.12	0.08	0.16	0.1	0.33		0.21	0.5	0.06	0.25	0.27	0.32	0.16
Krapje (CRO)	Natural	0	1	3	3	0	–	1	–	0	0	0	–	4
	Artificial	2	7	1	7	1	–	1	–	3	1	5	–	13
	Total	2	7	3	9	1	–	2	–	3	1	5	–	17
	Rarity	0.25	0.09	0.1	0.13	0.11		0.14		0.09	0.25	0.09		0.08
Orubica (CRO)	Natural	1	6	2	8	1	1	3	4	4	–	1	0	15
	Artificial	1	8	1	7	0	0	1	0	3	–	5	3	16
	Total	2	10	2	10	1	1	3	4	5	–	6	3	26
	Rarity	0.25	0.13	0.06	0.14	0.11	0.33	0.21	0.18	0.15		1.2	0.12	0.13
Slavonski Šamac (CRO)	Natural	0	9	3	5	0	–	0	4	3	–	4	2	6
	Artificial	1	7	3	5	1	–	1	0	3	–	7	3	8
	Total	1	12	3	6	1	–	1	4	5	–	7	5	11
	Rarity	0.12	0.16	0.1	0.08	0.11		0.07	0.18	0.15		0.12	0.2	0.05
Ušće Bosuta (SRB)	Natural	–	6	2	6	1	–	0	–	1	–	0	0	13
	Artificial	–	0	1	7	1	–	1	–	3	–	1	1	17
	Total	–	6	2	11	2	–	1	–	3	–	1	1	26
	Rarity		0.08	0.06	0.15	0.22		0.07		0.09		0.02	0.04	0.13
Sremska Mitrovica (SRB)	Natural	–	9	3	6	–	1	3	1	2	–	0	1	13
	Artificial	–	2	2	9	–	0	1	0	1	–	1	0	13
	Total	–	11	4	10	–	1	3	1	2	–	1	1	23
	Rarity		0.15	0.13	0.14		0.33	0.21	0.04	0.06		0.02	0.04	0.12

Šabac (SRB)	Natural	–	9	3	7	–	–	–	–	6	–	1	1	15
	Artificial	–	9	3	6	–	–	–	–	2	–	1	0	16
	Total	–	11	3	8	–	–	–	–	7	–	1	1	25
	Rarity		0.15	0.1	0.11					0.21		0.02	0.04	0.13
Ostružnica (SRB)	Natural	–	1	5	2	–	–	–	–	0	–	0	–	12
	Artificial	–	3	1	5	–	–	–	–	2	–	2	–	5
	Total	–	3	6	5	–	–	–	–	2	–	2	–	16
	Rarity		0.04	0.2	0.07					0.06		0.03		0.08
	Frequency %	55	100	100	100	66	33	77	55	100	33	100	77	100
	Rarity mean	0.19	0.10	0.10	0.10	0.16	0.33	0.17	0.19	0.10	0.33	0.23	0.14	0.10

Table 8 The importance of habitats of the Sava River by the characteristics of biodiversity of macroinvertebrates

Sites	Jarun (CRO)	Martinska Ves (CRO)	Krapje (CRO)	Orubica (CRO)	Slavonski Šamac (CRO)	Ušće Bosuta (SRB)	Sremska Mitrovica (SRB)	Šabac (SRB)	Ostružnica (SRB)
Index significance of habitat	1.4	1.3	1.1	1.3	1.2	1	1.1	0.7	0.6

Table 9 Species diversity of fish along the Sava River

	Number of species per family			
Fam/state	SLO	CRO-BIH		SRB
Petromyzontidae	1	1		2
Acipenseridae		1		1
Anguillidae		+		+
Clupeidae				+
Salmonidae	3 + 3[a]			
Thymalidae	1			
Esocidae	1	1	1	1
Umbridae		1[b]	1[b]	1[b]
Cyprinidae	26 + 3[a]	27 + 2[a]		28 + 4[a]
Balitoridae	1			
Cobitidae	4	5		2
Siluridae	1	1	1	1
Ictaluridae			2[a]	2[a]
Gadidae	1	1		
Gasterosteidae				+
Syngnathidae				+[a]
Percidae	5	7		8
Centrarchidae	1[a]	1[a]	1[a]	1[a]
Cottidae	1	1		
Gobiidae		3[a]	3[a]	4[a]
Total	45 + 6	46 + 6		46 + 13

[a]Nonnative species
[b]Habitats outside the main river course

characterized by the most diverse and the largest presence of allochthonous species of fish, such as Prussian carp (*Carassius gibelio*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), brown bullhead (*Ictalurus nebulosus*), and gobies (*Neogobius* spp.).

The number of species of macroinvertebrates and fish that are on the global and national endangered species lists when compared with the total number of species is different along the flow of the Sava River in different countries (Table 10, Fig. 5).

Croatia is the only country to have the red book of fish [40]. This document protects the largest number of fish species that inhabit the very flow of the Sava River, such as *Acipenser ruthenus*, *Salmo trutta*, *Hucho hucho*, *Umbra krameri*, *Carassius carassius*, *Leuciscus souffia*, *Leuciscus leuciscus*, *Idus idus*, *Chalcalburnus chalcoides*, *Cobitis elongata*, *Misgurnus fossilis*, *Gymnocephalus baloni*, *Gymnocephalus schroetzer*, *Zingel zingel*, and *Zingel streber* [40]. Unlike Croatia, the remaining countries in the basin of the Sava River do not have the red book of fish and other groups of aquatic organisms, so the endangerment of fish and other aquatic organisms can be found in other documents, such as the national legal documents and endangered species of plants and animals (Serbia, BIH, Slovenia) or specialized databases [41] and written books on fish [38]. Serbia is the only country

Table 10 The importance and conservation of habitats of the Sava River ecosystem according to the presence of endangered species of macroinvertebrates and fish

Taxon name	IUCN Red List [37] (global)	Bern Convention (1979) annexes	Habitats Directive annexes	IUCN Red List Croatia	IUCN Red List Serbia (BAES)	Official Gazette of the Republic of Serbia, No.5/10	Findings by country
Petromyzontidae							
Eudontomyzon danfordi	LC		II	NT	DD[a]		SRB[b], BIH[b], CRO[c]
Eudontomyzon mariae	LC	III	II	NT	LC[a]		SRB[b], CRO[b], SLO[b,c]
Acipenseridae							
Huso huso	CR A2bcd	III	II, V		EN A2cd[d]	+	SRB[e]
Acipenser ruthenus	VU A2cde		V	VU	VU[d]/LC[a]		SRB[b,c], BIH[b], CRO[c]
Acipenser nudiventris	CR A2cde	III	V		EN[a]	+	SRB[e]
Acipenser gueldenstaedtii	CR A2bcde		V			+	SRB[e]
Acipenser stellatus	CR A2cde	III	V			+	SRB[e]
Anguillidae							
Anguilla anguilla	CR A2bd +4bd					+	
Clupeidae							
Alosa caspia	LC		II,V		DD[a]		SRB[e]
Alosa immaculata	VU B2ab(v)		II,V			+	SRB[e]
Salmonidae							
Salmo trutta	LC			VU	VU		SRB[b] BIH[b] CRO[b], SLO[b,c]

Oncorhynchus mykiss[f]							BIH[b], CRO[b], SLO[b,c] SRB[b]
Salvelinus alpinus[f]	LC						SLO[c] SRB[b]
Salvelinus fontinalis[f]							SLO[b,c] SRB[b]
Hucho hucho	EN B2ab(ii, iii)	II, III	II	EN	EN A4dB1[d]/ VU[a]		SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Thymalidae							
Thymallus thymallus	LC	III	V		EN A4dB1[d]/ NT[a]		SRB[b], BIH[b], CRO[b], SLO[b,c]
Esocidae							
Esox lucius	LC				LR(lc)[a]		SRB[b,c], BIH[b,c], CRO[b,c], SLO[b,c]
Umbridae							
Umbra krameri	VU A2c	II	II	EN	CR B1a + 2b (ii)[d]/EN[a]	+	SRB[b] CRO[b]
Cyprinidae							
Abramis brama	LC				LR(lc)[a]		SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]
Abramis sapa		III		NT	LR(lc)[a]		SRB[b], BIH[b], CRO[c], SLO[b,c]
Blicca bjoerkna	LC				LR(lc)[a]		SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Abramis ballerus		III			LR(lc)[a]		SRB[b,c], CRO[c]
Vimba vimba	LC	III		VU	LR(lc)[a]		SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Tinca tinca	LC				LR(lc)[a]	+	SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Cyprinus carpio	VU A2ce			EN	LR(nt)[a]		SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]

(continued)

Table 10 (continued)

Taxon name	IUCN Red List [37] (global)	Bern Convention (1979) annexes	Habitats Directive annexes	IUCN Red List Croatia	IUCN Red List Serbia (BAES)	Official Gazette of the Republic of Serbia, No.5/10	Findings by country
Carassius carassius	LC			VU	EN A1be,B1b (i)[d]	+	SRB[b], BIH[b], CRO[c], SLO[b,]
Carassius gibelio[f]							SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]
Ctenopharyngodon idella[f]							SRB[c] CRO[b], SLO[b,c]
Scardinius erythrophthalmus					LR(lc)[a]		SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Aspius aspius	LC	III	II,V	VU	LR(lc)[a]		SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Rutilus pigus	LC		II		LR(lc)[a]		SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Rutilus rutilus	LC				LR(lc)[a]		SRB[b,c], BIH, CRO[b,c], SLO[b,c]
Rhodeus sericeus	LR/LC	III	II		LR(lc)[a]		SRB, BIH[b], CRO[b,c], SLO[b,c]
Pelecus cultratus	LC		II	DD	LR(lc)[a]		SRB[c], CRO[c]
Alburnus alburnus	LC				LR(lc)[a]		SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]
Leucaspius delineatus	LC	III			DD[d]	+	SRB[c]
Chalcalburnus chalcoides	LC	III	II	VU	DD[d]	+	SRB[b,c], BIH[b], CRO[b,c]
Alburnoides bipunctatus		III		LC	LR(lc)[a]		SRB[b], BIH[b], CRO[c], SLO[b,c]

Phoxinus phoxinus	LC				LR(lc)[a]		SRB[b], BIH[b], CRO[b], SLO[b,c]
Leuciscus souffia	LC	III	II	VU	DD[d]	+	SRB[b,e] BIH2, CRO[b,c], SLO[b,c]
Leuciscus leuciscus	LC			VU	DD[d]/LR(lc)[a]		SRB[b], CRO[c], SLO[b,c]
Squalius cephalus	LC				LR(lc)[a]		SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Chondrostoma nasus	LC	III			LR(lc)[a]		SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]
Idus idus	LC			VU	NT[d]/LR(lc)[a]		SRB[b], BIH[b], CRO[c], SLO[b,c]
Barbus barbus	LC		V		LR(lc)[a]		SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Barbus balcanicus	LC		V				SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Gobio gobio	LC			LC	LR(lc)[a]		SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Gobio uranoscopus	LC	III	II	NT	DD[d]		SRB[b] CRO[c], SLO[b,c]
Gobio albipinnatus	LC	III	II	DD			SRB[b], CRO[c], SLO[b,c]
Gobio kessleri	LC	III	II	NT	DD[a]		SRB[b] SLO[b,c]
Pseudorasbora parva[f]	LC						SRB[b,c], BIH[b], SLO[b,c]
Hypophthalmichthys nobilis[f]	DD						SRB[b], CRO[c]
Hypophthalmichthys molitrix[f]	NT						SRB[b]

(continued)

Table 10 (continued)

Taxon name	IUCN Red List [37] (global)	Bern Convention (1979) annexes	Habitats Directive annexes	IUCN Red List Croatia	IUCN Red List Serbia (BAES)	Official Gazette of the Republic of Serbia, No.5/10	Findings by country
Balitoridae							
Barbatula barbatula	LC						SRB[b], BIH[b], CRO[b], SLO[b,c]
Cobitidae							
Misgurnus fossilis	LC	III	II	VU	VU[d]/LR(lc)[a]	+	SRB[b] BIH[b], CRO[c], SLO[b,c]
Cobitis elongata	LC	III	II	VU	LR(lc)[a]	+	SRB[b], BIH[b], CRO[c], SLO[b,c]
Cobitis elongatoides	LC						BIH[b], CRO[b,c], SLO[b,c]
Sabanejewia aurata	DD	III	II		LR(lc)[a]		SRB[b] BIH[b], CRO[c], SLO[b,c]
Cobitis taenia	LC	III	II		LR(lc)[a]		SRB[b], BIH[b], CRO[c]
Siluridae							
Silurus glanis	LC	III			LR(lc)[a]		SRB[b,c], BIH[b], CRO[b,c] SLO[b,c]
Ictaluridae							
Ameiurus nebulosus[f]							SRB[b], CRO[b,c], SLO[b]
Ictalurus melas[f]							BIH[b], CRO[c]
Gadidae							
Lota lota	LC				LR(lc)[a]		SRB[c.b], BIH[b], CRO[b,c], SLO[b,c]

Gasterosteidae							
Gasterosteus aculeatus	LC				DD[a]		CRO[b]
Pungitius platygaster	LC	III					SRB[e]
Syngnathidae							
Syngnathus abaster[f]	LC	III					
Percidae							
Perca fluviatilis	LC				LR(lc)[a]		SRB[b,c], BIH[b], CRO[b], SLO[b,c]
Gymnocephalus cernuus					LR(lc)[a]		SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Gymnocephalus baloni	LC	III	II,V	VU	VU[d]/DD[a]	+	SRB[c] CRO[b,c]
Gymnocephalus schraetseri		III	II	CR	LR(lc)[a]		SRB[b], BIH[b], CRO[c], SLO[b,c]
Stizostedion lucioperca	LC				LR(lc)[a]		SRB[b,c], BIH[b], CRO[b,c], SLO[b,c]
Stizostedion volgense	LC	III		DD	LR(lc)[a]		SRB[b], CRO[c]
Zingel zingel	LC	III	II,V	VU	DD[d]/LR(nt)[a]	+	SRB[b], BIH[b], CRO[c]
Zingel streber	LC	III	II	VU	VU B1b(iii)E[d]/ DD[a]	+	SRB[b,c], BIH[b], CRO[c], SLO[b,c]
Centrarchidae							
Lepomis gibbosus[f]							SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Oreochromis niloticus							SLO[b]

(continued)

Table 10 (continued)

Taxon name	IUCN Red List [37] (global)	Bern Convention (1979) annexes	Habitats Directive annexes	IUCN Red List Croatia	IUCN Red List Serbia (BAES)	Official Gazette of the Republic of Serbia, No.5/10	Findings by country
Cottidae							
Cottus gobio	LC		II				SRB[b], BIH[b], CRO[b,c], SLO[b,c]
Gobiidae							
Neogobius fluviatilis[f]	LC	III					SRB
Neogobius melanostomus[f]	LC						SRB,CRO[c]
Neogobius gymnotrachelus[f]	LC						SRB, CRO[c]
Neogobius kessleri[f]	LC	III					SRB CRO
Proterorhinus marmoratus[f]	LC	III					SRB[c], CRO[c]

[a]Simonovic [38], Ribe Srbije
[b]Tributary of the Sava River
[c]The main stream of the river basin
[d]Baes.pmf.kg.ac.rs
[e]Species that have been confirmed in the past, but not found by the author
[f]Invasive species, + strictly protected species (Official Gazette of the Republic of Serbia, No.5/10) [39]

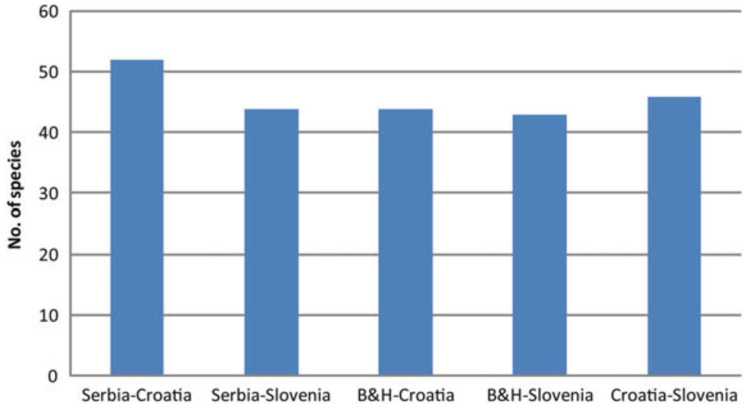

Fig. 3 The number of common fish species identified by countries that share with the Sava River

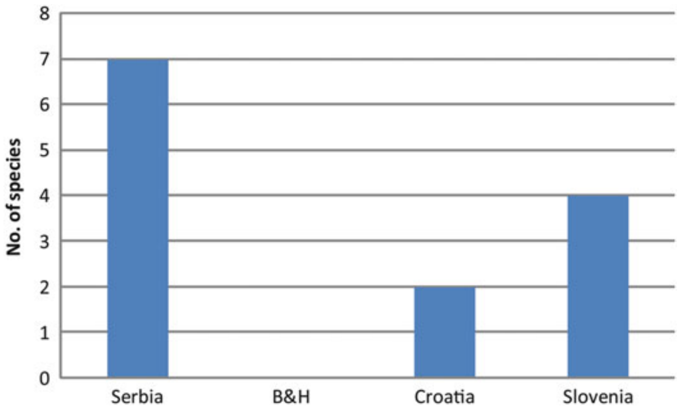

Fig. 4 The number of fish species recorded only in one country on the Sava River

to have a specialized database of aquatic ecosystems biodiversity (primarily including the diversity of macroalgae, macroinvertebrates, and fish) in which the endangerment of aquatic organisms on a national level is also shown [41]. By studying all these documents, it is clear that, in the territory of Serbia, the list of endangered species of fish that inhabit the Sava River contains fish that are not on the lists of other countries, like *Tinca tinca* and *Leucaspius delineatus*.

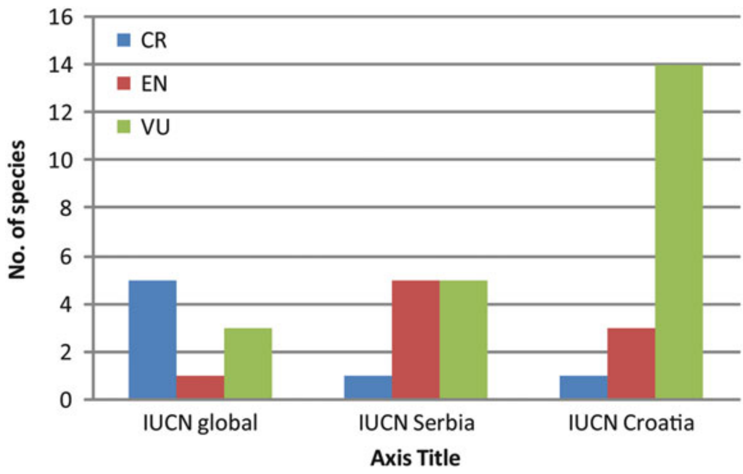

Fig. 5 The number of protected species of fish along the Sava River by country

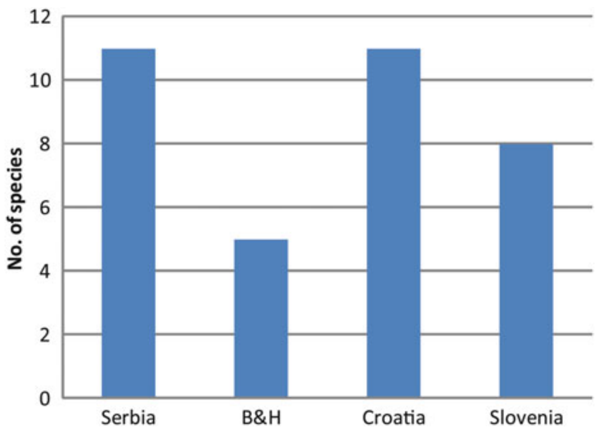

Fig. 6 The number of allochthonous species of fish along the flow of the Sava River by country

6 Protected Areas

The EU Water Framework Directive [3] is a fundamental tool for the implementation of all water-related EU Directives as well as a platform for the coordination of activities on the realization of other community legal instruments and global initiatives. Besides other issues, the WFD considers protected areas as areas that need extra protection.

The WFD and other related legal documents consider separately protected areas because they need extra protection for the conservation of important habitats and/or species, or they are distinguished as important to be protected based on other reasons covered by the community legislation (e.g., abstraction of drinking water, bathing waters, etc.; the WFD Article 6).

Within the Sava River basin, the related national legislation in non-EU countries is not fully harmonized with the EU standards. Slovenia delineated all areas identified in the WFD [3] [42]. The same applies in the case of Croatia. In Serbia, the new bylaw [43] identifies the sites and regulates the issue of management and financing of Pan-European Ecological Network.

Besides extensive data provided within the SRBMP [29], the protected and other important areas from the aspect of biodiversity conservation are widely discussed within the scope of project entitled "Protection of Biodiversity of the Sava River Basin Floodplains" [44].

Within the Sava River basin, eight national parks (Triglav, Plitvice, Sutjeska, Kozara, Una, Tara, Durmitor, and Biogradska gora) with a total area coverage of 216,308.51 ha and three parks of nature with a total area coverage of 90,921.00 ha are situated. Besides, seven Ramsar sites are situated within the basin area (Bardača Protected Area in Bosnia and Herzegovina; Lonjsko Polje, and Crna Mlaka in Croatia; Peštersko Polje, Obedska bara, and Zasavica in Serbia; and Cerkniško Lake in Slovenia), with a total area coverage of 71,673.00 ha.

In total, 112 [45] sites that are water relevant, with a total area coverage of 1,340,395.50 ha, are identified within the Sava River basin. Out of water-relevant [45] sites, 30 are important for the protection of avifauna, proposed to preserve the bird species enumerated in the Directive 92/43/EEC (Birds Directive) [46–48], with a total area coverage of 725,771.39 ha, while 91 sites are proclaimed as of community importance for the protection of the habitat types and the species enumerated in Directive 92/43/EEC (Habitats Directive)—total area coverage of 758,834.67 ha.

7 Threats of Biodiversity Along the Sava River

The Pan-European wetland ecosystems are exposed to direct or indirect anthropogenic influence. Uncontrolled exploitation of natural resources is associated with numerous harmful consequences. The most important among them involve:

- Severe degradation and fragmentation of habitats due to rapid development of (sub)urban, agricultural, and industrial regions and due to construction of dense transport networks
- Introduction of alien species (introduction of species into ecosystems from geographically remote regions)
- Permanent air, water, and soil contamination with pollutants (sulfur oxides, nitrogen, toxic heavy metals, biocidal substances, and persistent organic pollutants that have mutagenic, teratogenic, or carcinogenic effects)
- Increased level of ionizing and nonionizing radiation
- Stratospheric ozone depletion due to emission of partially or completely halogenated hydrocarbons

- Enormous emissions of the greenhouse gases that may induce climate change
- Soil erosion and degradation of soil quality
- Biodiversity reduction and genofond loss.

The synergic effect of these factors resulted in significant biodiversity reduction in both local and global levels [49]. Intense urbanization and rapid development of economic sectors (energy sector, mining, industry, agriculture, transport) are the main anthropogenic pressures on aquatic and wetland ecosystems along the Sava River.

Disappearance, degradation, and fragmentation of temporary denuded habitats are the main threats for aquatic and wetland vegetation. Stevanović [50] emphasized that the most of extinct and critically endangered plant taxa in Serbia belong to the group of aquatic and wetland species (*Trapa anosa* Janković, *Caldesia parnassiifolia* (L.) Parl., *Alisma parnassiifolium* L., *Juncus capitatus* Weigel, *Polemonium caeruleum* L., *Utricularia intermedia* Hayne, *Achillea ptarmica* L., *Cyperus rotundus* L., *Pilularia globulifera* L.). Drainage, irrigation, and amelioration of flooded regions as well as development of a complex flood defense system (dykes, levees, bank embankments, canals, pools, and ditches) resulted in a serious loss of wetlands and simultaneous expansion of arable land and (sub)urban regions. Inadequate water use (overexploitation of water resources for irrigation and industrial water supply) may result in permanent lowering of groundwater levels. Wetland ecosystems are sensitive to minor changes in the groundwater level. Overexploitation of water resources, when groundwater abstractions exceed the recharge and drainage of waterlogged agricultural soils, significantly reduces the depth of water table.

Water and soil contamination with pollutants is another threat of aquatic and wetland vegetation. A dense network of industrial towns along the Sava River requires permanent consumption of a huge amount of energy. Energy-supplying systems along the Sava River involve the nuclear power plant in Krško, numerous hydroelectric power plants along the Sava River and its tributaries, and thermoelectric power plants in Obrenovac. The power-generating units require the use of various natural resources (fossil fuels, water, radioactive elements, etc.) and therefore inevitably create harmful impacts on the environment. Different technologies used in the development of the energy sector have different environmental impacts.

Fossil fuel combustion has a number of adverse impacts to environment (e.g., the emission of sulfur dioxide, nitrogen oxides, aerosol particles, greenhouse gas compounds, and other air polluters; formation of ash deposit fields; dispersion of fly ash or dust particles; etc.). In addition, coal excavation leads to soil degradation. The “Nikola Tesla A” thermoelectric power plant near Obrenovac produces a huge amount of fly ash and other residues. Fly ash deposit is located in the vicinity of the power plant, on the right bank of the Sava River. Despite relatively successful programs of the restoration and revitalization, the fly ash deposit may have adverse impacts on neighboring terrestrial and aquatic ecosystems, due to leaching of toxic substances from the ash into soil and groundwater [51–54].

Despite the advanced safety systems, the use of nuclear energy is always linked with the risks of nuclear accidents. A major problem with nuclear power plants is the disposal of radioactive waste.

Hydroelectric power plants use renewable energy source. They belong to the group of clean technologies since they do not pollute air, water, and soil. However, the construction of hydropower plants has a number of adverse impacts on the environment, such as the submersion of fertile soil and complete loss of arable land, destruction of ecosystems, permanent loss of habitats of rare and endangered species, habitat fragmentation, creation of barriers for migratory species, etc. Dams of hydroelectric power plants slow down water velocity and form artificial water reservoirs. Slow water flows may cause eutrophication, increased sedimentation, and changes in the river bottom.

The Sava River runs through numerous towns with developed industrial facilities (Zagreb, Sisak, Slavonski/Bosanski Brod, Brčko, Sremska Mitrovica, Šabac, Obrenovac, Belgrade). **Waste waters** discharged from municipalities and industries along the Sava River and its tributaries were treated only at certain locations so the water quality used to be considerably endangered.

The most important water pollutants, with harmful effects on human health, biodiversity, and environment, are organic waste, persistent organic polluters, heavy metals, fertilizers, and radioactive elements.

The most important sources of the **organic waste** are domestic and industrial sewage. Immediately downstream of a sewage effluent, organic matter decomposition reduces the oxygen content of the water and results in the release of ammonium. Organic matter derived from diverse human activities is a major source of pollutant discharge to rivers. The decomposition and breakdown of the organic matter is mediated by microorganisms and takes place mainly at the surface of the sediment and vegetation in smaller rivers and in the water column in larger rivers. As the process requires the consumption of oxygen, severe organic pollution may lead to rapid deoxygenation of the river water and hence to the disappearance of fish and aquatic invertebrates.

Persistent organic polluters are the most dangerous organic compounds, since they have the most harmful effects on human health, biodiversity, and environment. This group of substances is heterogeneous and involves biocidal compounds (insecticides, pesticides, and herbicides such as hexachlorobenzene, heptachlor, heptachlor epoxide, aldrin, endrin, dieldrin, DDT, DDD, etc.), polychlorinated biphenyls, and polycyclic aromatic hydrocarbons.

Living organisms require some metals (in minute amounts) for their metabolic activities. However, excessive levels of metals can be damaging to the organism. Organisms have mechanisms to remove metals from metabolic processes. Problems arise when organisms are exposed to higher concentrations than usual, which they cannot remove rapidly enough to prevent damage. Water pollution by heavy metals is a serious problem since high concentration of mercury, lead, cadmium, copper, nickel, chromium, and other heavy metals is toxic for organisms.

The use of manure and fertilizers can lead to leaching of nitrate, ammonium, sulfate, potassium, and, to a lesser extent, phosphorus into the groundwater (and indirectly into surface water). Sewage and communal discharge may contaminate surface water by excessive amounts of nitrate and phosphorus. Water enrichment and overloading with nitrate and phosphorus initiate the eutrophication process. Eutrophication is the result of synergistic effects of multiple factors.

Inorganic phosphorus and nitrogen are the major limiting compounds for aquatic photoautotrophs (cyanobacteria, micro- and macroalgae, as well as angiosperms). High input of these compounds to waters may provoke a rapid phytoplankton production. Algal blooms (overgrowth of algal populations) may disturb the structure and functions of aquatic ecosystems.

Freshwater cyanobacteria produce several bioactive secondary metabolites with diverse chemical structure, which may achieve high concentrations in the water, when cyanobacterial blooms occur. Some of the compounds released by cyanobacteria have allelopathic properties, influencing the biological processes of other phytoplankton or aquatic plants. Allelopathy can influence the competition between different photoautotrophs for resources and change the structure of phytoplankton communities. Allelochemical compounds produced by dominant species eliminate weak competitors, reducing biodiversity of phytoplankton communities. Gross [55] described allelopathic mechanisms of cyanotoxins. Excessive growth of Cyanobacteria (previously misclassified as blue-green algae or Cyanophyta) can produce cyanotoxins in such concentrations that they are poisonous to fish, cattle, and humans. When dead phytoplankton sink to the bottom, their decomposition may reduce the oxygen concentration in the water to levels too low to support fish and benthic invertebrates. Enhanced biological production and other associated effects of eutrophication usually occur in lakes, reservoirs, coastal areas, and large, slowly flowing rivers.

Legal instruments and well-organized monitoring programs may control and reduce the emission of harmful pollutants in water from point sources (discharged from municipalities and industrial complexes). However, the nonpoint (diffuse) pollution is not traceable. The worst effect of diffuse pollution is eutrophication. Arable land around the Sava River and its tributaries is treated with different fertilizers. A huge amount of fertilizers is used in peri-Pannonian region. Leaching of nitrates and phosphates contributes to the eutrophication of water.

Eutrophication may accelerate succession processes in wetland ecosystems.

8 Invasive Species

Adverse impacts of fast-spreading introduced plant species (invasive species) on natural communities have been analyzed in numerous articles [56–58]. Invasive alien species involve taxa which are dispersed, deliberately or unintentionally, from their natural habitats and introduced in new ecosystems, where they have the ability to outcompete native species and to occupy new habitats. Most of invasive species belong to the group of "r-selected" taxa. The common characteristics of r-selected species are fast growth, quick sexual maturity, fast reproductive cycle, high production of seed, high dispersive potential, etc. [59]. As more powerful competitors, introduced taxa may threaten the existence of native (in some cases rare or endemic) species. Moreover, the introduction of new species may cause an introduction of organisms that are pathogenic to natives, but not to the introduced species. In such cases, the pathogenic disease may cause significant reduction in the biodiversity of native habitats.

The problem of introduced species has been emphasized in recent times because of the globalization of markets and increased trade, travel, and tourism. Considering such unfavorable trends, invasive alien species are recognized as one of the major threats to biodiversity. Parts of European inland waterways that are highly biologically contaminated are probably irreversibly changed with respect to the composition of fauna and flora. Alien species dominate in some communities. Large European rivers are main corridors for fast spreading of alien species. Monitoring of invasive species expansion is necessary for efficient protection of native flora and vegetation. Invasive species usually occupy ruderal, segetal, hygrophilous, and aquatic communities.

The significant sources of information on invasive species are published in numerous articles ([60–70]) and organized into DAISIE [71] and BAES (Biodiversity in Aquatic Ecosystems in Serbia) databases. The most frequent invasive alien plant species along the Sava River are *Elodea canadensis* Rich., *Impatiens balfourii* Hooker, *Impatiens glandulifera* Royle, *Xanthium strumarium* L. ssp. *italicum* (Moretti) D. Löve, *Echinocystis lobata* (Michx) Torrey et A. Gray, *Ambrosia artemisiifolia* L., *Amaranthus retroflexus* L., *Amaranthus albus* L., *Amaranthus blitoides* S. Watson, *Amaranthus deflexus* L., *Chamomilla suaveolens* (Pursh) Rybd, *Erigeron annuus* (L.) Pers., *Erigeron canadensis* L., *Helianthus annuus* L., *Helianthus decapetalus* L., *Helianthus scaberimus* Ell., *Helianthus tuberosus* L., *Solidago canadensis* L., *Conyza sumatrensis* (Retz.) E. Walker, *Iva xanthifolia* Nutt., *Solidago gigantea* Ait., *Lepidium virginicum* L., *Eleusine indica* L., *Paspalum paspaloides* (Mich.) Scriber, *Reynoutria japonica* Houtt., *Bidens frondosa* L., *Bidens bipinnata* L., *Solidago canadensis* L., *Xanthium spinosum* L., *Stenactis annua* (L.) Ness., *Chenopodium ambrosioides* L., *Kochia scoparia* (L.) Schrad., *Abutilon theophrasti* Medic., *Sorghum halepense* (L.) Pers., *Portulaca oleracea* L., *Asclepias syriaca* L., *Datura inoxia* Miller, *Galinsoga parviflora* Cav., *Phytolacca americana* L., etc. The most important invasive plants species that occupy wetland communities are *Echinocystis lobata* (Michx) Torrey et A. Gray and *Amorpha fruticosa* L. [72].

9 Discussion

Based on the data presented, the general impression is that indicative ecological status of the rivers within the Sava River basin ranges from high to poor, while the largest amount of water bodies has been assessed as moderate [29].

Based on this result, at considerable number of water bodies, improvement measures have to be designed and applied in order to achieve good ecological status. The majority of the measures have to be addressed to the reduction of organic and nutrient pollution [29].

The ecological status of large lowland rivers, such as the Sava River, which includes the quality and degree of pollution of the river water, can be more precisely and successfully detected if several biological methods are used simultaneously. By combining the widely applied saprobic indices, biotic indices, and diversity indices, as it was done in the Sava River, the results can be compared, whereby the ecological status, water quality, and degree of pollution of water are obtained as a score of similar values of the methods used. On the other hand, these researches have shown that the regional biotic index (BNBI), conceived for the detection of saprobity of rivers of the Balkan Peninsula, can be successfully applied to the ecosystems of large rivers. To make its use more effective, the database of indicator taxa from the group of macroinvertebrates was supplemented by primarily allochthonous gene from the groups Amphipoda and Mollusca.

Taking into account the characteristics of biodiversity of primarily macroinvertebrates and fish from the flow of the Sava River ecosystem, starting from the source to the mouth, three significant macrohabitats can be separated which are important for the sustainability of the entire ecosystem. The first "macrohabitat" includes the upper flow of the Sava River and its tributaries in that part. This macrohabitat is significant for the conservation of salmonid species of fish, such as the brown trout, hucho trout, and grayling [73–76], as well as the conservation of stenovalent forms of macroinvertebrates, primarily from the groups of Ephemeroptera, Plecoptera, and Trichoptera (EPT) [77, 78].

Part of the flow through Croatia and BIH (from Zagreb to the state border with Serbia) is the second and the biggest "macrohabitat," which is of central importance for the conservation of fish species from the families of Cyprinidae, Percidae, Esocidae, and Siluridae [79]. Besides the macrohabitat of the main flow of the Sava River, the basin area of this part of the river is very significant, namely, the large right tributaries of the Sava River which come from the territory of Croatia and Bosnia and Herzegovina, such as the Kupa, Una, Vrbas, Bosna, and Drina. The upper and middle flows of the rivers in question represent the most significant habitats for the conservation of biodiversity of salmonid species of fish, such as hucho trout, grayling, and brown trout, of not only the Sava River basin but the entire Danube River basin as well [80–82].

The third macrohabitat occupies the lower, mostly potamonic part of the Sava River, and it is significant for the conservation of biodiversity of potamonic communities. From the invertebrates group, the fauna of Mollusca, Oligochaeta, and Chironomidae is significant. Concerning fish, this macrohabitat is significant primarily for the conservation of population of sterlet (*Acipenser ruthenus*) and populations of commercially important species of fish, like carp, catfish, perch, and pike. Besides that, this area is significant for the conservation of fish species that have been declared endangered in the area of the middle flow (Croatia), such as *Gymnocephalus schraetser*, *G. baloni, Aspius aspius*, *Idus idus*, and *Vimba vimba*, whose populations are numerous in this part of the flow (especially *A. aspius* and *V. vimba*).

For the conservation of biodiversity of the Sava River, as well as the global conservation of the Danube River basin, the soundness of the riverside wetlands that have aquatic communication with the Sava River and extend along the flow is of great importance. Such habitats have been researched within the project "Protection of Biodiversity of the Sava River Basin Floodplains" (http://www.savariver.com/). As a result of the project, 49 sites (habitats) were allocated and included in the ecological network of significant habitats along the flow of the Sava River. In the future, the habitats should become a part of the ecological network of European Union program Natura 2000 [45].

On the territory of Slovenia, eight sites (habitats) were allocated, which include a part of the Sava River from the state border with Austria, Julian Alps, Sava Bohinjka, and Sava Dolinka as other smaller areas. The fact that the mentioned habitats are located within the first allocated "macrohabitat" of the Sava River, which includes the upper flow of the river, is significant from the aspect of this work. In this area, and within the allocated habitats, besides birds, amphibians, and reptiles, other aquatic organisms are allocated for protection. These also include freshwater crayfish *Austropotamobius torrentium* and *A. pallipes* as well as fish species *Eudontomyzon mariae*, *Eudontomyzon* spp., *Cottus gobio*, *Hucho hucho*, *Leuciscus souffia*, *Rutilus pigus*, *Barbus meridionalis*, *Barbus plebejus*, *Aspius aspius*, *Cobitis elongata*, *Cobitis taenia*, *Gobio uranoscopus*, *Rhodeus amarus*, and *Zingel streber*. Our research largely confirms the validity of the need of the allocated taxa for the conservation of their populations in this area. There is certain reservation concerning the taxonomic status of certain species, such as the white-clawed crayfish (*Austropotamobius pallipes*), two species of barbel *Barbus meridionalis* and *Barbus plebejus*, as well as two species of stork, Cobitis and Gobio.

The new research, based on molecular markers, indicates that white-clawed crayfish represents a complex of species and the area of Slovenia mainly in the rivers of the Adriatic River basin living species *Austropotamobius italicus* and that of *A. pallipes* primarily inhabits the Adriatic River basin [83].

On the other hand, according to Kottelat and Freyhof [84], the areal of distribution of fish species *Barbus meridionalis* and *Barbus plebejus* does not reach the territory of Slovenia and the basin area of the upper flow of the Sava River. Such similar taxonomic confusion must be dealt with further detailed research. By all means, as it has already been said, the upper flow of the Sava River is significant from the aspect of preserving benthic stenovalent invertebrate communities, primarily from groups Turbellaria, Plecoptera, Ephemeroptera, and Trichoptera.

The second recognized "macrohabitat" of the Sava River includes a total of 32 (16 CRO; 16 BIH) allocated flood areas which are significant for the conservation of biodiversity and the inclusion to Pan-European Ecological Network. In this part of the flow, the Sava River mostly has a character of middle and lower rhithron, although, at certain places through the flat part of Slavonia, the river also has the characteristic of a potamon (see chapter "Aquatic Macroinvertebrates of the Sava

River"). In the results of the mentioned IUCN project, which describes the biodiversity of flood areas along the flow of this part, the accent was put on the protection of the endangered taxa of vascular plants, birds, amphibian, reptiles, and mammals (*Lutra lutra* and *Castor fiber*). Out of fish species are protected only *Umbra krameri* (site Rača at the mouth of the Drina, BIH and site "Žutica" near nature park "Lonjsko Polje") and *Leuciscus souffia* (in a part of the 150 km course Save CRO), as well as a kind of lamprey *Eudontomyzon* sp. (in a part of the Sava River around 150 km of its flow and in nature park "Lonjsko Polje" CRO). Our research of biodiversity of primarily macroinvertebrates and fish of the main flow of the Sava River indicates the need to expand the list of taxa that need conservation. This stretch of the Sava River is characterized by the transition of rhithron and potamon general river type, which increases heterogeneity of this macrohabitat and, for the most part, significantly influences the biodiversity of both macroinvertebrates and fish. Research has shown that species diversity of macroinvertebrates and fish is the highest in this macrohabitat of the Sava River. Also, the mean index of significance of this macrohabitat (1, 2) is higher compared to the lower flow of the Sava River, where it was 0.85 (Table 8). Keeping those parameters in mind, it is suggested that the vitality of populations of certain species of macroinvertebrates is to be preserved at this macrohabitat. These species include clams of the genus *Unio*, snails of the genus *Theodoxus*, and a decapod crayfish *Astacus leptodactylus*. Besides invertebrates, conservation measures should include populations of fish such as *Gymnocephalus schroaster* and *G. baloni*, *Zingel zingel* and *Z. streber*, *Aspius aspius*, *Idus idus*, and *Vimba vimba*, as well as the population of a commercial species of fish, the carp (*Cyprinus carpio*).

The last "macrohabitat" in the lower flow of the Sava River basin (SRB) is characteristic of a potamon, which caused certain specificities of the biodiversity. Along the river banks of this fluvial macrohabitat, nine flood areas (sites) have been allocated, which are believed to be of significance to the global conservation of biodiversity of the Sava River. However, as in the previous case, primarily populations of vascular plants, mammals, birds, amphibians, and reptiles are recommended for conservation within these sites. Other groups of organisms that inhabit the riverbed itself and the aquatic environment of the sites in question are not recorded. Only the area of a special nature's reserve "Zasavica" is stated as significant for the conservation of the population of fish "crnka" *Umbra krameri* [85–87]. The results of biodiversity of the Sava River shown in this work, as well as in Simić et al. [88], Karadžić et al. [89], Ostojić et al. [90], Lucić et al. [91], Simonović et al. [92], Crnobrnja-Isailović et al. [93], indicate that it is necessary to perform measures of conservation in this part (macrohabitat) of the Sava River for certain species of fish that are rare or have completely vanished from other upstream areas. The conservation primarily relates to a population of globally endangered sturgeon species, sterlet (*Acipenser ruthenus*). Given the poor state noted in the population of this significant fish species, commercial fishing for this species must be banned, followed by other measures of conservation, such as revitalization and guarding its torus [94, 95]. Besides the starlet, the dominant commercial species of fish, namely, the carp (*Cyprinus carpio*) must be preserved from excess fishing. In the flood areas around the lower flow of the Sava River, it is necessary to preserve populations of endangered species of fish, such as *Carassius carassius*, *Misgurnus fossilis*, and *Tinca tinca*.

Factors that threat the biodiversity of ecosystem of the Sava River are numerous and complex and can globally be analyzed through the complex of factors from the acronym "HIPPO" [96]. Habitat alteration occurs in the entire flow of the Sava River and its basin area. The most significant changes in the habitat of the Sava River are riverbed regulation (done by the construction of embankments as a defense from floods), gravel exploitation from the riverbed, and fragmentation of the river flow (17 dams). Invasive species research shows that the lower and middle flows of the river are most affected by allochthonous and allochthonous-invasive species. Besides the transfer of allochtonous species, the transfer of "foreign genes" into indigenous populations, also done. This way, the transfer of grayling genes from the Adriatic basin into the genome of indigenous populations of brown trout of the Danube River basin in the river Gradac (a tributary to the Kolubara, the Sava River basin) was discovered [97–99]. Pollution—The analysis of water quality and ecological status of the Sava River indicates that the river is loaded mostly by organic polluters and that the effect of pollution is mostly exhibited in the lower flow. Population growth—Population density is unevenly distributed along the river, the most densely populated places (more than 10^6 inhabitants) and places where the number of inhabitants is increasing are in the lower flow (Belgrade) and at the beginning of the middle flow (Zagreb 250 to 10^6 inhabitants). In the remaining parts of the flow of the Sava River, population density is lower or considerably lower than 250,000 inhabitants. Overexploitation—Parts of the flow through Croatia and Serbia where commercial fishing alongside recreational fishing occurs are especially stricken by this factor. Research has shown that restrictions of both kinds of fishing are necessary, especially in the part of flow through Serbia (see chapter "Aquatic Macroinvertebrates of the Sava River" on fish). Alongside the exploitation of fish resources, control and reastrictions in the usage of other resources of biodiversity such as: forests (wood), reed, protection of pastures from overgrazing etc., also done.

The previous discussion indicates a significant complexity of preserving the biodiversity of large rivers. It has been shown that in order to successfully preserve the biodiversity of large rivers, it is necessary to view the fluvial ecosystem as a complex that is composed of three dependent ecological entities—the main riverbed, flood areas, and tributaries. The researches have confirmed it to a large degree by supplementing the research of biodiversity of flood areas of the Sava River and giving the characteristics of biodiversity of the riverbed of the Sava River and, to an available measure, the biodiversity of its basin.

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