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Contribution to the improvement of diatom-based assessments of the ecological status of large rivers – The Sava River Case Study

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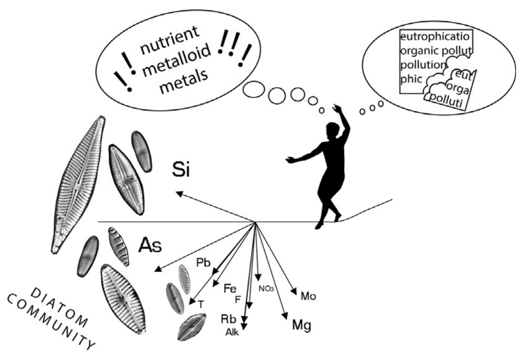
HIGHLIGHTS

- Benthic diatoms in the Sava River under anthropogenic pressures were studied.
- In large rivers, diatoms are indicators of multiple pressures.
- Arsenic and silicon are important factors determining diatom communities.
- Diatom indices are appropriate metrics for overall degradation of large rivers.

GRAPHICAL ABSTRACT



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ABSTRACT

The Sava River Basin is a major drainage basin of southeastern Europe, significantly influenced by anthropogenic activities. Our study was focused on diatom communities as an indicator of the ecological status of running waters. We investigated over 937 km of the Sava River at 19 sampling sites. Benthic diatom communities and 17 diatom indices were analyzed along with a large set of environmental parameters. CCA revealed that the most important elements along the spatial gradient were As and Si. Our results show that the species *Navicula recens* (Lange-Bert.) Lange-Bertalot and *Eolimna minima* (Grunow) Lange-Bertalot are very abundant at downstream localities where the highest concentrations of As were measured. The number of motile diatoms increased along the nutrient gradient, i.e. with Si availability. Correlations between diatom indices and selected environmental factors showed that temperature, As, Si and Fe are in significant negative correlation with most diatom indices. Analysis revealed the influence of As and metals in water on diatoms, although their concentrations did not exceed environmental standards. While our findings do not confirm that diatom indices reveal the intensity of pressures solely caused by nutrient and/or organic pollutants, they suggest that in moderately polluted large rivers benthic diatoms are good bioindicators of multiple pressures, and that diatom indices could serve as indicators of the level of overall degradation of an ecosystem.

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1. Introduction

The Sava River Basin, with a total area of 97,713.20 km^2, is a major drainage basin of southeastern Europe. Its water resources to a great extent constitute the total freshwater for most of the countries that share its basin area (Sava River Basin Management Plan, 2014). The Sava is a subalpine river in the upper stretch and a lowland river along most of its watercourse. Anthropogenic pressures change downstream. They range from hydropower plants and heavy industry in the upper reaches to agricultural areas, industry and large municipalities in the middle and lower reaches. The effects of a wide range of stressors are evident in large rivers such as the Sava. To improve the efficiency of monitoring systems, the procedures utilized for ecological status assessment of the Sava in countries through which it flows take into consideration the phytobenthos as an important element of biological quality (WFD, 2000).

Diatoms are accepted indicators of nutrient and organic load and have been widely used in water quality assessment (Berhon et al., 2011; Fore and Grafe, 2002; Lane et al., 2007; Raunio and Soininen, 2007; Rott et al., 1997, 1999; Sládecěk, 1986). The ecological status of large rivers based on benthic diatoms is assessed in many countries in Europe (Schöll et al., 2012). All applied methods reveal the effects of eutrophication and/or organic pollution. The most complete survey of large river benthic algae (diatoms and other algae groups) and the assessment of the ecological status based on benthic diatoms were performed at six-year intervals, in 2001, 2007 and 2013, within the Joint Danube Surveys. The results of the investigation presented in Makovinska and Hlubikova (2015) show that the diatom-based assessment is the most effective when nutrients and organic pollution are dominant stressors, as is the case in the River Danube. Nonetheless, the authors suggested that these results should be combined with other benthic algal groups in order to obtain a more reliable final assessment. Aside from water quality, periphytic diatoms can be used to assess landscape disturbances in large rivers (Kireta et al., 2012), as well as metal pollution in lowland rivers (De Jonge et al., 2008).

Previous algological investigations of the Sava River were mainly based on phytoplankton communities (reviewed in Simić et al., 2015), until recent research into the qualitative composition of phytobenthos along the entire flow of the Sava was undertaken (Simić et al., 2015). Our study, which was conducted within the GLOBAQUA project (Navarro-Ortega et al., 2015; Paunović et al., 2016), covered two seasons of research, at high and low water levels, at localities situated on the upper, middle and lower stretches of the river. The identified benthic diatoms were analyzed along with a large set of environmental parameters. Factors linked with eutrophication and organic pollution are expected to determine the diatom community and consequently diatom-based metrics (Lecointe et al., 1993). The present study, which was based on the identification of the most important environmental factors affecting diatom communities and their correlation to diatom indices, is a significant step forward in the study of large rivers such as the Sava.

In this paper we hypothesized that: (i) benthic diatom communities are a good indicator of the presence of multiple stressors in large river ecosystems; (ii) the main determinants that shape the benthic diatom communities are related to organic pollution and nutrient load; (iii) diatom community descriptors could be used as indicators of the influence of metals and metalloids; (iv) differences in hydrological conditions produce changes in the composition of benthic diatom communities and diatom indices.

2. Materials and methods

2.1. Study area

The River Sava is 945 km long. It originates in Slovenia from the Sava Dolinka and the Sava Bohinjka. It flows through Croatia, along the border of Bosnia and Herzegovina with Croatia to Serbia where it meets the Danube as one of its largest tributaries. According to its geomorphological features, the Sava can be divided into three sectors: the upper stretch in the hilly-mountainous part of Slovenia (which is 265 km long and includes the Sava Dolinka), the middle stretch from the Slovenian-Croatian border up to the confluence with the River Una (129 km long), and the longest lower stretch (597 km long), with properties of a typical lowland river (Sava River Basin Management Plan, 2013). Anthropogenic activities exert a significant influence on the river: hydropower plants are situated in the upper stretch (Sava River Basin Management Plan, 2013), areas of intense agriculture along the middle stretch, while the lower reaches are mainly affected by high pollution from industrial processing and untreated municipal wastewater discharges (Markovics et al., 2010). Inland navigation in the lower stretch is also significant (Sava River Basin Management Plan, 2013). Detailed characteristics of the Sava River Basin, such as landscape types, climatic features, geology, land use and other anthropogenic influences are described in Milačič et al. (2015).

2.2. Collection of samples and supporting data

Investigation of the Sava River was carried out within the objectives of the GLOBAQUA project (Navarro-Ortega et al., 2015). Sampling of the phytobenthos component was performed in September 2014 and in September 2015 at a total of 19 sites along 937 km of the watercourse. The locations of the sampling sites are presented in Fig. 1; the names of the locations and their main characteristics are shown in Table 1.

The water level of the Sava differed during the investigations, although it was expected to be within the usual low water range for September. However, September of 2014 was characterized by periods of heavy rainfall which led to mid to high waters and flooding (Hydrological Year Book, 2015; Meteorological and Hydrological Bulletin No. 9, 2014; High water levels in rivers in 2014. Preliminary report, 2015). During the field investigation, the water stage reached the levels of first flood alert, as recorded at the Jasenovac locality in the middle stretch, towards downstream localities. September of 2015 was hydrologically characterized by low waters (Hydrological Year Book, 2016; Meteorological and Hydrological Bulletin No. 9, 2015; Our Environment, 2015).

The sampling sites were selected with the aim of detecting the influence of the main municipal centers, industry, damming (site 6, Vrhovo) and agriculture along the watercourse (for details see Milačič et al., 2015).

Phytobenthos samples (26 samples, 13 samples per season, Table 1) were collected from stones in the euphotic zone of the Sava, following the instructions provided in EN 13946 (2003). When stones were lacking in periods of high waters, some samples were taken from the available hard substratum, such as branches or artificial surfaces.

Measurements of physicochemical parameters were performed during the same period as the phytobenthos samples.

The riverbed substrate type was classified based on the predominant fraction as follows: 1 – rocks, large stones and boulders; 2 – pebbles, gravel and coarse sand; 3 – fine sand and mud (Table 1). This classification was shown to be useful in the case of large lowland rivers (Simonović et al., 2017).

2.3. Material and data processing

Phytobenthos material was processed following the procedure described by Krammer and Lange-Bertalot (1986). Permanent diatom microscope slides were prepared using Naphrax medium, and observed with the Axio Lab1 Carl Zeiss microscope (Axiocam ERc 5s camera with ZEN software) at 1000 x magnification. Qualitative and quantitative analyses of the samples were performed as described in EN 14407 (2004). Diatoms were classified according to Hofmann et al. (2013) and Lowe et al. (2014). The relative abundance (%) of the identified