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A WATER QUALITY ASSESSMENT BASED ON BENTHIC DIATOMS OF THE TIMOK RIVER BASIN (EASTERN SERBIA) UNDER MULTIPLE ANTHROPOGENIC PRESSURES

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Abstract

The study is focused on diatom communities as indicators of the water quality of aquatic ecosystems. The study included watercourses of the Timok River Basin in eastern Serbia. Sampling of algological material and physico-chemical measurements were conducted at 30 locations in September 2016. Non-metric multidimensional scaling (NMDS) showed that conductivity had an effect on the spatial variability of the diatom communities. At locations under influence of acid mine drainage along the Borska river, its tributaries and the Timok river, the diversity of benthic diatoms decreased and the abundance of metal tolerant taxa *Achnanthidium minutissimum*, *Nitzschia capitellata* and *Nitzschia palea* increased. Our study revealed that the combined effect of different pollutants significantly impacted on diatom assemblages; diatoms are good bioindicators of multiple pressures; and diatom indices with different types of pollution, may show an unreliable picture of the actual state, therefore, biological and physico-chemical parameters should also be observed when interpreting the results of a solely diatom-based assessment of the ecological condition of freshwaters.

Key words: biomonitoring, benthic diatoms, diatom indices, water quality, Timok river

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Introduction. All freshwater ecosystems around the world are strongly influenced by different types of pollution caused by accelerated industrialization and urbanization. Physicochemical analyses can only assess the level of pollution, while the degree of impact on the environment and living organisms is determined by biological monitoring [1]. Aquatic organisms such as diatoms respond to various anthropogenic disturbances in their environments [1] and provide a more realistic picture of the overall state of an aquatic ecosystem. Diatoms have a number of properties that make them extremely good indicators, such as: global distribution, inhabiting all types of aquatic ecosystems, constant presence throughout the year and short life cycle, which allows them to respond quickly and detect newly-occurring changes in the physical and chemical quality of water [2]. Diatoms have consequently become part of the monitoring of rivers and streams in many countries [3]. The EU Water Framework Directive (WFD) 2000/60/EC is a piece of legislation for the protection of all water bodies in the European Union [3]. It proposes diatoms in water quality assessments for the monitoring of biological integrity [4]. In Serbia, benthic diatoms are also a mandatory biological element that must be analyzed to assess the ecological status of surface and groundwater using two diatom indices, Specific Polluosensitivity Index (IPS) and Index of European Economic Community (CEE) [5]. The aim of the study was to determine the most significant environmental factors affecting the composition and distribution of dominant taxa of the epilithic diatom community. We also wanted to test how effective diatom indices (IPS and CEE) were in assessing the ecological status of rivers in Serbia.

Materials and methods. ***Study area.*** The Timok is the largest river in eastern Serbia. It flows into the Danube at 845.65 river km. The Timok is formed near Zaječar by the confluence of the Beli Timok and the Crni Timok. Beli Timok is formed near Knjaževac, where Svrljiški and Trgoviški Timok join. The Timok river receives water drained from the basin under a wide range of stressors. The main sources of pollution are untreated municipal sewage from the towns of Bor, Zaječar and Knjaževac; agriculture; and the input of acid mine drainage (AMD) from the industrial mining complex of Bor in the Borska river [6], which is the main tributary flowing into the Timok river.

Sampling collection. Algological investigation of the Timok river was conducted concurrently at 30 sampling sites in September 2016 (Fig. 1). Material for analysis was collected according to Taylor et al. [7], such as the processing of samples for the preparation of diatom permanent slides [7]. Diatom species were identified according to Lange-Bertalot et al. [8]. The physical and chemical parameters for the purpose of this study: water temperature (°C), pH value, electrical conductivity (µS/cm), specific conductivity (S/m) and salinity (‰) were measured directly in the field using a multiparameter device (PCSTester 35K).

Data processing. The variability of diatom community structure was visualized using non-metric multidimensional scaling (NMDS). The hierarchical cluster analysis group average was applied to confirm the grouping tendency in the ordination space. This iterative ordination method was followed by a distance-based permutational multivariate analysis of variance (PERMANOVA), which tested the significant differences in community structures. Indicator species analysis [9] was applied to define the indicator diatom taxa responsible for obtained ordination and classification patterns of sampling sites. Significant species with IndVal of more than 25% were identified using the Monte Carlo significance test [10]. To test the relationship between community structure and environmental parameters, biologic environmental gradient (BIO-ENV) analysis [11] was performed. Diatom indices were calculated using the `OMNIDIA` 5.3 software package [12].

Results and discussion. The BIO-ENV method revealed that out of five environmental parameters, only conductivity, ($\rho = 0.277$, $p = 0.046$) represented the main driver for community structuring in the Timok river. Rivers and streams greatly differ in terms of ionic concentration and composition, mainly due to natural sources of variability – differences in lithology, climate, vegetation, or due to anthropogenic activities [13]. In the investigated Timok Basin, the main stressors originated from agricultural runoff or wastewater from municipalities, industry and mines. Pollution was especially evident downstream of the industrial area, which led to significantly increased conductivity (above 1000 µS/cm) and salinity (above 0.5‰). Triggered changes can be the main, or one of the main factors that shape diatom community [13].

Using NMDS analysis the ordination pattern of sampling sites in the two-dimensional space showed the tendency of grouping, where the Group average formed three clusters (Fig. 2). The diatom community significantly differed (Pseudo-F = 5.222, $p < 0.001$) in structure among three groups derived from the hierarchical cluster analysis. The IndVal listed 3, 5 and 15 significant diatom indicators for I, II and III groups (Table 1). The first cluster includes moderately polluted locations which are moderately influenced by agriculture and urban wastewater. The cluster is represented by both frequent and abundant species in the investigated area, usually present in freshwaters with higher trophic levels and moderate to high conductivity (*C. placentula* Ehrenberg), or in the broad trophic spectrum (*A. pediculus* (Kützing) Grunow and *A. copulata* Kützing (Schoeman)) [8]. Exceptions to this are locations BR2 and BR7, which are severely affected by wastewater from the industrial mining complex and city of Bor [6] and location BR3 impacted by wastewater of the quartz sand processing plant. These are peripherally located in ordination space within the first cluster, with high conductivity values measured and a dominance of species *A. pediculus*. The second cluster includes heavily polluted locations, which receive municipal wastewater, and heavily polluted water from the industrial mining complex of Bor. It is represented by alkaliphilic species of the *Nitzschia* genus (*Nitzschia capitellata* Hustedt and *Nitzschia palea* (Kützing) W. Smith), frequently present in waters with a high conductivity, and in β-mesosaprobic and polysaprobic waters [14]. *A. minutissi-*

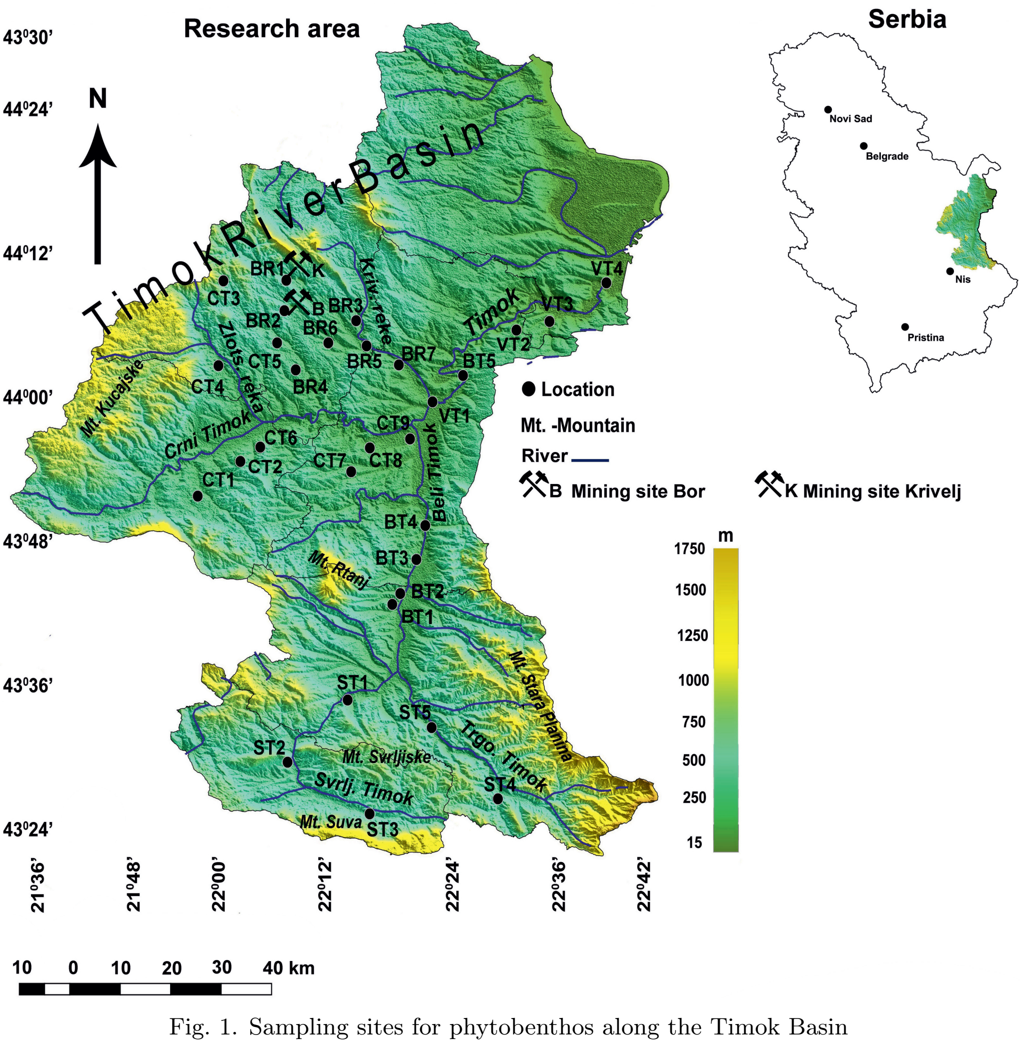

Fig. 1. Sampling sites for phytobenthos along the Timok Basin

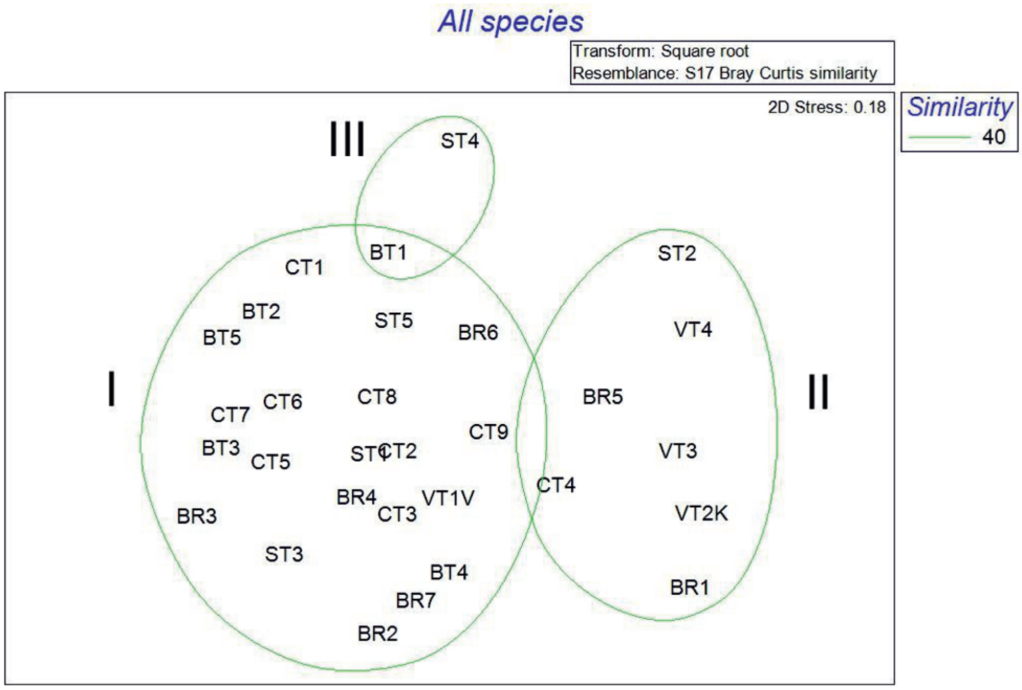

Fig. 2. The distributional pattern of sampling sites based on diatom community data. Green circles represent groups derived from the hierarchical cluster analysis, Group average, applying an arbitrary cut at 40% similarity

Table 2

Values of the IPS and CEE diatom indices at the sampling sites along the Timok River Basin (classes of ecological status: high – blue, good – green, moderate – yellow, poor – orange and bad — red) [5]

Localities	ST1	ST2	ST3	ST4	ST5	BT1	BT2	BT3	BT4	BT5	CT1	CT2	CT3	CT4	CT5	CT6	CT7	CT8	CT9	BR1	BR2	BR3	BR4	BR5	BR6	BR7	VT1	VT2	VT3	VT4
IPS index	15.2	5.7	12.9	16.2	16	14.1	16.6	14.9	18.1	11.6	12.2	12.2	16	16.4	14.6	15	13.9	13	15.4	17.1	16.6	15.4	15.9	11.8	12.1	16.5	12.1	10.4	7.5	4.1
CEE index	16	7.5	14.1	13.2	16.4	13.5	16	15.6	17.5	13	12.6	14.9	16	15.1	15.6	15.6	14.9	14.1	15.3	15.3	15.3	16.2	16	14.1	13.4	16.4	13.7	14.3	11.6	6.3

T a b l e 1

Representative and significant diatom taxa for the groups based on hierarchical cluster analysis, Group average. Listed taxa have IndVal values of more than 25%, being significant for groups with frequency and abundance > 50%

Taxa	Group	IndVal
Amphora pediculus	I	86.7
Amphora copulata	I	85.1
Cocconeis placentula	I	80.1
Nitzschia capitellata	II	84.1
Nitzschia linearis	II	67.4
Nitzschia palea	II	63
Achnanthidium minutissimum	II	58.5
Nitzschia recta	II	55.8
Navicula amphiceropsis	III	100
Navicula veneta	III	99.6
Cymbella compacta	III	90.5
Denticula kuetzingii	III	89.1
Navicula capitatoradiata	III	88.6
Diatoma ehrenbergii	III	85.7
Cymbella perparva	III	83.2
Diatoma vulgaris	III	78.5
Melosira varians	III	76.5
Cymbella excisa	III	74.5
Reimeria sinuata	III	66.2
Sellaphora capitata	III	47.9
Sellaphora pupula	III	47.1
Cymbella tumida	III	46.7
Ulnaria ulna	III	35.9

mum (Kützing) Czarnecki, a representative species of the second cluster, is a widespread and tolerant taxon that can be found in acidic, base, oligo- to hyper-eutrophic waters [14]. The third cluster includes two geographically close locations, from relatively unpolluted areas, with a wide range of indicator species, of which the most abundant are *C. compacta* Østrup and *D. kuetzingii* Grunow. These species are β-mesosaprobes, indicators of meso-eutrophic habitats [14].

Besides an evident increase in nutrient and organic load from anthropogenic sources, acid mine drainage from industrial mining has a strong negative effect on the ecosystem of the Borska river and thus of the Timok river. According to FILIMON et al. [15], concentrations of Cu, Zn, Pb, and As systematically exceeded the target values for metal loadings in aquatic sediments of the Borska river. Diatoms are distinguished as indicators of metal pollution evident from a decrease in species richness and/or the presence of teratologies [16]. The number of deformed valves in the samples from Borska river, its tributaries, and the

Timok was negligible, while a decrease in the number of taxa in comparison to other investigated rivers was evident. In periphyton communities, stress caused by higher metal concentrations leads to a significant decline in sensitive taxa and increases in tolerant ones [2]. In almost all locations along the Borska river, its tributaries and the Timok, species *A. minutissimum*, *N. capittelata* and *N. palea* (representatives of the second NMDS cluster), were central taxa of the diatom community. Evidence of high abundances of *A. minutissimum* in rivers heavily-polluted with heavy metals has been studied by many authors [16,17]. Shifts in diatom community structure have seasonal patterns according to long-term surveys of metal-polluted streams, where resistance of *N. palea* (abundant in summer and autumn), has been reported [16]. Both *A. minutissimum* and *Nitzschia* spp. have almost completely dominated epiphyton following accidental spillage from mine ponds [18], which is in agreement with our data.

The water quality of the Timok River Basin based on IPS and CEE diatom indices is significantly diminished downstream the city of Bor, as well as on the Timok river downstream of Zaječar and after receiving the Borska river (Table 2). At locations under the influence of AMD from the industrial mining complex of Bor (locations BR1, BR2, BR6 and BR7), IPS and CEE index values suggest generally good water quality, while species composition, diversity and high conductivity suggest disturbed conditions. The IPS index shows a higher sensitivity, unlike the values of the CEE index, which almost indicates the same water quality at all locations (Table 2). Individual taxa may respond differently to water chemistry in different geographical areas, therefore, diatom indices that are created and defined in specific geographical areas and are adapted to the specific hydrological conditions of a particular country can give unreliable estimates for ecological status when applied outside the region for which they were originally developed [19]. Our result has shown that the use of CEE indexes in this way is not reliable in assessing the ecological status of rivers in Serbia.

Conclusions. Our study showed that different types of pollution shape the diatom community, and that diatoms are good indicators of multiple pressures. Conductivity is the most significant environmental parameter affecting taxon composition and distribution of diatom communities and there is an interdependency between increased conductivity and the relative abundance of taxa characteristic for heavily polluted locations. At locations under the influence of acid mine drainage, the diversity of benthic diatoms decreased and the abundance of metal-tolerant taxa increased. Although diatom indices can be used to determine general degradation and its degree in rivers, they do not show the real condition in cases where pressure deriving from acid mine drainage exists. This study highlights how biological and physico-chemical parameters along with other features of diatom assemblage (abundance of tolerant diatoms, diversity of diatoms, etc.) must be observed when interpreting the results of a solely diatom indices-based assessment of the water quality of freshwaters.

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