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Benthic diatoms of the Vrla River (Serbia) and their application in the water ecological status assessment

by

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Abstract

Epilithic diatoms from the Vrla River (Serbia) have been used to assess the ecological status of water. A total of 227 diatom taxa belonging to 50 genera were identified in the Vrla River during six research seasons with 13 dominant species recorded. *Gomphonema* (30 species), *Navicula* (28) and *Nitzschia* (26) were the most species-rich genera, followed by *Pinnularia* (12) and *Encyonema* (11). One taxa was recorded as new to Serbia – *Geissleria acceptata*. CCA grouped the diatom taxa into three main groups. The first group included taxa found at most of the sampling sites, the second group involved diatom taxa significantly positively correlated with the oxygen, while the third group showed positive correlation with temperature. RDA showed that some diatom taxa, including: *Cocconeis placentula* var. *placentula*, *C. placentula* var. *lineata*, *C. pseudolineata* and *Mayamaea atomus* var. *permitis*, are significantly positively correlated with temperature, while others, for example *Achnanthidium minutissimum*, *Hannaea arcus*, *Nitzschia pura* are mostly correlated with total phosphorus, alkalinity and water hardness. The ecological status of the Vrla River ranged from moderate, good to high. It was shown that according to the diatom indices, the ecological status of water downstream and upstream of a trout fish pond was slightly different.

Key words: new record, *Geissleria acceptata*, diatom indices, ecological status of water, Vrla River

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Introduction

The best way to assess the ecological status of water is to combine physicochemical and biological analysis. Chemical analyzes provide only an insight into the current state of the ecosystem and hence they may yield a false picture of the ecosystem state. Aquatic organisms, such as diatoms, aquatic invertebrates, fish and macrophytes are a very useful tool for evaluating the ecological status of surface waters because they are sensitive to many changes in the environment (Szczepocka et al. 2014). Diatoms are obligatory indicators in the water ecological status assessment in the EU countries (Torrisi & Dell'Uomo 2006; Szulc & Szulc 2013; Szczepocka et al. 2014; Bąk et al. 2014).

Diatoms are excellent biological indicators in the water quality monitoring, especially eutrophication, increased pollution and acidification (Furse 2006). They are often the most diverse group of photoautotrophs in freshwaters (King et al. 2006), occurring in all river sections and responding to changes in water quality (Torrisi & Dell'Uomo 2006). Benthic diatoms have become an inevitable part of water ecological status monitoring in the last decade in many countries of Europe (Kelly et al. 1995; Solak & Àcs 2011; Ivanov et al. 2003; Eloranta & Soinien 2002).

Even though the diatoms are one of the most important and influential organisms, and consequently excellent indicators of water ecological status, the applicability of diatom indices for the assessment of the ecological status of flowing waters in Serbia is still insufficiently explored. Andrejić (2012) conducted an assessment of the ecological status of the Nišava River and its tributaries Jerma and Temska based on the Trophic Diatom Index (TDI). The Diatom Pollution Index (DAIpo) is used as an ecological indicator of organic pollution in the Djetinja River (Krizmanić et al. 2013). Vidaković (2013) determined the ecological status of the Raška River based on 17 indices calculated with the help of the software package OMNIDIA (Lecointe et al. 1993). Benthic diatoms were used by Vasiljević et al. (2014) in the ecological status assessment of small streams in hilly terrains of Eastern Serbia. Four diatom indices (DAIpo, BDI, TDI and IPS) were used to assess the ecological status of the Danube-Tisza-Danube (DTD) hydrosystem in Northern Serbia (Jakovljević et al. 2014).

The main objective of this study was to evaluate the ecological status of the Vrla River based on the diatom indices and to test their use for the assessment of the ecological status of flowing waters in Serbia. Furthermore, the objective was to present the floristic richness of diatoms developing in the Vrla River. No data have been published to date on the diatom flora in the Vrla River.

Study area

The Vrla River is a right-hand tributary of the South Morava River in the southeast of Serbia. It is about 28 km long with a basin of about 500 km^2. The spring is situated between Vardenik Mountain and Vlasina Lake. The Vrla River flows into the South Morava River in the center of a little town called Vladičin Han, at an altitude of 323 m a.s.l. While the basin has characteristics of an uncultivated mountainous land in its upper parts, a cement-mortar cover with stone chips protects the riverbed against erosion processes in its urban areas. The Vrla River is often considered to be part of the Vlasina hydrosystem due to its proximity to the spring of the Vlasina River and Vlasina Lake (Đeković et al. 2010). Three of the four hydropower stations of the Vlasina-Vrla hydrosystem were built in the Vrla River valley. Samples were collected from 6 sampling sites along the river, at an altitude ranging from 961 to 1056 m a.s.l. (Fig. 1).

The dominant substrate at the first sampling site were stones and rocks, whereas gravel occurred only occasionally. While stones covered with mosses were the dominant type of substrate at the second sampling site, stones and gravel prevailed at the third site. Silt occurred along the left bank as a result of the deposition of sediments originating from a pond. The bottom of the river at the fourth sampling site consisted of rocks and gravel, and silt occurred on the right bank. Rocks, stones, sand and gravel were the dominant type of substrate at the fifth and the sixth sampling site. A trout fish pond was built in the upper part of the investigated section of the Vrla River, between the second and the third sampling site.

Materials and Methods

Sampling was conducted at 6 sampling sites (VR1-VR6) along the Vrla River, during the six seasons (May, July, September, November 2011 and March and May 2012).

Water temperature-T, conductivity, pH, ammonium ions, nitrates, oxygen-O_2, biochemical oxygen demand (BOD5), alkalinity, total phosphorus-TP, orthophosphates-OP, and water hardness-WH were measured at each site. Water temperature, pH and conductivity were measured with a Lovibond Multimeter WTW 340i. Ammonium ions, nitrates, oxygen, alkalinity, total P, orthophosphates and total hardness were measured

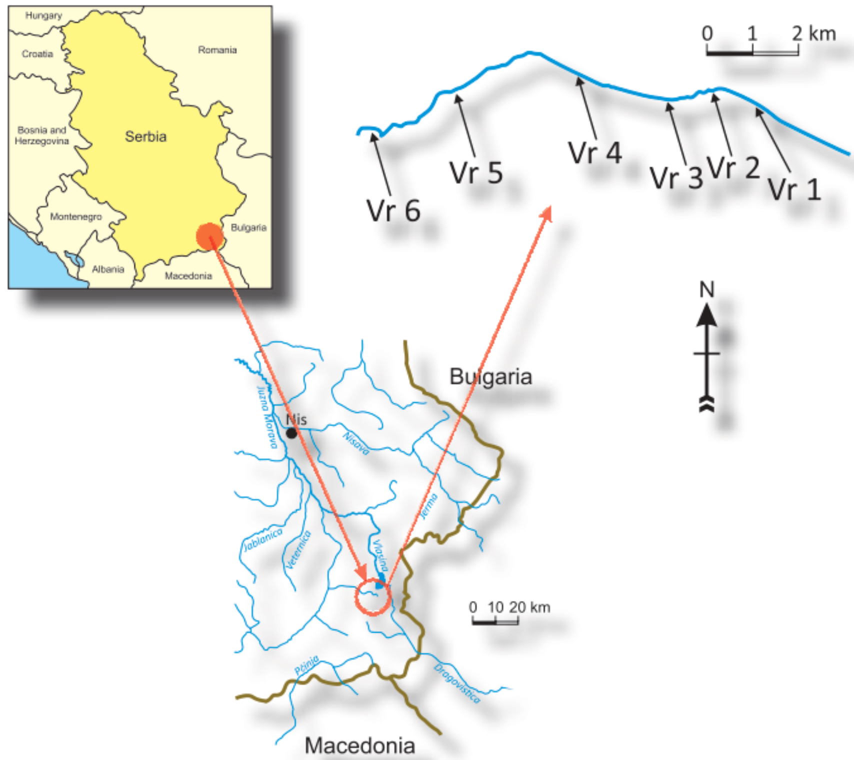

Figure 1

Distribution of the sampling sites (VR1-VR6) along the Vrla River

using a Lovibond photometer PC Multidirect. These parameters were measured in situ except BOD5 which was measured in a laboratory.

Diatoms were collected from stones by cleaning them with a toothbrush. The material was immediately fixed with formaldehyde and preserved to the final concentration of 4%. Prior to the preparation of permanent slides, algological samples were treated with a standard method using cold acid (Krammer & Lange-Bertalot 1986) and then mounted in a synthetic medium Naphrax®. Algological material was observed using a light microscope Zeiss AxioImager.M1 with a digital camera AxioCam MRc5 and AxioVision 4.8 software.

The following literature was used for the identification of diatoms: Krammer & Lange-Bertalot 1986, 1988, 1991, 1991a; Krammer 1997, 2000, 2002; Reichardt 1999; Lange-Bertalot 2001; Hofmann et al. 2013.

Four hundred valves were counted on each slide to determine the relative abundance of each taxa. Calculation of diatom indices was performed using the Omnidia software (Lecointe et al. 1993) and the ecological status of the Vrla River was assessed based on the indicator values of identified taxa. Six diatom indices were taken into consideration to determine the ecological status of the Vrla River: IPS, SLA, DESCY, CEE, IBD and TDI. These indices have been chosen because they are routinely used to assess the biological quality of European rivers in national networks. The IPS is used in Luxembourg and Spain, the SLA in Hungary and Portugal, the DESCY in Belgium and Luxembourg, the CEE in France, Belgium, Luxembourg, Andorra, Spain, Greece, Hungary and Portugal, the IBD in France, Luxembourg, Hungary and Portugal and the TDI in England (Lek et al. 2005). Furthermore, the legal regulation of the Republic of Serbia prescribes the use of IPS and CEE for the ecological status assessment of surface waters. All these indices evaluate the water ecological status in a range between 1 and 20, except for TDI whose values range from 1 to 100 (Descy 1979; Sládecek 1986; Descy & Coste 1991; Nieuwenhuis et al. 2005; Coste et al. 2009), where 1 indicates a very bad and 20 a very good water ecological status, and in the case of TDI – 1 indicates a very good and 100 a very bad water ecological status (Kelly & Whitton 1995).

Statistical analyses

Statistical analyses were performed using CANOCO for Windows Version 5 (Ter Braak & Šmilauer 2012). Canonical correspondence analysis CCA was used to visualize the relationships between the identified diatom taxa and the measured physicochemical data (explanatory environmental variables) with sampling sites and sampling seasons included as supplementary variables. The presence-absence data were used as a measure, so a unimodal method was applied. Two hundred twenty six diatom taxa were included in the analysis, but due to the high number of taxa, only 47 of the best fitting taxa (which percentage of variation is best explained by the ordination space) were shown on the graphs. The redundancy analysis (RDA) was performed to examine the potential effects of the measured environmental variables on the dominant diatom taxa with sampling sites and sampling seasons used as supplementary variables. RDA was used since the gradient was 1.6 SD units long. The dominant taxa were represented as a percentage calculated based on the valve percentage representation of each taxon relative to the total of 400 valves on each permanent slide. RDA with the option center and standardize was used and response data (diatom taxa) were log transformed. The statistical significance of each variable in both analyses (for the level of 0.01) was assessed using the Monte Carlo unrestricted permutation test involving 4999 permutations.

Results

Taxonomic analysis

A taxonomically diverse diatom flora was identified in 36 analyzed samples, including 227 diatom taxa belonging to 50 genera. *Gomphonema* (30 species), *Navicula* (28) and *Nitzschia* (26) were the most species-rich genera, followed by *Pinnularia* (12) and *Encyonema* (11). A considerable number of genera (18 or 36%) were represented by only one species. *Achnanthidium minutissimum* (Kützing) Czarnecki, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Amphora pediculus* (Kützing) Grunow, *Geissleria acceptata* (Hustedt) Lange-Bertalot & Metzeltin, *Gomphonema elegantissimum* Reichardt & Lange-Bertalot, *Navicula antonii* Lange-Bertalot, *Nitzschia abbreviata* Hustedt, *Planothidium lanceolatum* (Brébisson) Bukhtiyarova and *Reimeria sinuata* (Gregory) Kociolek & Stoermer occur in all seasons and in all samples. *Amphora inariensis* Krammer, *Cocconeis placentula* var. *lineata* (Ehrenberg) van Heurck, *Diatoma mesodon* (Ehrenberg) Kützing, *Hannaea arcus* (Ehrenberg) Patrick and *Navicula gregaria* Donkin were absent in only one sample. Thirteen taxa were defined as dominant. Their percentage contribution was 10% or more at least at one site (Table 1).

A taxon new to Serbia was found in the analyzed material – Geissleria *acceptata* (Hustedt) Lange-Bertalot & Metzeltin. Valves are elliptical to linear-elliptical; length 6.34-12.41 μm, breadth 3.89-4.64 μm, striae 16-18 per 10 μm which corresponds with literature data (Lange-Bertalot, 2001) (Fig. 2). It was found in all samples, in abundance ranging from 0.3 to 4.5% in the Vrla River. The pH values in the analyzed material varied from 7 to 8.8, the conductivity – from 58 to 115 μS cm^{-1} and the water hardness – from 0.12 to 12.24 °dH.

Physicochemical analysis of water

The values of physicochemical parameters of the Vrla River are presented in Table 2. The water temperature during the sampling period ranged from 2 to 17.3°C, pH ranged from 7 to 8.8 and conductivity from 50 to 110 μS cm^{-1}. Water hardness varied from 0.12 to 12.24, which means that water can be described as soft, moderately hard, hard to very hard depending on the season.

Canonical Correspondence analysis (CCA) was used to evaluate the relationship between the examined environmental parameters and diatom taxa recorded in the Vrla River (Fig. 3).

Table 1

Dominance in diatom communities in the Vrla River at sites VR1-VR6 in 2011-2012, in six seasons

% occurrence: 0 (white) | <10 (green) | 10≤20 (blue) | 21≤40 (olive)

Season and site	05.2011						07.2011						09.2011						11.2011						03.2012						05.2012					
Taxa	VR1	VR2	VR3	VR4	VR5	VR6	VR1	VR2	VR3	VR4	VR5	VR6	VR1	VR2	VR3	VR4	VR5	VR6	VR1	VR2	VR3	VR4	VR5	VR6	VR1	VR2	VR3	VR4	VR5	VR6	VR1	VR2	VR3	VR4	VR5	VR6
Achnanthidium minutissimum	10≤20	21≤40	10≤20	10≤20	10≤20	21≤40	10≤20	10≤20	10≤20	10≤20	10≤20	<10	<10	<10	10≤20	10≤20	<10	<10	<10	<10	<10	10≤20	21≤40	<10	<10	<10	10≤20	10≤20	10≤20	10≤20	10≤20	10≤20	10≤20	10≤20	<10	<10
Achnanthidium subatomus	10≤20	<10	<10	<10	<10	<10	<10	10≤20	<10	<10	10≤20	<10	<10	<10	<10	<10	<10	<10	10≤20	<10	<10	<10	<10	<10	<10	<10	<10	<10	10≤20	<10	<10	<10	<10	<10	<10	<10
Cocconeis placentula var. *lineata*	<10	0	<10	<10	<10	<10	<10	<10	<10	<10	0	10≤20	21≤40	10≤20	10≤20	10≤20	21≤40	21≤40	<10	10≤20	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Cocconeis placentula var. *placentula*	<10	0	0	0	0	0	<10	<10	<10	0	<10	<10	10≤20	<10	<10	<10	10≤20	10≤20	<10	<10	<10	<10	<10	<10	<10	0	<10	<10	0	0	0	<10	<10	<10	0	<10
Cocconeis pseudolineata	<10	0	<10	0	0	0	<10	<10	<10	<10	<10	10≤20	21≤40	10≤20	<10	<10	<10	<10	<10	<10	<10	<10	<10	10≤20	<10	<10	<10	<10	<10	0	<10	0	<10	<10	<10	<10
Gomphonema elegantissimum	10≤20	10≤20	<10	<10	<10	<10	10≤20	10≤20	<10	<10	10≤20	10≤20	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Gomphonema pumilum var. *rigidum*	<10	<10	<10	<10	0	0	<10	<10	0	<10	<10	<10	<10	<10	0	0	0	0	<10	10≤20	<10	<10	<10	0	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Hannaea arcus	<10	<10	<10	10≤20	10≤20	<10	0	<10	<10	<10	0	<10	0	<10	0	0	<10	<10	<10	<10	<10	<10	<10	<10	<10	10≤20	<10	21≤40	10≤20	<10	<10	<10	<10	<10	<10	<10
Mayamaea atomus var. *permitis*	<10	<10	<10	<10	0	0	<10	<10	<10	10≤20	<10	<10	0	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	0	<10	<10	0	0	0	<10	<10	<10	<10	<10	<10
Nitzschia abbreviata	<10	<10	10≤20	<10	<10	10≤20	<10	<10	21≤40	21≤40	21≤40	10≤20	0	<10	<10	10≤20	10≤20	21≤40	<10	<10	10≤20	10≤20	21≤40	21≤40	<10	<10	<10	<10	<10	<10	<10	<10	<10	10≤20	10≤20	<10
Nitzschia archibaldii	<10	0	<10	<10	0	0	0	0	<10	<10	0	0	0	0	10≤20	<10	<10	0	0	0	<10	<10	<10	<10	0	<10	<10	0	0	0	0	0	<10	0	<10	<10
Nitzschia pura	<10	<10	10≤20	10≤20	10≤20	<10	0	<10	0	0	0	<10	0	0	0	0	<10	0	<10	<10	<10	<10	0	0	10≤20	10≤20	<10	<10	<10	<10	<10	<10	0	<10	<10	<10
Planothidium lanceolatum	<10	<10	<10	<10	<10	<10	10≤20	10≤20	<10	<10	<10	<10	<10	10≤20	<10	<10	<10	<10	<10	<10	10≤20	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	10≤20	10≤20	<10	<10	10≤20

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Table 2

Chemical water parameters of the Vrla River at the studied sites (VR1-VR6) in 2011-2012

Parameter	VR1	VR2	VR3	VR4	VR5	VR6
Temperature (°C)	2.2-13.0	2.3-12.4	2.0-15.0	2.3-15.2	2.5-15.5	2.0-17.3
Conductivity (µS cm^{-1})	50-90	50-80	60-100	60-90	60-90	70-110
pH	7.0-8.4	7.1-8.6	7.0-8.8	7.1-8.7	7.0-8.7	6.9-8.3
Ammonia (mg l^{-1})	<0.01-0.15	<0.01-0.13	<0.01-0.26	<0.01-0.16	<0.01-0.18	<0.01-0.10
Nitrates (mg l^{-1})	1.7-3.4	1.5-2.7	1.6-2.9	1.5-3.1	1.5-2.8	1.3-2.1
Oxygen (mg l^{-1})	9.4-15.1	9.2-14.8	9.0-13.9	9.0-14.2	9.1-14.3	8.8-13.3
BOD5 (mg l^{-1})	0.4-8.6	<0.2-6.6	<0.2-6.7	0.3-6.1	0.2-6.2	0.3-6.0
Alkalinity (Mmoll^{-1})	0.4-2.6	0.4-2.3	0.7-2.6	0.5-2.6	0.4-2.8	0.5-2.5
Total P (mg Pl^{-1})	0.03-0.06	0.02-0.07	0.02-0.09	0.02-0.05	0.02-0.07	0.02-0.07
Orthophosphates (mg Pl^{-1})	0.02-0.04	0.01-0.04	0.01-0.05	0.01-0.03	0.01-0.03	0.01-0.03
Water hardness (°dH)	0.12-12.01	0.15-11.79	0.22-11.79	0.16-11.79	0.16-12.24	0.16-12.24

CCA showed high statistical significance (F = 1.4, P = 0.0002). The first CCA axis shows the highest positive correlation with oxygen (r = 0.4399), and the second CCA axis showed a positive correlation with temperature (r = 0.6015). Conductivity showed a positive correlation with both CCA axes, with slightly higher correlation with the second axis (first axis: r = 0.4462, second axis: r = 0.5159). Alkalinity and water hardness were positively correlated with the third CCA axis (not shown on the ordination diagram). The temperature was dependent on the season and the lowest value was recorded in S5 and the highest one in S3 (at VR6). In general, considering average values of temperature at the sampling sites during all seasons, VR3 had the lowest, while VR6 the highest average temperature. Oxygen showed a reverse pattern and was negatively correlated with temperature (the angle between the vectors of these two variables is nearly 180 degrees indicating almost perfect negative correlation). Conductivity was low during S6 (lowest at VR1) and the highest in S3 and S5. Sampling site VR6 showed the highest values of conductivity in all seasons (70-110 µS cm^{-1}). High values of alkalinity and very high values of water hardness (WH) compared to other seasons were also recorded during S1 at sampling sites VR5 and VR6. Orthophosphates (OP) and NO_3 had the highest values recorded at VR3 in S1 and S2, respectively.

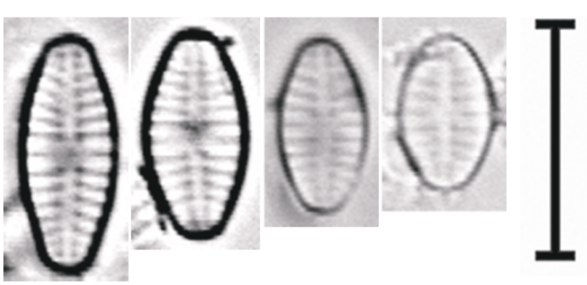

Figure 2

LM micrographs of *G. acceptata*. Scale bar = 10 µm

CCA divided the diatom taxa into three main groups. The first group is composed of diatom taxa (for example *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Sellaphora radiosa* (Hustedt) H. Kobayasi, *Fragilaria recapitellata* Lange-Bertalot & Metzeltin, *Psammothidium daonense* (Lange-Bertalot) Lange-Bertalot) found at most of the sampling sites (in the center of the ordination diagram) (Fig. 3). The second group (the right side of the ordination diagram) is represented by taxa found only at sampling site VR6 in S5 (for example *Amphora copulata* (Kützing) Schoeman & Archibald, *Gomphonema acuminatum* Ehrenberg, *Gyrosigma sciotoense* (Sullivant) Cleve, *Nitzschia recta* Hantzsch ex Rabenhorst, *Staurosirella pinnata* var. *intercedens* (Grunow) Hamilton, *Surirella brebissonii* var. *kuetzingii* Krammer & Lange-Bertalot). These taxa apparently tend to occur at the higher values of oxygen and conductivity. The third group of diatom taxa is placed in the upper part of the ordination diagram and it is positively correlated with temperature and conductivity. These taxa, including *Cymatopleura solea* var. *apiculata* (W. Smith) Ralfs, *Gomphonema gracile* Ehrenberg, *Navicula amphiceropsis* Lange-Bertalot & Rumrich, *Cymbopleura cuspidata* (Kützing) Krammer,

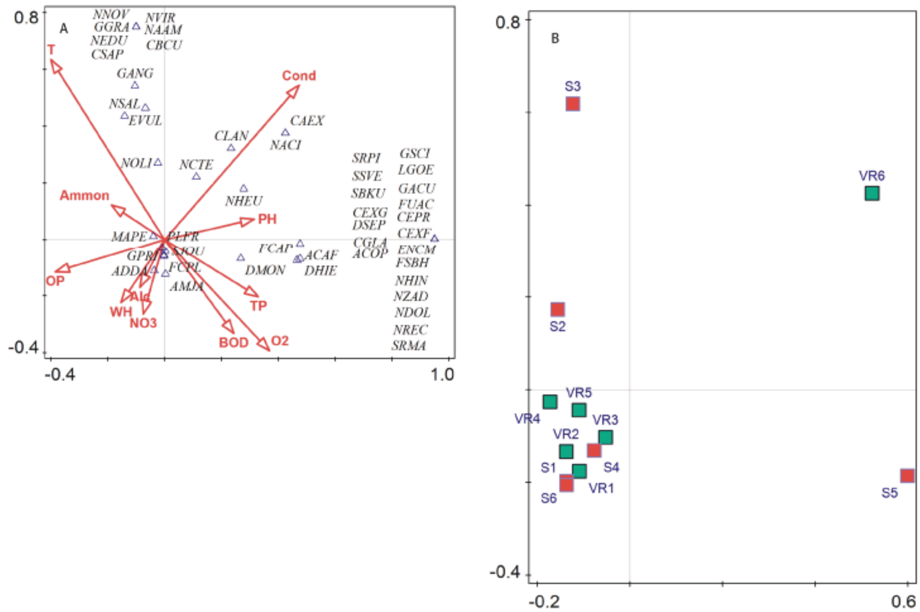

Figure 3

Canonical correspondence analysis (CCA) biplot ordination on the basis of the measured environmental variables (temperature-T, conductivity, pH, total phosphorus-TP, O2, biochemical oxygen demand-BOD, NO3, alkalinity, water hardness-WH, orthophosphates-OP and ammonium) and diatom taxa recorded in the Vrla River, with sampling sites (VR1-VR6) and sampling seasons (S1-S6) as supplementary variables (F=1.4, P=0.0002). Due to the large number of data, the graphs A and B are not overlapped, and diatom taxa and explanatory variables (environmental data) are shown on graph A, while sampling sites and sampling seasons used as supplementary variables are shown on graph B for better visualization. NNOV – *Navicula novaesiberica*, GGRA – *Gomphonema gracile*, NEDU – *Neidium dubium*, CSAP – *Cymatopleura solea* var. *apiculata*, NVIR – *Navicula viridula*, NAAM – *Navicula amphiceropsis*, CBCU – *Cymbopleura cuspidata*, GANG – *Gomphonema angustatum*, NSAL – *Navicula salinarum*, EVUL – *Encyonema vulgare*, NOLI – *Navicula oligotraphenta*, NCTE – *Navicula cryptotenella*, CLAN – *Caloneis lancettula*, CAEX – *Cymbella excisa*, NACI – *Nitzschia acicularis*, NHEU – *Nitzschia heufleriana*, MAPE – *Mayamaea atomus* var. *permitis*, PLFR-*Planothidium frequentissimum*, GPRI – *Gomphonema pumilum* var. *rigidum*, ADDA – *Psammothidium daonense*, SJOU – *Sellaphora radiosa*, FCPL – *Fragilaria recapitellata*, AMJA – *Achnanthidium minutissimum* var. *jackii*, FCAP – *Fragilaria capucina*, DMON – *Diatoma moniliformis*, ACAF – *Achnanthidium affine*, DHIE – *Diatoma hyemalis*, SRPI – *Staurosirella pinnata* var. *intercedens*, SSVE – *Staurosira venter*, SBKU – *Surirella brebissonii* var. *kuetzingii*, CEXG – *Cymbella exigua*, DSEP – *Diploneis separanda*, CGLA – *Handmannia glabriuscula*, ACOP – *Amphora copulata*, GSCI – *Gyrosigma sciotoense*, LGOE – *Luticola goeppertiana*, GACU – *Gomphonema acuminatum*, FUAC – *Fragilaria acus*, CEPR – *Cymbella excisa* var. *procera*, CEXF – *Cymbella excisiformis*, ENCM – *Encyonopsis microcephala*, FSBH – *Fallacia subhamulata*, NHIN – *Navicula hintzii*, NZAD – *Nitzschia adamata*, NDOL – *Nitzschia oligotraphenta*, NREC – *Nitzschia recta*, SRMA – *Martyana martyi*

Navicula novaesiberica Lange-Bertalot are found only at sampling site VR6 in S3. Sampling site VR6 was characterized by high values of conductivity in all seasons (70-110 μS cm^{-1}). Apparently, some diatom taxa such as *Navicula cryptotenella* Lange-Bertalot, *Cymbella excisa* Kützing, *Nitzschia acicularis* (Kützing) W. Smith show a high correlation with conductivity, and they occurred in different seasons at sampling site VR6 only.

Potential effects of the measured environmental variables on the dominant diatom taxa were investigated using RDA (Fig. 4).

The first RDA axis is positively correlated with temperature (r = 5.050), and negatively correlated mainly with total phosphorus (r = -0.5651), alkalinity (r = -0.5106) and water hardness (r = -0.5776). The second RDA axis is negatively correlated with pH (r = -0.6019) and conductivity (r = -0.6497). The other measured environmental variables were weakly correlated (positively or negatively) with both RDA axes. *Cocconeis placentula* var. *lineata*, *Cocconeis placentula* var. *placentula* Ehrenberg and *Cocconeis pseudolineata* (Geitler) Lange-Bertalot (all abundant in S3), *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot (abundant in S2 and S6), as well as *Nitzschia abbreviata* and *Nitzschia archibaldii* Lange-Bertalot are positively correlated with temperature and with the first RDA axis. All above-mentioned diatom taxa, except *Mayamaea atomus* var. *permitis* were found in higher percentage at sampling site VR6, so they are also correlated with conductivity. *Hannaea arcus* and *Nitzschia pura* Hustedt were the most abundant in S1 and S5 and most positively correlated with pH and variables that show a negative correlation with the first RDA axis (alkalinity, water hardness and total phosphorus). *Achnanthidium minutissimum*, *Achnanthidium subatomus*, *Gomphonema elegantissimum*, *Gomphonema pumilum* var. *rigidum* Reichardt & Lange-Bertalot were also correlated with

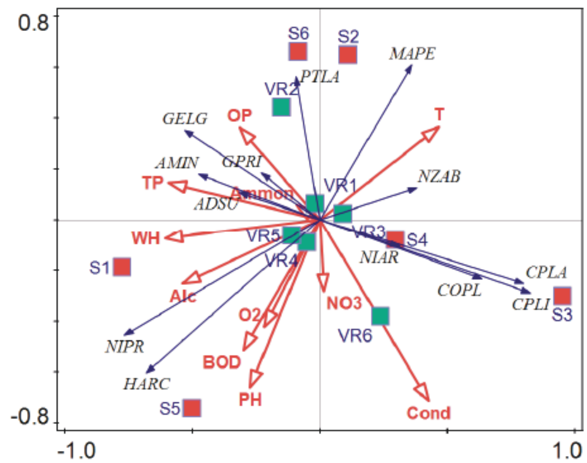

Figure 4

Redundancy analysis (RDA) of dominant diatom taxa and measured environmental variables

(temperature-T, conductivity, pH, total phosphorus-TP, O_2, biochemical oxygen demand-BOD, NO_3, alkalinity, water hardness-WH, orthophosphates-OP and ammonium) with sampling sites (VR1-VR6) and sampling seasons (S1-S6) as supplementary variables. AMIN – *Achnanthidium minutissimum*, HARC – *Hannaea arcus*, NIPR – *Nitzschia pura*, ADSU – *Achnanthidium subatomus*, GELG – *Gomphonema elegantissimum*, GPRI – *Gomphonema pumilum* var. *rigidum*, PTLA – *Planothidium lanceolatum*, COPL – *Cocconeis pseudolineata*, MAPE – *Mayamaea atomus* var. *permitis*, CPLA – *Cocconeis placentula* var. *placentula*, CPLI – *Cocconeis placentula* var. *lineata*, NIAR – *Nitzschia archibaldii*, NZAB – *Nitzschia abbreviata*

TP, WH and Alc (and also with OP), but together with *Planothidium lanceolatum* were most abundant at VR2 where very low conductivity was determined, so they all showed a negative correlation with conductivity. Roughly speaking, RDA divided the taxa into two groups: those found on the left side of the ordination diagram correlated mainly with TP, WH and Alc (some of them with OP and pH) and those placed at the right side of the ordination diagram correlated mainly with temperature.

Diatom indices

Values of the six diatom indices indicated a similar ecological status of the water at the studied sites (Table 3). The correlation between the selected indices is shown in Table 4. The highest correlation is between CEE, IPS and IBD (Table 4). These three indices had the highest values at sampling sites VR1 and VR2, while the lowest at VR5 or VR6 (Table 3). DESCY was slightly different and its maximum value was calculated at sampling sites VR6 and VR5, and the lowest at VR2 (Table 3). Values of the CEE, IPS and DESCY indices clearly indicated good (class II) ecological status (Table 3) of the Vrla River at all the studied sites. Values of the IBD index for sampling sites VR1 and VR2 indicated high (class I) ecological status (Table 3). Also, chemical analysis of pH, ammonia, OP and TP indicated good and high ecological status of the Vrla River (Table 2). The highest value of the diatom index SLA was determined at sampling site VR1, and the lowest one at VR3 and VR4 (Table 3). Values of the SLA index for sampling sites VR2, VR3, VR4 and VR6 indicated a moderate ecological status or class III (Table 3), although these values were close to values indicating a good ecological status. The highest value of TDI was at sampling site VR3 (Table 3). Based on the TDI index, the Vrla River was characterized by moderate concentration of nutrients (class III) (Table 3). Table 3 clearly shows the ecological status of the Vrla River which ranges from moderate, good to high. Also, the percentage contribution of species characteristic of organic pollution (%PT) was calculated. This is related to TDI. The 20% contribution of these species indicates organic pollution and very high values of PT (over 40%) indicate the risk of eutrophication (Kelly et al. 1995). %PT was over 20% at sampling sites VR3, VR4 and VR5 in season S1, at VR3 in season S3 and at VR2 in season S5.

Table 3

Average values of diatom indices and water quality classes at the studied sites (VR1-VR6) of the Vrla River in 2011-2012

Site	Values of diatom indices / Water Quality Class											
	IPS	SLA	DESCY	CEE	IBD	TDI	IPS	SLA	DESCY	CEE	IBD	TDI
VR1	16.55	13.38	15.71	15.31	17.15	55.1	II	II	II	II	I	III
VR2	16.56	12.78	15.21	15.35	17.33	55.03	II	III	II	II	I	III
VR3	15.43	12.63	15.55	13.91	14.76	56.56	II	III	II	II	II	III
VR4	15.30	12.56	15.23	13.81	14.00	49.28	II	III	II	II	II	III
VR5	15.25	13.28	15.76	13.63	13.76	47.73	II	II	II	II	II	III
VR6	15.36	12.88	16.05	14.66	13.73	52.88	II	III	II	II	II	III

Table 4

Correlation coefficients for selected diatom indices (IPS, SLA, DESCY, CEE, IBD and TDI)

	IPS	SLA	DESCY	CEE	IBD	TDI
IPS	1					
SLA	0.318	1				
DESCY	-0.287	0.535	1			
CEE	0.905*	0.268	-0.019	1		
IBD	0.986*	0.243	-0.378	0.842*	1	
TDI	0.585*	-0.153	-0.051	0.615*	0.637*	1

Legend: *statistically significant correlation coefficients

Discussion

Taxonomic analysis and physicochemical analysis of water

Great diatom species richness was documented during the floristic survey of the Vrla River. Species diversity determined with the use of the software Omnidia was in the range of 3.06 to 4.77. Clean rivers with a low level of pollution, such as the Linda River (Szczepocka et al. 2014) and the Pilica River (Szulc 2007), are also characterized by high species diversity. A similar species richness was observed in rivers located (like the Vrla River) in the southeast of Serbia: the Nišava, the Jerma and the Temska River (Andrejić et al. 2012). Furthermore, the most species-rich genera were the same as in our study. Species of the genera *Navicula* and *Nitzschia* occur frequently in Central Europe (Noga et al. 2013a) and Euro-Asia (Solak et al. 2012). Similar diatom communities were recorded in other rivers of Serbia but also in some rivers in other parts of Europe and Euro-Asia (Nikitović 1998; Tomašević 2000; Jurišić 2004; Torrisi & Dell'Uomo 2006; Andrejić 2012; Solak et al. 2012; Várbíró et al. 2012; Noga et al. 2013b).

Achnanthidium minutissimum has a much wider range of tolerance to various environmental factors as compared to other *Achnanthidium* species. Teratological forms of *Achnanthidium minutissimum*, *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst, *Fragilaria rumpens* (Kützing) Carlson, *F.* cf. *crotonensis* Kitton were found in streams polluted by acid mine drainage, which is related to high levels of metals (Silva et al. 2009; Luis et al. 2015). *A. minutissimum* is one of the most metal-tolerant species (Cantonati et al. 2014).

Achnanthidium subatomus achieves its optimum in oligotrophic, calcareous waters with moderate to very low content of electrolytes (Hofmann et al. 2013). *Cocconeis placentula* var. *lineata* and *C. placentula* var. *euglypta* (Ehrenberg) Grunow have a wider ecological range than *C. pediculus* Ehrenberg, especially with regard to the electrolyte content and the trophic situation. These species are found even in streams with a lower content of electrolytes, dominated by silicates (Hofmann et al. 2013).

Gomphonema elegantissimum is widespread in karst regions in the Balkan Peninsula and fairly common in other limestone areas of Central Europe (Reichardt 1997).

Geissleria acceptata is a new taxon in the diatom flora of Serbia. It is the only *Geissleria* species that can have or lack an isolated pore (Novais et al. 2013). It is probably a cosmopolitan species, found in meso- to eutrophic waters, but also in oligosaprobic waters with a moderate electrolyte content (Lange-Bertalot 2001), same as waters of the Vrla River. The species occurs mostly in rivers and streams of a silicate mountain range, but it was also frequently found in alkaline lakes of the lowlands (Hofmann et al. 2013). *G. acceptata* is generally considered to be a representative of good ecological status (Lek et al. 2005). Based on the results obtained in this study, the ecological status of the Vrla River is assessed as good, so our research confirms the occurrence of *Geissleria acceptata* in specific conditions.

Seasonal and spatial variations in water temperature affect the distribution of benthic diatoms in rivers. In general, the diversity of taxa increases in temperatures of 25-30°C and drops in temperatures above 30°C. In nature, however, seasonal and spatial dynamics of diatoms does not depend only on water temperature (Stevenson et al. 1996). In the Vrla River, *Achnanthidium minutissimum* was the most numerous at sites VR5 and VR6 in S1 and at VR5 in S4, where the measured temperature was <14°C. In our study, RDA showed that some species of the genus *Cocconeis* (placed on the right side of the ordination diagram) were positively correlated with temperature (*C.*

placentula var. *lineata*, *C. placentula* var. *placentula* and *C. pseudolineata*), while diatom taxa on the left side show correlation with many environmental variables, including those related to nutrients. The trophic level of water significantly influences the structure of benthic diatom communities. The significance of different nitrogen compounds is demonstrated by multivariate analyses (Vilbaste & Truu 2003), but in our case, the nitrogen compound (ammonia) was weakly correlated with the two RDA axes, and its arrow was short, meaning that it has a lesser effect on the dominant diatom taxa presented on the ordination diagram. Regarding phosphorus, OP and TP were more significant (especially TP) and correlated with many dominant diatom taxa. According to Pringle (1990), an addition of inorganic and organic phosphorus resulted in an increase in the number of motile diatoms, mainly *Nitzschia* and *Navicula* species on sandy substrates and *C. placentula* var. *placentula* and *A. minutissimum* on glass slides. Our study also showed that *Nitzschia pura* and *A. minutissimum* are positively correlated with the concentration of total phosphorus and orthophosphates and they can be found at places with a higher level of phosphates. Research on the effects of nutrient enrichment on stream algae suggested that diatom species richness declines (Peterson et al. 1985) and algal biomass increases (Bothwell 1989) along with increasing phosphorus. According to Chételator et al. (1999), diatoms reach the maximum growth rate at the total phosphorus concentration of 0.5 mg l^{-1}. Even though there have been already many studies focusing on nutrients conducted worldwide, Bellinger et al. (2006) concluded that more controlled studies (experiments) are needed to better understand how nutrients (in addition to other factors) affect algal species.

The first group of diatom taxa on the CCA ordination diagram involves diatom taxa that occurred at most of the sampling sites in more than one season and shows correlation to one or more different variables. More than half of the recorded taxa were grouped in the center of the ordination diagram, but we used 47 best fitted taxa in CCA analysis. A similar composition of communities at most of the sampling sites was characteristic of this river in general. The second group showed correlation with the higher oxygen levels. According to Van Dam et al. (1994), all above-mentioned diatom taxa (*Amphora copulata*, *Gomphonema acuminatum*, *Gyrosigma sciotoense*, *Nitzschia recta*, *Staurosirella pinnata* var. *intercedens*, *Surirella brebissoni* var. *kuetzingii*) prefer water with a moderate to high oxygen level. Diatom taxa from the third group showed correlation with temperature and conductivity. For example, *Navicula novaesiberica* lives in a wide range of temperatures (Noga & Siry 2010). Some diatoms from this group prefer water with a lower oxygen level, as reported for *Cymatopleura solea* var. *apiculata* (Van Dam et al. 1994). *Navicula cryptotenella* is a good indicator of β-mesosaprobic or even better water quality and does not occur in waters with very low or very high electrolyte concentrations. This species is sensitive to pollution (Lange-Bertalot 2001), similarly to *Nitzschia acicularis* (Krammer & Lange-Bertalot 1988). *Cymbella excisa* occurs mostly in mesotrophic to slightly eutrophic waters with a moderate electrolyte content (Hofmann et al. 2013).

Cocconeis placentula was reported by Van Dam et al. (1994) as a species occurring in environment with a higher trophic level (eutrophic taxon), higher pH and moderate nitrogen content. Belal (2012) reported that *C. placentula* had a high optimum of total phosphorus (511.5 mg l^{-1}) in the River Nile. Values of total phosphorus in our localities were significantly lower. *Nitzschia archibaldii* has ecological optimum in oligo- to mesosaprobic waters with a medium electrolyte content, the same as *Nitzschia abbreviata* (Hofmann et al. 2013). According to RDA results obtained in our study, *N. archibaldii* preferred sampling sites with higher levels of conductivity. *A. minutissimum* was absent or occurred with low abundance in rivers polluted by nutrients in Appalachia. It was shown that higher abundance of *A. minutissimum* was mostly associated with low nutrient and ionic content (Ponader & Potapova 2007). This species is classified as a species with preferences for higher trophic levels (7 level, ammonium-nitrogen content) and moderate pH (3 level) (Van Dam et al. 1994). Hofmann et al. (2013) defined *Hanneae arcus* and *Nitzschia pura* as oligosaprobic species. In our study, *H. arcus* was positively correlated with pH, alkalinity, water hardness, total phosphorus and BOD5. *Achnanthidium subatomus* was mostly found in nutrient-poor rivers within a narrow pH range from 8 to 8.3, but over a wide range of conductivity levels, with a preference for higher values. Nutrients and pH were major factors affecting the distribution of this species, although the measured chemical characteristics were poor predictors of its relative abundance (Ponader & Potapova 2007).

Diatom indices

Values of the diatom indices show that there are no major variations in the ecological status of water at the studied sites. The obtained values of the CEE, IPS and IBD indices for sampling sites VR1 and VR2 compared to VR3, VR4 and VR5 could be explained by the fact that VR3 is located 50 m below the discharge pipe of the trout pond through which the water is discharged directly into the recipient (i.e. the river). Furthermore, a high correlation was determined between IPS and IBD based on the research conducted in the Pilica River (Poland) (Szulc & Szulc 2013). Our study showed that the organic pollution indices, i.e. SLA and IPS, indicate a better ecological status as compared to the trophic index TDI. Similar results were obtained during the survey of the Matysówka (Noga et al. 2013a) and Baryczka stream (Noga et al. 2013b) in Poland. The highest TDI value was recorded at sampling site VR3 (near the pond) and the lowest one at VR5. In accordance with this, we obtained the expected results for %PT. There is a possibility of organic pollution at sampling sites VR3, VR4 and VR5. This is not surprising because these sites are located downstream of the trout fish pond.

The study supports the use of diatom indices, especially IPS, DESCY and CEE, to monitor rivers in Serbia. Values of IPS, DESCY and CEE indices were most similar indicating a good ecological status of the Vrla River which is confirmed by chemical data. Altogether 104 diatom taxa (45.81% of the total number of species found in the Vrla River) were used in the calculation of the 17 indices using the Omnidia software. Differences in the values of particular indices were not significant. Statistical analyzes show that the biological methods are the best tool for the water ecological status assessment. They accurately show the condition of the ecosystem, contrary to physical and chemical parameters which can indicate the current state but not necessarily the true one. The ecological status assessment of rivers can be used for many purposes (e.g. warning and sustainable development of the region) (Ivanković et al. 2011). The applicability of diatom-based indices depends on the local characteristics of the river and the type of substrate (Kwandrans et al. 1998) but also the similarity in species composition in the study area and the taxa used in each index.

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