The application of benthic diatoms in water quality assessment (Mlava River, Serbia)

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Abstract–The main objective of this study was to assess the ecological status of the Mlava River based on epilithic diatoms and to test the use of diatom indices as a tool for estimating the quality of flowing waters in Serbia. Quantitative analysis showed that in April *Achnanthidium minutissimum* was dominant at each site, except at the fifth site, where *Amphora pediculus* was dominant. In July and September, *Achnanthidium minutissimum, Achnanthidium pyrenaicum, Amphora pediculus, Denticula tenuis, Diatoma vulgaris, Gomphonema elegantissimum, Cocconeis pseudolineata* and *Cocconeis placentula* var. *lineata* dominated. Detrended correspondence analysis (DCA) was used to detect the major patterns of variation in species composition. The first DCA axis summarizes the distribution of the diatom community, mainly through temperature, conductivity, oxygen and water hardness gradient. The second DCA axis was weakly correlated with few variables. Based on the average values of the pollution sensitivity index (IPS), commission for economical community metric (CEE) and biological diatom index (IBD), the water of the Mlava River belonged to water class I during all three seasons. Values of the diatom-based eutrophication/pollution index (EPI-D) indicated class II water quality. According to calculated trophic diatom index (TDI) values, water of the Mlava River was characterized by intermediate nutrient concentrations during three seasons. Principal components analysis was used to represent the correlation between diatom indices, and the highest correlation among the selected diatom indices is seen between EPI-D, IPS and IBD.

Key words: biomonitoring, diatoms, indices, Mlava River

Introduction

The best way to evaluate the ecological status of waters is the simultaneous use of physico-chemical and biological analysis. Use of only physico-chemical methods gives unreliable results, because water quality may change over a short period of time. The really current state of the ecosystem can only be obtained by using biological methods. According to the Water Framework Directive (WFD 2000/60/EC), biological indicators play a key role in the assessment of ecological status. Biological assessment is expressed according to a numerical scale between zero and one – the 'ecological quality ratio' (EQR). Values of this scale ranged from zero to one, with high status represented by values close to one and bad status by values close to zero. 'Macrophytes and phytobenthos' are together one of the biological quality elements (BQEs) for assessment of the ecological status of European rivers and lakes. In total, 66 macrophyte and phytobenthos assessment systems (32 for macrophytes, 30 for phytobenthos and 4 combined) have been developed and intercalibrated (Poikane et al. 2016). When it comes to phytobenthos, benthic diatoms are a valuable tool in water quality assessment and monitoring because they occur in most aquatic ecosystems, at any time, are easy to collect and sensitive to physical, chemical and biological changes in the water (Vasiljević et al. 2014). However, there are some difficulties in the use of diatoms in water quality assessment. One disadvantage is the large number of taxa involved, which is partly resolved by using an index based on genera (Rumeau and Coste 1988). Also, considerable taxonomic expertise is required (Ács et al. 2004). In addition, pH, conductivity, total phosphorus, temperature, alkalinity, altitude, nitrates, calcium, biological oxygen demand (BOD), chlorophyll *a* and substrate type are major environmental factors that affect diatom distribution in streams, in addition to light availability, total phosphorus and grazing pressure (Toman et al. 2014).

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Diatoms have been used for water evaluation purposes for more than a decade in many countries of Europe (Austria, Switzerland, Germany, Belgium, France, Poland, Finland, Luxemburg, United Kingdom, Spain, Portugal, Italy). According with the requirements of the WFD, the epilithic diatoms were investigated for ecological status assessment of the Aksu and Isparta streams, as well as the Porsuk and Karasu rivers in Turkey (Solak 2011). Ecological status assessment, based on diatom indices, is still a new topic in Turkey and the number of studies on this topic is constantly on the increase (Solak and Àcs 2011). Diatom indices have been widely used for Polish rivers and streams. Algological investigation of 38 Polish rivers was conducted and it was found that generic diatom index and pollution sensitivity index (IPS) indices are the most appropriate for Polish conditions. Many rivers were characterized by high and good ecological status (Solak 2011). Diatom studies have been sporadic and focused on large rivers in Hungary. Ács et al. (2009) investigated 398 streams and found that more than half had good ecological status based on the values of IPS, Austrian saprobic index and Austrian trophic index. The implementation of EU standards has been started and these countries possess information on ecological status of their most important rivers (Solak 2011).

The Official Gazette of the Republic of Serbia (»Sl. glasnik RS«, No. 74/2011) prescribes the use of two diatom indices: IPS (Coste in Cemagref 1982) and commission for economical community metric (CEE) (Descy and Coste 1991). The WFD has not been sufficiently integrated in Serbia. Examination of diatom indices applicability in the assessment of the ecological status of flowing waters in Serbia started during 2012 (Andrejić 2012). In that study, the trophic diatom index (TDI) was used in assessment of the water quality of the Nišava River and its tributaries the Jerma and Temska. After that, the ecological status of the Djetinja River was determined using the diatom pollution index (DAIpo) (Krizmanić et al. 2013). In all, 17 diatom indices were calculated with the help of a software package OMNIDIA (Lecointe et al. 1993) based on which the quality of the Raška River was estimated (Vidaković 2013). Water quality assessment of the study area of the Danube-Tisza-Danube (DTD) hydrosystem has been evaluated by four diatom indices (Watanabe's index-DAIpo, biological diatom index-IBD, TDI and IPS) (Jakovljević et al. 2014).

Serbia is in the early stage of preparation in the field of environment and climate changes (Denić et al. 2015). It is necessary to audit the »Ordinance on the parameters of the ecological and chemical status of surface waters and the parameters of the chemical and quantitative status of groundwater«, (»Sl. glasnik RS«, br. 74/2011) in the part of the parameters list and class boundaries for individual parameters of the biological quality elements. Based on the category of ecological status expressed through ecological quality ratios (EQR) values (WFD CIS Guidance No. 7. 2003), excellent ecological status is achieved when the EQR value is greater than 0.80. In all, 6 samples over 3 years are needed for reliable ecological status classification (WFD-UKTAG 2014).

The aim of this study was to assess the ecological status of the Mlava River based on epilithic diatoms and to test the use of diatom indices as tool for estimating the quality of flowing waters in Serbia.

Materials and methods

The Mlava River is located in the northwestern part of Eastern Serbia with a course of 120 km, covering a drainage area of 1830 km^2, and flows into the Danube. It is one of the longest rivers in Eastern Serbia. The Mlava River is particularly specific in physical-geographical characteristics (geological structure of the terrain and water level of the basin) (Manojlović et al. 2012). The upper part of the river flows through a gorge and the downstream part through a wide valley (Babić Mladenović 2009). A trout fish pond was created in the upper part of the investigated section of the Mlava River, between the first and second sampling site. Some basic data about the sites are given in Tab. 1.

The sampling was conducted during three seasons (April, July and September 2011) from 5 localities (ML1-ML5), along the Mlava River. Benthic diatoms were collected from the stones by scraping with a toothbrush. The material was preserved in 4% formalin. Water temperature, conductivity, pH, ammonium ions, nitrates, oxygen, biochemical oxygen demand, alkalinity, total phosphorus, orthophosphates and water hardness were also determined in the water samples from each sampling site.

Diatom frustules were cleaned using the standard method with concentrated sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$) and oxalic acid to remove the organic content (Krammer and Lange-Bertalot 1986). When pH was approximately 7, the material was mounted in Naphrax® mounting medium and permanent slides were made. Microscopic analysis of the permanent slides was performed using a Zeiss Axio-Imager M1 microscope with a digital camera AxioCam MRc5 and AxioVision 4.8 software.

Tab. 1. Characteristics of the 5 investigated sites (ML1-ML5), along the Mlava River (Serbia).

Site	ML1	ML2	ML3	ML4	ML5
Altitude [m]	314	311	310	297	296
Water depth [m]	0.29	0.17	0.2	0.16	0.23
Flow width [m]	12	7.27	9.63	6.81	7.16
Flow velocity [m s^{-1}]	0.1	0.44	0.39	0.65	0.37
Watercourse bottom	gravel: 40%	stones: 75%	stones: 75%	stones: 70%	stones: 75%
Coordinates	44°11.493N 021°47.012E	44°11.842N 021°46.516E	44°11.781N 021°46.158E	44°12.133N 021°45.131E	44°13.488N 021°44.635E

Relative abundance of each diatom taxa was determined by counting 400 valves in each slide. Seventeen diatom indices are calculated based on the indicator values of identified taxa using the OMNIDIA software (Lecointe et al. 1993), and five were taken into consideration for the water quality assessment. These were: IPS (Coste in Cemagref 1982), eutrophication and/or pollution index – diatom based – EPI-D (Dell'Uomo 2004), CEE (Descy and Coste 1991), IBD (Coste et al. 2009) and TDI (Kelly and Whitton 1995). IPS, EPI-D, CEE and IBD are scaled from 1 to 20, while TDI is scaled from 1 to 100. Higher values of the first four indices indicate water quality improvement while higher values of TDI indicate bigger trophy of water (Tab. 2) (Lecointe et al. 1993).

Statistical analyses, detrended correspondence analysis and principal component analysis, were done using CANOCO for Windows Version 5 (Ter Braak and Šmilauer 2012).

Results

Qualitative and quantitative analysis of diatoms

An investigation of the benthic diatoms from the Mlava River during three seasons resulted in the description of 86 diatom taxa, belonging to 27 genera. The greatest taxa richness was recorded within the following genera: *Navicula* Bory sp. (12), *Gomphonema* Ehrenberg sp. (11), and *Nitzschia* Hassall sp. (8). Samples from the third sampling site (ML3) had the highest diversity of benthic diatoms in all seasons. Forty-five taxa were recorded in April, 39 taxa in July and 30 in September. The lowest number of diatom taxa was recorded at the site ML1. In April, 16 taxa were recorded, 25 taxa in July and 17 in September. According to the frequency of the taxa, *Amphora pediculus* (Kützing) Grunow, *Denticula tenuis* Kützing, *Cocconeis placentula* var. *lineata* (Ehrenberg) van Heurck, *Planothidium frequentissimum* Lange-Bertalot, *Reimeria sinuata* (Gregory) Kociolek & Stoermer, *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot and *Encyonema minutum* (Hilse) D.G.Mann were the taxa that occurred at the most investigated sites. *Cymbella excisa* Kützing, *Surirella brebissonii* var. *kuetzingii* Krammer and Lange-Bertalot, *Nitzschia pusilla* Grunow, *Cymbella excisiformis* Krammer and *Fragilaria parasitica* var. *parasitica* (W. Smith) Grunow were documented at only one site (Fig. 1).

Quantitative analysis showed that in April *Achnanthidium minutissimum* (Kützing) Czarnecki was dominant at
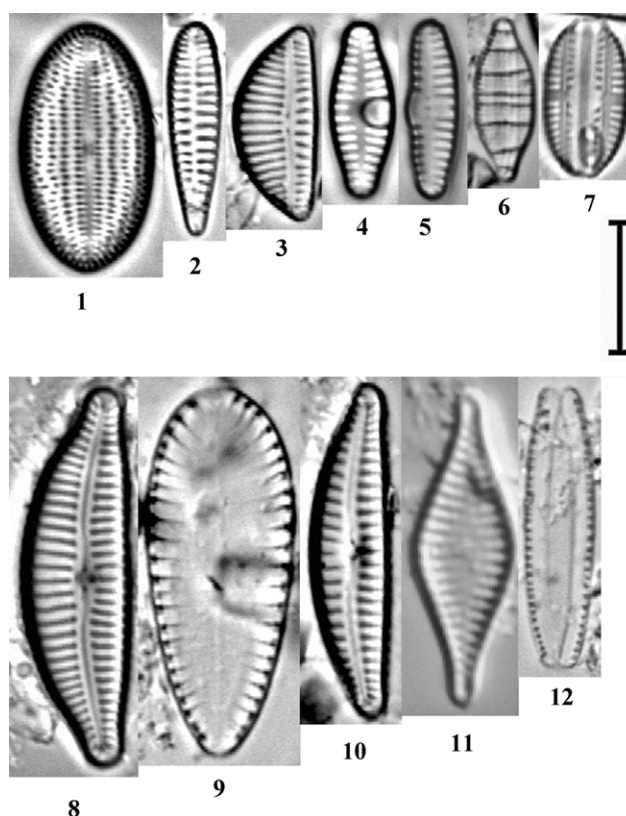
Fig. 1. Light micrographs of diatom species that occurred at most investigated sites (1–7); species that occurred at only one site (8–13). 1 – *Cocconeis placentula* var. *lineata*; 2 – *Rhoicosphenia abbreviata*; 3 – *Encyonema minutum*; 4 – *Planothidium frequentissimum*; 5 – *Reimeria sinuata*; 6 – *Denticula tenuis*; 7 – *Amphora pediculus*; 8 – *Cymbella excisa*; 9 – *Surirella brebissonii* var. *kuetzingii*; 10 – *Cymbella excisiformis*; 11 – *Fragilaria parasitica* var. *parasitica*; 12 – *Nitzschia pusilla*. Scale bar = 10 μm.

each locality, except at the fifth site, where *Amphora pediculus* was dominant. Also, *Planothidium frequentissimum*, *Gomphonema micropus* Kützing, *Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi, *Amphora pediculus* and *Reimeria sinuata* (Gregory) Kociolek & Stoermer were dominant taxa during April. *A. minutissimum, A. pyrenaicum* (Hustedt) H.Kobayasi, *A. pediculus*, *Denticula tenuis*, *Diatoma vulgaris* Bory, *Gomphonema elegantissimum* E.Reichardt & H.Lange-Bertalot, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot and *Cocconeis placentula* var. *lineata* dominated in July and September. The percentage participation of dominant taxa was 10% or more at least in one site.

Tab. 2. Class boundary limits for diatom indices. PS – pollution sensitivity index, EPI-D –diatom-based eutrophication/pollution index, CEE – commission for economical community metric, IBD – biological diatom index, TDI – trophic diatom index.

Water quality class	Ecological status	IPS, EPI-D, CEE, IBD	TDI	Trophic status	average % of taxa used for the index calculation
I	high	17–20	<20	oligotrophic	100 (IPS)
II	good	13–16	20–40	oligo-mesotrophic	73.91 (EPI-D)
III	moderate	9–12	40–60	mesotrophic	74.23 (CEE)
IV	poor	5–8	60–80	eutrophic	93.61 (IBD)
V	bad	1–4	80–100	hypertrophic	82.49 (TDI)

Tab. 3. Chemical water parameters of the Mlava River at the studied sites (ML1–ML5). BOD – biological oxygen demand.

Parameter	ML1	ML2	ML3	ML4	ML5
Temperature [°C]	10.4–11.4	10.5–15.2	12.8–16.8	10.6–14.6	10.8–15
Conductivity [µS cm^{-1}]	378–435	374–490	355–506	340–490	351–475
pH	7.14–7.38	7.53–7.8	7.62–7.95	7.94–8.12	7.97–8.04
Ammonia [mg L^{-1}]	<0.0189–0.0471	0.143–0.4556	0.246–0.379	0.1648–0.3396	0.0505–0.176
Nitrates [mg L^{-1}]	6.85–7.5	6.1–8	6.8–8.2	6.8–8.8	3.5–8.8
Oxygen [mg L^{-1}]	11.1–12.2	7.7–10.8	9.4–11.2	10.6–10.9	9.1–10.8
BOD [mg L^{-1}]	1.65–5.1	1.4–5.7	1.55–5.25	2–5.4	2.25–5.15
Alkalinity [mmol L^{-1}]	3.6–4	3.6–4.1	1.29–4	3.4–4.2	3.5–4.2
Total phosphates [mg P L^{-1}]	0.0335–0.052	0.0544–0.0817	0.0389–0.0942	0.0252–0.1128	0.0282–0.1151
Orthophosphates [mg P L^{-1}]	0.0192–0.036	0.033–0.0344	0.0275–0.039	0.0134–0.0468	0.0171–0.0392
Water hardness [°dH]	11.4–12.06	1.27–12.16	1.29–12.06	1.3–12.27	1.3–12.15

Diatom-environmental relationships

The values of physico-chemical parameters of the Mlava River are presented in Tab. 3.

Detrended correspondence analysis (DCA) was used to detect the major patterns of variation in species composition (Fig. 2). The eigenvalues of the first and second axis were 0.7398 and 0.3210 respectively. The high eigenvalue of the first axis indicates its good explanatory power. The first DCA axis summarizes the distribution of the diatom community, mainly through temperature, conductivity, oxygen and water hardness gradient. Sites, sampling time and taxa from the left side of the ordination diagram have a tendency toward higher oxygen and water hardness, but lower temperature and conductivity level, while sites, sampling time and taxa from the right side of the diagram show the opposite. As for the second axis, it is more difficult to interpret, since there are several variables weakly correlated with it, for example nitrates and orthophosphates. Higher levels of orthophosphates correspond to the lower part of the ordination diagram, and higher levels of nitrates to the upper part of the diagram. Correlation coefficients of the selected variables and the two DCA axes are given in On-line Suppl. Tab. 1. It is also worth saying that the sampling site ML1 had the lowest values of ammonia and total phosphorous and highest values of oxygen and water hardness. The Species Response curves that explain difference for the selected species distribution along the first DCA axis using generalized linear model are shown in On-line Suppl. Fig. 1. Since the first DCA axis shows the highest negative correlation with oxygen and highest positive correlation with temperature, species response curves of selected taxa show that *Achnanthidium minutissimum*, *Planothidium frequentissimum* and *Gomphonema micropus* are more abundant at higher oxygen levels, while *Cocconeis placentula* var. *lineata* and *Cocconeis pseudolineata* are more to be found at higher temperatures.
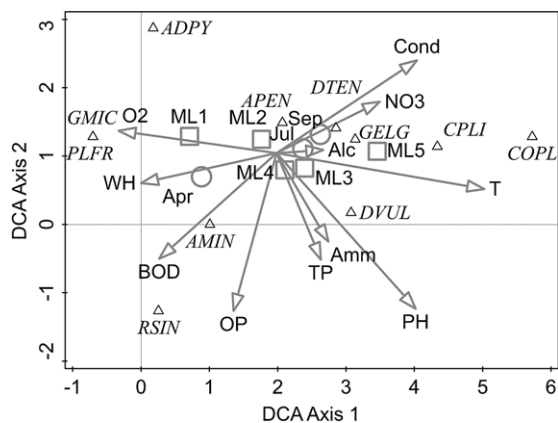

Fig. 2. Detrended correspondence analysis (DCA) of dominant diatom taxa (ADPY – *Achnanthidium pyrenaicum*, GMIC – *Gomphonema micropus*, PLFR – *Planothidium frequentissimum*, AMIN – *Achnanthidium minutissimum*, RSIN – *Reimeria sinuata*, APEN – *Amphora pediculus*, DTEN – *Denticula tenuis*, GELG – *Gomphonema elegantissimum*, DVUL – *Diatoma vulgaris*, CPLI – *Cocconeis placentula* var. *lineata*, COPL – *Cocconeis pseudolineata*) in the Mlava River (Serbia) in the ordination space of first and second axis with locality (squares; ML1–ML5), sampling season (circles; Apr, Jul, Sep) and physico-chemical characteristics (oxygen level-O₂, water hardness-WH, biochemical oxygen demand-BOD, orthophosphates-OP, ammonia-Amm, total phosphorus-TP, pH, temperature-T, nitrates-NO₃, conductivity-Cond and alkalinity-Alc) as a supplementary variable.

Diatom indices and water quality

The values of the five diatom indices used indicated a similar ecological status of the water at the studied sites. PCA was used to represent the correlation between diatom indices, where the highest correlation is seen between EPI, IPS and IBD. Also, CEE is correlated with them. These three indices had the highest values at sampling site ML2 during September. CEE had the highest value at sampling site ML1 during September and TDI at sampling site ML5 during April (Fig. 3).

Based on the average values of the IPS, CEE and IBD diatom index, water of the Mlava River belong to water class I during all three seasons, which corresponded to high ecological status. Values of the EPI-D index indicated class II water quality (good ecological status), although these values were on the border with values indicating class I water quality. According to calculated TDI values, water of the Mlava River is mesotrophic with moderate nutrient concentrations and corresponds to water class III during all sea-
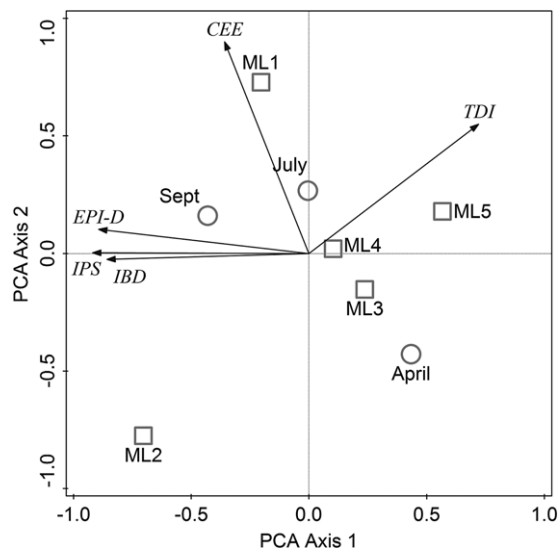
Fig. 3. Principal components analysis (PCA) of selected diatom indices (EPI-D – eutrophication and/or pollution index – diatom based, IPS – specific pollution index, IBD-biological diatom index, CEE – commission for economical community metric, TDI – trophic diatom index) with sampling time (April, July and September) and sampling site (ML1–ML5) as supplementary variables.

sons. TDI values during the first (April) and second season (July) were on the border with values indicating the high nutrient concentrations. Values of all five indices increase in the second and third season (September) (On-line Suppl. Tab. 2).

Discussion

Some of the most significant factors affecting the distribution of benthic diatoms in the rivers are the physico-chemical characteristics of water, type of substrate, water velocity, amount of light and presence of predators (Stevenson et al. 1996). The influence of the physico-chemical characteristics of water has been widely studied by many authors. Patrick (1973) recorded that acidic waters do not support an abundance of *Bacillariophyceae*, but in alkaline waters with pH above 8.0, their density is much higher. Some studies indicate that pH in the neutral range (7.0–8.0), like the values recorded in our study (7.14–8.12), supports a good population of diatoms (Kumar and Oommen 2011). Optimal temperatures for benthic diatoms are in the range from 25 to 30 °C with diversity declining at the temperatures above 30 °C. According to Rusanov et al. (2009), conductivity related to geology, as well as total phosphorus, were the most important variables related to changes in the diatom assemblage structure. Phosphates, nitrates and silica are generally considered the most important nutrients in primary production, and it is documented that diatoms reach their maximum growth rate at a concentration of total phosphorus by 0.5 mg L^{-1} (Chételator et al. 1999).

The DCA performed showed that the highest correlations with the first DCA axis were with temperature and oxygen. Even though the temperature range in our study

was quite low (10.4–16.8), DCA showed that some species of the genus Cocconeis (*Cocconeis placentula* var. *lineata* and *Cocconeis pseudolineata*) have a tendency toward higher temperatures (they are placed at the right side of the ordination diagram). Andrejić (2012) reported that optimal temperatures for *Cocconeis* genus are > 25 °C. It is known that *Cocconeis placentula* var. *lineata* has a wide ecological range, especially with regard to the electrolyte content and the trophic situation, even found in poorer electrolyte, silicate-dominated streams (Hofmann et al. 2013). One of the indicators of oxygen-rich waters, *A. minutissimum,* (Noga et al. 2014) is one of the most frequent taxa in epilithic diatom communities in streams and rivers in general (Virtanen and Soininen 2011). It is a cosmopolitan species that has a wide ecological spectrum recorded in water bodies with the conditions from oligo- to eutrophic with a wide range of pH (4.3–9.2). There are records of numerous populations that develop in high mountain streams (Van Dam et al. 1994, Kawecka 2012). It is also one of the most common and most frequently noted diatoms in the Podkarpacie region, Poland, especially in the upper courses of rivers and streams (Noga 2012, Pajączek et al. 2012). This diatom requires a continuously high concentration of dissolved oxygen. Other taxa requiring a fairly high oxygen level (above 75% saturation) (Van Dam et al. 1994) are *Planothidium frequentissimum* and *Gomphonema micropus.* DCA showed good correlation between all these three taxa and higher oxygen level, but also with higher water hardness. It is worth mentioning that *Planothidium frequentissimum* was an indicator of high levels of organic pollution and electrolyte contents in rivers in the Northeast of Spain and in France (Tornés et al. 2007, Rimet 2009) and appeared in samples with a phosphates concentration up to 5 mg L^{-1} and nitrates concentration up to 35 mg L^{-1}. In our samples, orthophosphates concentration was much lower and nitrates concentration was similar and varied 3.5–8.8. *Amphora pediculus* is an alkaliphilous species, often found in waters with moderate conductivity and tolerant to increased concentrations of organic nitrogen (Van Dam et al. 1994). It colonized oligosaprobic and mesosaprobic habitats (Hofmann et al. 2013) corresponding to those of the studied sites of the Mlava River. Based on PCA analysis conducted by studying diatoms of the Nišava River, it was observed that *A. pediculus* is positively correlated with nutrients (Andrejić 2012). It can be seen from our study that this species is correlated with nitrates but also with conductivity.

Achnanthidium pyrenaicum and *Reimeria sinuata* are placed on the opposite sides of the DCA second axis, but it is not sure which factors contribute the most to their position along the second axis. It is known that *A. pyrenaicum* is an alkaliphilous taxon mainly occurring at pH > 7 and a nitrogen-autotrophic taxon, tolerating elevated concentrations of organically bound nitrogen (Van Dam et al. 1994, Noga et al. 2014). *A. pyrenaicum* had an optimum in oligo- to mesotrophic calcium rich waters with medium to high concentration of electrolytes (Krammer and Lange-Bertalot 1991). It was very abundant in the territory of the Podkarpacie Province, Poland, in the upper course of the Wisłok River (Noga 2012).

Many taxa already mentioned above occurred at most of the sampling sites and were the dominant taxa in the diatom community. *Achnanthidium minutissimum*, *Achnanthidium pyrenaicum* and *Amphora pediculus* were dominant taxa in all three seasons. In general, in our study a great diversity of diatom species was documented and a similar diversity was found in an investigation of the Porsuk River in Turkey (Solak 2011) and the Raška River in Serbia (Vidaković 2013). *Navicula*, *Gomphonema* and *Nitzschia* are usually the most numerous genera in rivers in Europe, and in Serbia as well (Solak 2011, Andrejić 2012, Noga et al. 2013, Vidaković 2013, Vasiljević et al. 2014).

As mentioned, the lowest number of diatom taxa was recorded at ML1. Results showed that the sampling site ML1 had the lowest values of ammonia and total phosphorous and highest values of oxygen and water hardness (seen also in DCA ordination diagram), as expected, due to the fishpond located directly after the ML1. The higher number of diatom taxa from the second locality was caused by the higher level of nutrients, especially phosphates.

The water quality studies of the Mlava River during April, July and September 2011 indicated good and high water qualities. This was confirmed by most of the measured physico-chemical characteristics which indicated class I water quality. Diatom indices calculation showed that at the studied sites there are no major variations in water quality, regardless of the trout pond which is located between the first and second locality. Based on PCA analysis, a correlation was noticed between the selected indices, indi-

cating their applicability to the rivers in Serbia. The EPI-D index was used in monitoring seven rivers located in the central eastern Apennine sector (Italy) (Torrisi and Dell' Uomo 2006). As in our study, a high correlation was shown between this index and the IPS and IBD indices. IPS and CEE are the most sensitive to eutrophication and organic pollution (Descy and Coste 1991). CEE had the highest value at sampling site ML1, which can be explained by the position of the pond (between the first and the second locality), although these values still are within the first class quality. According to Szabó et al. (2004), among IPS, IBD, CEE and EPI-D diatom indices, which are good for Hungarian rivers, IPS is probably the best. The suitability of the index is dependent on the percentage of taxa used for the index calculation. In our study, the average percentage of taxa used for the IPS calculation is 100, so IPS is the most suitable index for this reason. TDI is not correlated with the other four indices in our study, which is expected, since it is the only index in which the values range from 1 to 100 while the values of all other indices range from 1 to 20. Values of all five indices increase in the second and third season which indicates the improvement of water quality. The data provided by this study can be used for the development of a biomonitoring tool for the rivers in Serbia.

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