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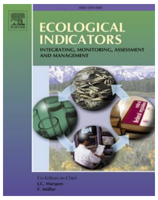

Epilithic diatoms in environmental bioindication and trout farm's effects on ecological quality assessment of rivers

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ABSTRACT

The main aim of this study was to investigate the application of benthic diatoms and the efficiency of diatom indices in water ecological assessment of rivers in Serbia, as well as the influence of trout farms on rivers water quality. Research was conducted in one year period along the Crnica and Radovanska rivers (Eastern Serbia), in 11 sampling sites (taking into account the position of trout fish farms that are built on both rivers). Results showed that fish farms affect the physical and chemical parameters, as well as diversity of the epilithic diatom assemblage along the rivers. Canonical correspondence analysis (CCA) indicated that among tested physical and chemical parameters, conductivity "EC", water hardness "WH", sulfates "SO_4^{2-}", ionized ammonia fraction "NH_4^+", nitrates "NO_3^-", "pH", mud, stone and depth were the most important variables affecting the distribution of diatom species. A total of 206 epilithic diatom taxa were recorded in the Radovanska and 170 in the Crnica River. Based on diatom indices, trout fish farms have no significant negative impact on the water quality of the investigated rivers (indices are not enough sensitive). Redundancy analysis (RDA) showed a high degree of correlation between the majority of indices which mostly indicated to good water quality of studied rivers. TID and TDI were the only two indices indicating moderate and poor water quality of both rivers. Water quality based on the TID_{RS}, the first proposed diatom index for Serbia was differ in two classes in most sites compairing with TID.

1. Introduction

The importance of diatoms in rivers and streams is primarily reflected through their fundamental role in food chains, oxygenation of surface waters and biogeochemical cycles. They are significant factors of biodiversity and genetic diversity in aquatic ecosystems (Smol and Stoermer, 2010). Analysis of diatom assemblages is a very useful tool in preserving and sustainable management of water resources (Mogna et al., 2015). Many countries for the assessment of water quality take into account only diatoms (Poikane et al., 2016). The two most important features of diatoms that make them ideal bioindicators capable to indicate early pollution are a rapid response to environmental changes and narrow ecological valence toward specific environmental factors (Karacaoğlu and Dalkıran, 2017). By analyzing diatoms, a picture of general condition, ecological integrity and water quality of a particular aquatic ecosystem can be obtained (Taylor et al., 2007). They are extremely good indicators of trophic state, acidity and organic pollution of water. The diatom assemblage structure is strongly affected by the concentration and ratio of nutrients in water. Many studies have shown that changes in biomass and diversity of diatoms reflect changes in the concentration of nitrogen and phosphorus in rivers and streams (Bere et al., 2016).

Discharge waters from fish farms have a major impact on the water quality of rivers, changing the concentration of ions and dissolved

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oxygen. This further leads to many other changes that affect different groups of organisms (Stojanović et al., 2017). Globally, production of farmed fish has increased intensively in recent decades (Naylor et al., 2000). In Serbia, in the 19th century, there was only one larger fish farm, while during the 20th century, this number was constantly growing (Gavrilović and Dukić, 2002). There are currently about 130 trout farms in the country (Božanić et al., 2018). The increasing anthropogenic influence has led to the creation and application of diatom indices worldwide. They can be defined as a way of expressing changes in water quality (Watanabe et al., 1986; Kelly and Whitton, 1995; Lobo et al., 2004; Hurlimann and Niederhauser, 2006; Coste et al., 2009).

Diatom indices values should be carefully interpreted in the water quality assessment of surface waters, taking into account many factors, such as the region where the particular index was originally used, the choice of substrates from which the sampling is carried out, as well as the degree of expertise of the person performing the analysis (Solak and Àcs, 2011). Differences between diatom indices occur due to diatom different sensitivity to the type and degree of pollution (different indices are designed to detect different types of pollution) (Wang et al., 2014). Also, differences in the indicator taxa lists that are taken into account in their calculation (different taxa are used to calculate different indices depending on the region where they are designed) have a major impact on the results of calculating diatom indices. Therefore, their applicability in regions different from their place of origin must be investigated and tested in detail (Xue et al., 2019).

Water Framework Directive (WFD) has been adopted by the European Union countries as a reaction to the predicted loss of biodiversity and increased anthropogenic factors (WFD, 2000). It is a pioneering legislation that aims to protect and promote sustainable all surface waters (rivers, lakes, transitional and coastal waters), as well as groundwater (Carvalho et al., 2019). The main purpose of the WFD is to establish a Europe-wide implementation of a comprehensive framework to protect the sustainability of water resources (Pander and Geist, 2013). This can be achieved by integrating chemical, physical and chemical and biological quality components (WFD, 2000). All member states and candidate countries are obligated to include the WFD into national legislation.

Compared to most European countries, benthic diatoms have recently been used in the ecological status assessment of surface waters in Serbia. Therefore, this study was conducted to test the diatom indices and the influence of trout farms on rivers water quality in Serbia. There are no data on previous research of diatoms from the Radovanska River and very scarce data about diatoms from the Crnica River (Ranković et al., 1995), so this research is of great importance also from a floristic point of view. Also, one of the aims was to identify the environmental variables that can explain the distribution and community composition of epilithic diatom assemblages in the Radovanska and Crnica rivers.

2. Material and methods

2.1. Description of investigated rivers and sampling sites

The Radovanska River is a typical mountain river in Eastern Serbia. It is formed by connecting three streams at 642 m above the sea level. The length is about 20 km, with an average drop of 32.33‰. Also, it is an underground river that sinks in the gorge, into the Pećur cave (Gavrilović and Dukić, 2002). The fish farm is located just below the source of the Radovanska River, at 820 m above sea level. The total production area is 1643 m^2, and the total production capacity of juveniles and fish for consumption is 60 t. Trout farming pools are placed in four levels, and there are a total of eight. This fish farm is specific because it uses renewable sources for obtaining electricity throughout the year, in the form of water and solar energy (Stojanović, 2017). For the purposes of this research, 5 sites were selected along the investigated part of the Radovanska River. The first site (RR1) was located at the source from which the trout fish farm is supplied with water, and four sites downstream from the trout farm (Fig. 1).

The Crnica river is the tributary of Velika Morava River. It has a length of 31.8 km and a drainage area of 338 km^2. The Crnica River springs in Sisevac, on the western side of the Kučajske Mountains, at 380 m above sea level. Near the main spring (karst spring “Staro Vrelo”), there are three more karstic springs that are used for water supply of surrounding villages (Gavrilović and Dukić, 2002). The temperatures of these waters range from 24.7 °C to 29 °C (Milić, 2006). The fish farm on the Crnica River is one of the leading producers of California trout in Serbia, whose annual production is 70 t of fish and 600.000 juveniles. The production area of the fish farm is 4200 m^2, with a total of 43 pools of different dimensions (Stojanović, 2017). In the dry season, when the amount of water drops drastically, the fish farm includes a pump that allows the return of water from the farm outlet, in order to compensate for the required amount of water. For the purposes of this research, 6 sites were selected along the investigated part of the Crnica River. The first two sites were located before the fish farm, and four sampling sites downstream from the farm (Fig. 1). The basic characteristics of sampling sites along the Radovanska and Crnica rivers are shown in Table 1.

2.2. Dynamics and sampling method

The sampling was conducted 6 times along each river during one year from April 2011 to May 2012, in 11 sites. Algological samples (66) were collected from each site by selecting 5 medium-sized stones from constant parts of the rivers. The epilithic diatom assemblage was scraped with a toothbrush in plastic bottles of 100 ml volume and was conserved by formaldehyde to a final concentration of 4% in each sample.

The physical and chemical parameters for the purposes of this research were measured directly in the field (water temperature “t_w”, depth and breadth of the riverbed, velocity “V”, flow rate “Q”, substrate type, oxygen “O_2”, “pH”, conductivity “EC”) or in the laboratory of the Institute of Chemistry, Technology and Metallurgy of the University of Belgrade (anions concentrations (sulfates “SO_4^{2-}“, nitrates “NO_3^-“ and chlorides “Cl^-“), total phosphorus “P_u”, orthophosphates “PO_4^{3-}“, ionized ammonia fraction “NH_4^+”, un-ionized fraction “NH_3” and water hardness “WH”). The “t_w” was measured using a temperature probe that is an integral part of the PCE-PCD measuring device and velocity using a measuring instrument - speedometer (GEOPACKS Stream Flowmeter, UK). The depth and width of the riverbed were measured using a meter. The flow rate “Q” was measured by dividing the river profile at the sampling sites into several subsections, and then the depth and velocity within each section were measured. The flow rate “Q” was then calculated by multiplying the area of a given section by the mean value of the velocity (measured with a speedometer) between two measurement points. The analysis of the type of substrate was performed by visual assessment, and the composition of the substrate was classified into five classes (Stojanović et al., 2019). The “O_2”, “pH” and “EC” were measured using the PCE-PHD device. The data of physical and chemical parameters were used from previous publication (Stojanović et al., 2019), with difference that mean values of these parameters were calculated for six occasions when the diatoms were sampled.

2.3. Laboratory method and microscopic analysis

Diatom frustules were cleaned using the method with concentrated sulfuric acid (H_2SO_4), potassium permanganate (KM_nO_4) and oxalic acid ($C_2H_2O_4$) to remove the organic content (Krammer and Lange-Bertalot, 1986) and the material was mounted in Naphrax®.

Microscopic analysis of the permanent slides was performed using a Zeiss Axio-Imager M1 microscope with a digital camera AxioCam MRc5 at a 1600x magnification and Differential Interference Contrast (DIC). AxioVision4.8 computer software was used to process diatom micrographs.

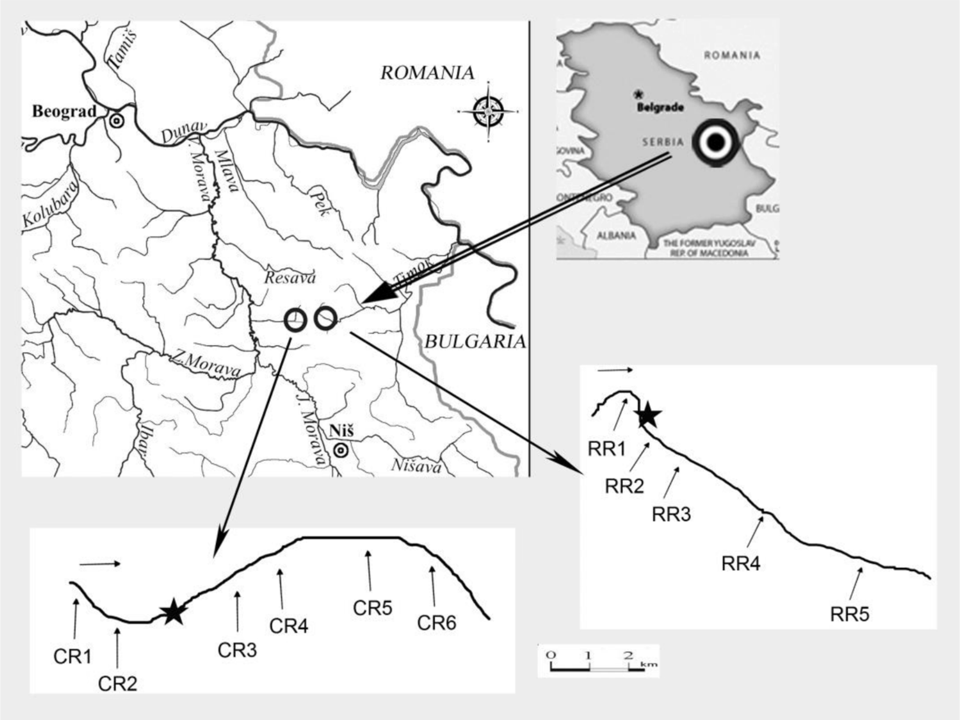


Fig. 1. Sampling sites (RR1-RR5, CR1-CR6) and fish farms (*) along the Radovanska and Crnica rivers. RR1 (43° 53.802 N 21° 47.062 E), RR2 (43° 53.666 N 21° 47.274 E), RR3 (43° 53.458 N 21° 47.484 E), RR4 (43° 53.066 N 21° 47.946 E), RR5 (43° 52.597 N 21° 48.867 E), CR1 (43° 57.327 N 21° 35.406 E), CR2 (43° 57.393 N 21° 35.256 E), CR3 (43° 57.296 N 21° 34.919 E), CR4 (43° 57.279 N 21° 34.688 E), CR5 (43° 57.273 N 21° 34.119 E), CR6 (43° 57.396 N 21° 33.872 E).

Table 1
The basic characteristics of sampling sites along the Radovanska (RR1-RR5) and Crnica rivers (CR1-CR6).

Sampling sites	Altitude (m.a.s.l.)	Location of the investigated sites
RR1	415	100 m upstream from the trout farm
RR2	380	200 m downstream from the trout farm
RR3	374	500 m downstream from the trout farm
RR4	353	1.6 km downstream from the trout farm
RR5	336	3.1 km downstream from the trout farm
CR1	348	250 m upstream from the trout farm
CR2	345	20 m upstream from the trout farm
CR3	342	20 m downstream from the trout farm
CR4	340	400 m downstream from the trout farm
CR5	336	1.3 km downstream from the trout farm
CR6	334	1.7 km downstream from the trout farm

2.4. Data analysis

The identification of diatom taxa was carried out based on different morphometric characteristics of the cell wall. Taxa identification was performed using appropriate literature (Krammer, 2000; 2002;; Lange-Bertalot, 2001; Lange-Bertalot et al., 2017; Levkov, 2009; Levkov et al., 2016) and classification according to the online database “AlgaeBase” (Guiry and Guiry, 2017). After qualitative analysis, a quantitative analysis was carried out within the diatom epilithic assemblage. It was presented in the form of the percentage representation of each taxon in the sample by counting 400 valves per slide.

The software package OMNIDIA 6.0.4 was used to calculate seventeen diatom indices (Lenoir and Coste, 1996). The water quality assessment of the investigated rivers was performed according to the boundaries of water quality classes based on the values of diatoms indices (Leclercq and Maquet, 1987; Prygiel and Coste, 2000). Table with full names of indices, abbreviations and the type of pollution indicated by each index is given in Supporting Information (SI) (Table S.2).

For the studied rivers, based on the preliminary list of indicators (Vidaković, 2019) formed on the basis of the total phosphorus values and the number of valves, the values of $\mathrm{TID_{RS}}$ index are calculated. It is a first, last year proposed diatom index for assessing the trophic status of running waters in Serbia (Vidaković, 2019):

$$\mathrm{TIDrs} = \frac{\sum_{i=1}^{n} x_i s_i v_i}{\sum_{i=1}^{n} x_i v_i}$$

with: x_i-relative abundance of taxon *i*, s_i-indicator value of taxon *i*, v_i-indicator weight of taxon *i*.

The $\mathrm{TID_{RS}}$ index was tested for the first time and its values were compared with the TID values, which was developed for the rivers of Austria (Rott et al., 1999), but is also widely used in Germany, Hungary, Poland and Slovenia to assess the trophic status of surface waters.

Ecological status assessment of the studied rivers was carried following the legislation of Serbia (Official Gazette of Republic of Serbia, 2011). Based on it, ecological status/potential of surface waters in Serbia are determined based on biological, chemical and physical and chemical, and hydro-morphological parameters. IPS and CEE diatom indices (or IPS only) are biological parameters of the ecological status assessment of rivers based on phytobenthos in the Republic of Serbia. The investigated parts of the Crnica and Radovanska rivers were classified into appropriate water bodies and watercourses types (Official Gazette of Republic of Serbia, 2010).

2.5. Statistical analyses

Canonical correspondence analysis (CCA) was performed using software CANOCO for Windows, Version 5.0 (Ter Braak and Šmilauer, 2012). Using CCA, the relationship between recorded diatom taxa (presence/absence) in the Crnica and Radovanska rivers and environmental parameters (physical/river parameters, chemical parameters and data referring to the dominant substrate type) was assessed. Significant variables in each group of parameters were selected by applying the option “Interactive forward selection”, where the statistical significance of each variable was tested by the Monte Carlo permutation test ($p < 0.05$). Only those variables that showed significance were included in the final CCA diagram. Tested physical/river parameters were river depth and width, “t_w”, “pH”, “EC”, “WH”, “V”, “Q”, where “EC”, “pH”, “WH” and depth showed significance; after chemical parameters testing (“O_2”, “P_u”, “PO_4^{3-}“, “NH_4^+”, “NH_3”, “NO_3^-“, “Cl^-“, “SO_4^{2-}“) significant were “NO_3^-“, “NH_4”, “SO_4^{2-}“, considering substrates (mud, gravel, sand, stone, rock), mud and stone were singled out as significant. As supplementary variables, the terms indicating rivers and variables that refer to the position of sampling sites with fish farm (before (BFF) and the after fish farm (AFF)) were included. CCA with option “downweight rare species“ was used and 25 the best fitted taxa is shown on the ordination diagram.

To assess how much variability in our data described every mentioned group of variables (by taking into account all variables), variation partitioning (simple and conditional effect) was performed. The amount of variability, as well as the significance of each group of variables (fraction) was presented.

The correlation between diatom indices, ecological parameters (physical and chemical parameters of water) and sampling sites was shown using redundancy analysis (RDA). The analysis was made for each river separately, taking into account all indices and all physical and chemical parameters of water to examine the relationship with all, but it was also emphasized which factor was the most significant after using the “interactive forward selection” option.

3. Results

3.1. Influence of the trout farms on physical and chemical parameters

The values of most physical and chemical parameters along the investigated part of the Radovanska River were different between sites located upstream and those located downstream from the fish farm (Table 2). The water temperature of the Radovanska River was quite low with relatively small annual variations. The impact of the fish farm is evident due to an increase in the average temperature at site RR2 (after discharge) compared to site RR1. “O_2” was reduced after the discharge of wastewater from the farm compared with site RR1, and downstream these concentrations were gradually increasing. “P_u”, “PO_4^{3-}“, “NH_4^+”, and “NH_3” have significantly increased after the discharge of wastewater from the fish farm, while downstream these values gradually decreased. Only “pH” continued to grow downstream from the fish farm, so the highest values were recorded at site RR5. “EC”, “WH”, “NO_3^-“, “Cl^-“ and “SO_4^{2-}“ varied in very narrow range at investigated sites. Considering hydrological parameters, there was an slight increase in depth downstream from site RR1. The “V” varied slightly at all sites and based on these values, the Radovanska River was classified into moderately fast rivers. At site RR1, the dominant type of substrate was mainly made of stones, subdominant gravel, while the rocks, sand and mud in form of fine particulate organic matter (FPOM) made up about 5% of the substrate. At site RR2, the quantity of mud and rocks increased significantly, although the stones continued to be the dominant type of substrate. Downstream from the site RR2, there was a decrease in the amount of mud, gravel, and sand, while the amount of stone substrate increased.

Observing the physical and chemical parameters of the Crnica River at different sites, the significant effect of the fish farm on the majority of abiotic factors was noticed (Table 2). One group of factors (“t_w”, “pH”, “WH”, “NO_3^-“, “Cl^-“, “SO_4^{2-}“) was quite constant and their values did not vary significantly depending on the site, while the values of the second group of factors (“O_2”, “P_u”, “PO_4^{3-}“, “NH_4^+”, “NH_3”) changed significantly at site CR3 (after the discharge of wastewater from the fish farm). “t_w”, “EC” and “WH” were quite constant and their values varied in a narrow range. The total annual variation in “t_w” was 6.3 °C. “O_2” and “NH_4^+” significantly changed at sites after discharge (sites CR3-CR5). “NH_4^+” at the discharge point increased >10 times compared with sites before discharge, and then gradually decreased downstream, although the values were still several times higher than values at sites upstream from the fish farm. “pH” was rising downstream without changes in the specified pattern at the sites around discharge. “P_u”, “PO_4^{3-}“ and “NH_3” were significantly increased after the discharge, but unlike “O_2” and “NH_4^+”, there was no pattern of their downstream decrease. The “V” was the lowest at site CR1, and was increasing downstream. The biggest change in velocity was recorded at site CR2, where water became two times faster than at site CR1. “Q” significantly increased after the discharge of wastewater from the fish farm. The stone was the dominant type of substrate at all sites, while sand was the least represented. It can be seen increased representation of mud at site CR3, and then decreased significantly.

3.2. Diatom assemblages structure and influence of the trout farms on diatom diversity

An investigation of the epilithic diatoms from the Radovanska River

Table 2
The mean values of physical and chemical parameters at sampling sites of the Radovanska (RR1-RR5) and Crnica rivers (CR1-CR6).

Parameter	Unit	RR1	RR2	RR3	RR4	RR5	CR1	CR2	CR3	CR4	CR5	CR6
t_w	(°C)	9,2 ± 0,5	10,4 ± 0,9	10,6 ± 1,0	11,1±1,0	11,4±1,3	10,76 ± 0,30	12,44 ± 0,57	12,77 ± 0,92	12,46 ± 0,83	12,50 ± 0,90	12,2±0,82
O_2	(mg/l)	11,6±0,3	9,2 ± 0,6	9,8 ± 0,6	10,6 ± 0,3	10,4 ± 0,3	9,91 ± 0,49	10,7±0,40	9,15±0,43	9,55±0,40	10,44 ± 0,33	10,89 ± 0,45
pH		7,45±0,09	7,73±0,08	7,87±0,09	8,05±0,11	8,16±0,09	7,22±0,11	7,39±0,98	7,65±0,07	7,69±0,06	8,01±0,12	8,07±0,15
EC	(µS/cm)	384±28	408±35	404±34	389±21	410±25	468,9 ± 11,6	477,4 ± 15,1	464,4 ± 13,5	465,0 ± 13,6	461,7 ± 14,7	456,6 ± 13,1
P_u	(µg/l)	29,7±7,9	79,0±15,1	54,2±7,5	46,6±8,4	49,0±6,3	25,7±6,9	28,0±8,8	48,9±11,7	76,6±27,5	68,3±26,8	63,3±23,2
PO_4^{3-}	(µg/l)	23,0±4,7	51,4±10,7	37,4±7,1	34,9±7,5	30,8±4,3	14,2±4,6	13,6±4,7	21,7±4,0	31,4±8,0	26,2±3,9	25,1±5,1
NH_4^+	(µg/l)	77±43	541±104	339±99	248±148	92±41	42,3±16,6	46,9±19,4	354,5 ± 53,6	264,6 ± 32,9	193,0 ± 22,8	166,1 ± 36,6
NH_3	(µg/l)	0,33±0,16	5,70±1,42	5,45±2,02	5,09±2,47	3,18±1,72	0,13±0,06	0,27±0,14	4,07±1,05	3,14±0,60	5,65±1,62	8,46±4,90
WH	(dH)	1,08±0,10	1,09±0,11	1,08±0,12	1,08±0,15	1,11±0,11	1,31±0,09	1,31±0,11	1,27±0,11	1,28±0,11	1,29±0,10	1,27±0,1
NO_3^-	(mg/l)	5,27±0,65	5,58±0,32	5,92±0,33	6,25±0,58	6,08±0,57	4,92±0,39	4,58±0,42	4,71±0,41	4,74±0,35	5,05±0,31	5,18±0,28
Cl^-	(mg/l)	0,96±0,13	0,99±0,08	0,97±0,08	1,20±0,26	1,01±0,12	1,09±0,11	1,02±0,12	0,93±0,10	0,99±0,14	1,17±0,18	1,05±0,11
SO_4^{2-}	(mg/l)	8,81±1,30	9,55±0,79	9,42±0,79	9,95±0,66	9,86±0,89	11,53 ± 0,64	11,50 ± 0,57	11,43 ± 0,81	11,38 ± 0,66	11,33 ± 0,65	11,40 ± 0,63
Depth	(m)	0,14±0,01	0,174 ± 0,02	0,19±0,02	0,20±0,02	0,18±0,01	0,18±0,01	0,14±0,03	0,20±0,03	0,16±0,02	0,17±0,02	0,21±0,02
Width	(m)	5,14±0,55	5,04±0,48	5,34±0,51	6,40±0,44	5,96±0,49	6,08±0,71	2,39±0,44	6,12±0,14	7,47±0,75	6,68±0,67	4,69±0,38
Velocity	(m/s)	0,36±0,03	0,38±0,03	0,37±0,05	0,36±0,05	0,39±0,05	0,12±0,05	0,24±0,05	0,37±0,02	0,37±0,03	0,43±0,06	0,33±0,04
Flow	(m^3/s)	0,27±0,07	0,35±0,07	0,39±0,10	0,43±0,12	0,39±0,07	0,16±0,07	0,15±0,08	0,42±0,07	0,43±0,10	0,49±0,11	0,44±0,09
FPOM	(%)	0,7±0,7	24 ± 6	7 ± 3	8 ± 3	3 ± 1	9,17±3,27	5±2,24	16,67 ± 3,07	3,33±1,67	5,83±2,39	5,00±2,24
Gravel	(%)	15 ± 3	13 ± 3	11 ± 2	11 ± 2	17 ± 5	16,67 ± 3,33	12,50 ± 1,71	12,50 ± 2,50	11,67 ± 1,67	12,5±2,14	9,17±1,54
Sand	(%)	1 ± 1	9 ± 3	6 ± 3	9 ± 3	6 ± 3	3,33±2,11	5,00±2,24	3,33±2,11	2,50±1,71	6,67±4,01	2,50±1,71
Stones	(%)	79 ± 5	34 ± 6	55 ± 5	55 ± 7	66 ± 6	58,33 ± 4,01	68,33 ± 2,79	60,83 ± 5,07	75,00 ± 3,16	68,33 ± 4,59	66,67 ± 3,57
Rocks	(%)	4 ± 1	19 ± 5	21 ± 3	18 ± 3	9 ± 2	12,50 ± 2,50	9,17±2,39	6,68±1,05	7,50±1,12	6,67±1,05	16,67 ± 3,33

resulted in the description of 206 diatom taxa (Table S.1 - SI). *Navicula* (30 species) was the most species-rich genus, followed by *Nitzschia* (20 species) and *Gomphonema* (16 species). The most frequent diatom taxa (recorded in all samples) in the Radovanska River were *Amphora pediculus* and *Reimeria sinuata*. Taxa found in 96% of all samples were *Achnanthidium minutissimum*, *Cocconeis lineata*, *C. pseudolineata*, *Gomphonema elegantissimum*, *Meridion circulare*, *Planothidium dubium*, *P. lanceolatum* and *P. frequentissimum*. The highest number of taxa (133) was documented at site RR3, and the lowest number (79) at site RR1. Besides, the highest values of diversity (Shannon-Wiener diversity index) and evenness (Pielou's evenness index) were determined at sites RR3 and RR4 and the lowest values at site RR1 (Fig. 2). *Denticula tenuis*, *Achnanthidium minutissimum* and *Amphora pediculus* were predominant with annual average relative abundance of 16.44, 14.05 and 12.15%, respectively. Taxa with annual average relative abundance of > 5% are also included *Cocconeis lineata* (11.25%), *Meridion circulare* (8.68%) and *Achnanthidium pyrenaicum* (5.32%).

A slightly smaller number of epilithic diatom taxa was recorded in the Crnica River (170 taxa) comparing with the Radovanska River (Table S.1 - SI). *Navicula*, *Nitzschia*, and *Gomphonema* were also the most species-rich genera, with the difference that the greatest taxa richness was recorded within the *Nitzschia* genus (21 species), followed by *Navicula* (20) and *Gomphonema* (19). The most frequently observed diatom taxa in the Crnica River (recorded in all samples) were: *Achnanthidium minutissimum*, *A. pyrenaicum*, *Amphora pediculus*, and *Cocconeis lineata*. Some of the most frequently observed diatoms (in 97% of all samples) were: *Gomphonema elegantissimum*, *Meridion circulare* and *Reimeria sinuata*. The largest number of taxa was recorded at site CR6 (115), and the lowest at site CR1 (69). Besides, the highest values of diversity and evenness were determined at site CR6 and the lowest values at site CR3 (Fig. 2). *Achnanthidium minutissimum* was predominant with annual average relative abundance of 21.88%. Taxa with annual average relative abundance of > 5% are also included: *Achnanthidium pyrenaicum* (9.33%), *Amphora pediculus* (8.54%), *Gomphonema elegantissimum* (8.19%), *Cocconeis lineata* (7.89%) and *Meridion circulare* (6.11%).

3.3. Diatom-environmental relationships

The relationship between documented taxa in the Radovanska and Crnica rivers and preselected environmental parameters (physical/river parameters, chemical parameters and data referring to the dominant substrate type) as described above is shown in Fig. 3, where 25 the best fitted taxa together with 9 explanatory and two supplementary variables is shown on CCA ordination diagram that explained 22.5% variability in our data (F = 1.8, P = 0.002). Variables oriented toward the left side of the ordination diagram ("EC", "WH", "SO$_4^{2-}$", Stone) showed a positive correlation with *Fragilaria vaucheriae*, *Navicula pseudoppugnata* and *Gomphonema olivaceum*, taxa that were most frequently found in the Crnica River. On the other side, variable "NO$_3^-$" on the right side of the ordination diagram was positively correlated with many taxa more frequently documented in the Radovanska River. Variables "NH$_4^+$", "pH", Mud and Depth were positively correlated with taxa in the upper part of the ordination diagram. We emphasise that the higher values of these variables were recorded at sampling sites after fish farm (AFF) and all taxa shown on this part of the ordination diagram were more frequently found at these sampling sites and on mud substrate. One big group of diatoms placed in the central part of the diagram did not show specific relation to any variable, except it is slightly negatively correlated with "NH$_4^+$", "pH", Mud, and Depth.

The results of the variation partitioning that included three groups of variables (physical/river parameters, chemical parameters and data referring to the dominant substrate type) are shown in Table 3. Physical/river parameters explained 14.7% variability in the data independently of others (fraction "a") and in total (fractions a + d + f + g) 16.6%. Chemical variables in total (fractions b + d + g + e) explained 17.2% of the variability, and independently 14.7% (fraction "b"), the same as the first group. Group of variables that refer to substrates explained 9.7% altogether (c + e + f + g), and independently, 8.0% of variability (fraction "c") in the data. Comparing these three groups, chemical parameters in total explained the highest portion of variability in our data and were followed by physical/river parameters and substrates. The significance of the mentioned fractions, as well as their mentioned variability is represented in Table 3.

3.4. Diatom indices and ecological status assessment of investigated rivers

Along the investigated part of the Radovanska River most indices values had a narrow range, ie. there was no noticeable decline in water quality at sites downstream from the farm. Based on the majority of diatom indices, the water quality of the Radovanska River during the investigated period was assessed as good at most sampling sites (Table 4). Indices that indicated the high water quality of the Radovanska River in all or most sites were IBD, DESCY and CEE, while TID and TDI indicated moderate and poor water quality. In only one sample the percentage of mobile pollution-tolerant taxa was 27.9%, while in all

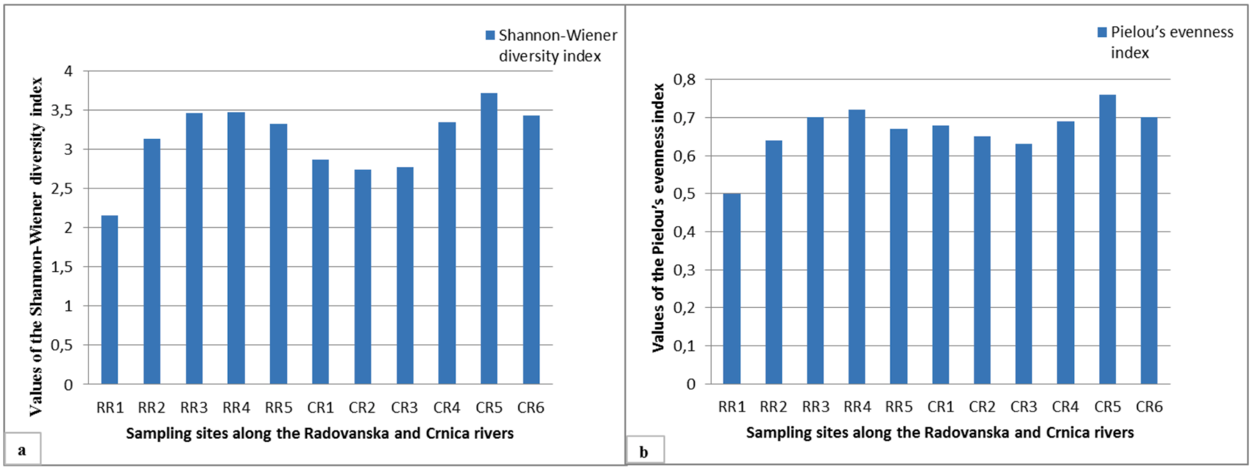


Fig. 2. a) Diversity (Shannon-Wiener diversity index) and b) evenness (Pielou's evenness index) in the Radovanska and Crnica rivers (RR1-RR5: sites along the Radovanska River, CR1-CR6: sites along the Crnica River).

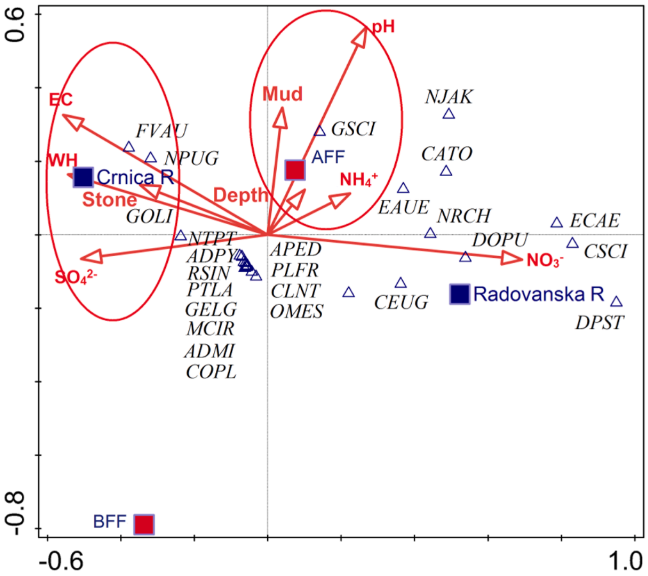


Fig. 3. CCA showing the relationship between documented taxa in the Radovanska and Crnica rivers and preselected environmental parameters (physical/river parameters, chemical parameters and data referring to the dominant substrate type). AFF - after fish farm, BFF - before fish farm. *Fragilaria vaucheriae* - FVAU, *Navicula pseudoppugnata* - NPUG, *Gomphonema olivaceum* - GOLI, *Navicula tripunctata* - NTPT, *Achnanthidium pyrenaicum* - ADPY, *Reimeria sinuata* - RSIN, *Planothidium lanceolatum* - PTLA, *Gomphonema elegantissimum* - GELG, *Meridion circulare* - MCIR, *Achnanthidium minutissimum* - ADMI, *Cocconeis pseudolineata* - COPL, *Amphora pediculus* - APED, *Planothidium frequentissimum* - PLFR, *Cocconeis lineata* - CLNT, *Odontidium mesodon* - OMES, *Cocconeis euglypta* - CEUG, *Discostella pseudostelligera* - DPST, *Diploneis oculata* - DOPU, *Navicula jakovljevicii* - NJAK, *Gyrosigma sciotoense* - GSCI, *Cyclotella atomus* - CATO, *Encyonema auerswaldii* - EAUE, *Navicula metareichardtiana* - NRCH, *Encyonema caespitosum* - ECAE, *Cymbella subcistula* - CSCI.

other samples this number was <20%. Similar to the Radovanska River, most diatom indices indicated good and high water quality along the investigated part of the Crnica River (Table 4). Also, TID and TDI were the only two indices indicating moderate and poor water quality. In five samples % PT values were >20%.

The RDA analysis of the relationship between diatom indices and physical and chemical parameters in the Radovanska River is presented in Fig. 4 and describes 66.9% of the data variability. The RDA showed that one group of indices (lower left quadrant of the ordinate diagram) is positively correlated with “NH_4^+”, “NH_3”, “P_u”, “PO_4^{3-}“ and “WH”. The second group of indices (located in the upper left quadrant of the ordinate diagram) was best positively correlated with “SO_4^{2-}“, “Q”, “V” and Depth, but also with “pH” and “Cl^-“. WAT singled out from all other indices and showed a positive correlation with “O_2” and “NO_3^-“. IPS and CEE were positively correlated, with the difference that IPS best correlated with “NH_4^+” and “NH_3”, and CEE with “PO_4^{3-}“, “WH”, “t_w”, “P_u”, and “EC”. The “interactive forward selection” option was distinguished three most important factors: “P_u” ($r = -0.48$) and “NH_4^+” ($r = -0.54$), which are negatively correlated with the first RDA axis, and “SO_4^{2-}“ positively correlated with the second RDA axis ($r = 0.60$).

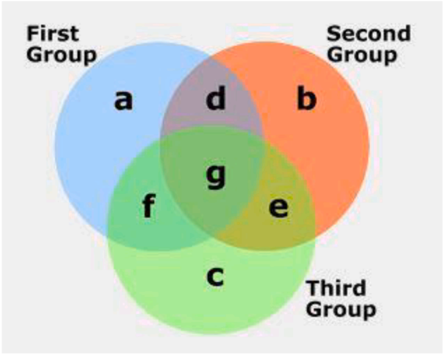
The RDA analysis of the relationship between diatom indices and physical and chemical parameters in the Crnica River describes 57.2% of the data variability. RDA diagram (Fig. 5) for this river also showed that a large number of indices were mutually correlated and oriented to the right side of the diagram. The whole group of indices showed correlations with “EC”, “t_w”, and “P_u”. Indices in the upper right quadrant of the ordination diagram also showed correlation with “WH”, while those in the lower right quadrant correlated with “pH”, “NH_3” and “NH_4^+”. Three indices were separated from this group. DI-CH positively correlated with the last three mentioned parameters, but also with the “V”, “Q” and Depth. DESCY and IDP, except with the “V”, “Q” and Depth, also showed correlation with “NO_3^-“, “SO_4^{2-}“ and “Cl^-“. IPS and CEE were mutually correlated, with the difference that the CEE was the most positively correlated with the “EC”, and IPS with “t_w”. The “interactive forward selection” option found that the most significant parameter was “NO_3^-“, which was negatively correlated with the first RDA axis ($r = -0.53$).

Values of the TID_{RS}, the first proposed diatom index for Serbia, were calculated using the taxa and indicator values from the proposed preliminary list (Vidaković, 2019). It is noticed that the TID_{RS} and TID values differ in two water quality classes in most sites (Fig. 6). The exceptions are RR5 sampling site, where they differ for one water quality class and RR1 where they indicate the same quality class.

According to the legislation of the Republic of Serbia (Official Gazette of Republic of Serbia, 2011), the investigated parts of the Radovanska and Crnica rivers belong to the types 6 and 3 of water bodies (water body codes: RAD_1, CRN_3), respectively. The type 6 of water bodies refers to small watercourses outside the Pannonian Plain area that are not covered by types 3 and 4, as well as watercourses not covered by another ordinance. The type 3 refers to small and medium watercourses, altitudes up to 500 m, with the dominance of a large surface. For type 6 rivers, the IPS is used as the only biological parameter in the assessment of the ecological status based on phytobenthos in Serbia and for type 3, the IPS and CEE indices are used (Table 5). Observing the chemical and physical and chemical element, the investigated parts of both rivers belonged to the IV class of ecological status

Table 3

Variation partitioning that included three groups of variables (physical/river parameters, chemical parameters and data referring to the dominant substrate type) (conditional (a, b, c) and simple effects (a + d + f + g, b + d + g + e, c + e + f + g)) representing explained variation and significance tests for each fraction. a – fraction that physical/river parameters explained independently; b – fraction that chemical variables explained independently; c – fraction that substrates explained independently; d – fraction that physical/river and chemical parameters explained together; e – fraction that chemical parameters and substrates explained together; f – fraction that physical/river parameters and substrates explained together; g – fraction that all parameters explained together.



Tested fraction	Explained variation (%)	F	P
a	14.7	1.3	0.002
b	14.7	1.3	0.002
c	8.0	1.2	0.036
a + d + f + g	16.6	1.4	0.002
b + d + g + e	17.2	1.5	0.002
c + e + f + g	9.7	1.3	0.006
a + b + c + d + e + f + g	40.1	1.4	0.002

Table 4
Water quality assessment of the Radovanska and Crnica rivers at sampling sites (RR1-RR5, CR1-CR6) according to the mean values of diatom indices (high water quality - blue, good - green, moderate - yellow, poor - orange).

Index/Site	RR1	RR2	RR3	RR4	RR5	CR1	CR2	CR3	CR4	CR5	CR6
IBD	17.6	19.2	18.8	17.7	17.4	17.8	18.1	19	18.6	18.5	19.5
IPS	16	17.8	17.2	16.8	16	16.5	17.3	17.6	17.3	16.7	17.7
GDI	13	14.8	14	13.1	12.3	14.3	16.2	14.2	13.4	13.6	14.9
DESCY	17.7	18.5	18.1	18.1	19.1	17.3	16.1	16.5	17.2	17.6	16.9
SLA	14.1	17	16.1	15.4	14.5	14.8	15.4	15.9	15	14.6	15.3
IDSE	15.7	17.1	16.4	15.9	15.4	16.1	16.6	16.3	15.9	16.1	16
IDAP	15.4	15.9	15.7	15.8	15.1	16.7	17.8	17.1	16.3	16.1	16
EPID	16	16.6	16.3	15.9	15.2	15.8	16.5	16.4	15.9	15.8	16.1
LOBO	10.8	16.5	15.3	17.5	15.9	16.3	16	16.3	17.3	16	17.7
DICH	12.5	17.7	16.9	15.1	13.3	13.4	15	16.1	14.7	14.9	16
TID	8.7	12.5	11.8	9.3	8	9.3	10.5	11.7	10.1	9.2	10
SID	15.1	16.3	16	15.1	14	14.4	15	15.2	14.7	14.5	14.6
CEE	17.4	17.3	16.8	17	16.5	17.5	18.6	16.1	16.3	17	16.7
WAT	16.9	14.8	14.7	15.6	16.5	16.3	17	17.2	16.8	16.5	16.5
TDI	11.5	14.3	12.7	10.6	8.1	10.5	11.4	10.6	10.8	9.2	8.7
IDP	13.3	15	14.5	12.7	12.6	14.2	14.4	14.7	14.3	13	13.7
SHE	14.6	15.8	15.6	15	14.4	14.9	15.9	15.5	14.9	14.9	15.2

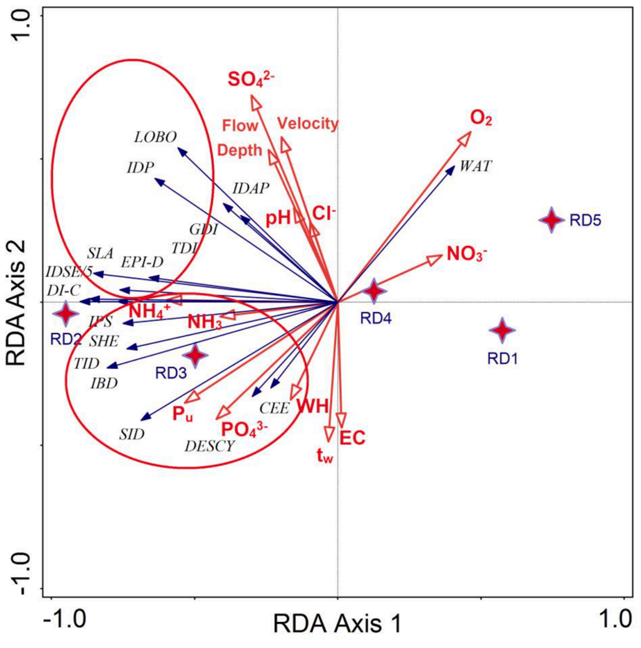


Fig. 4. RDA diagram showing relationship between diatom indices and physical and chemical parameters in the Radovanska River at sampling sites (RR1-RR5).

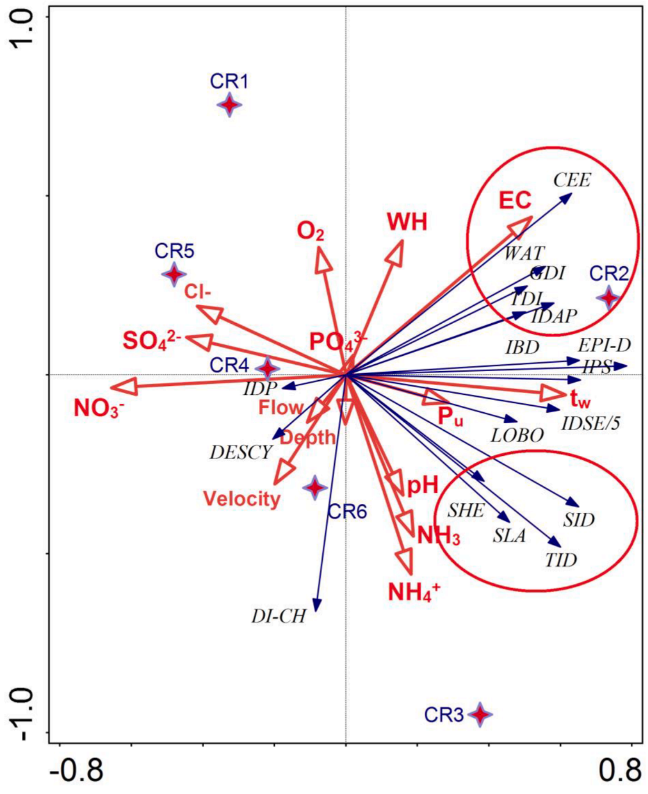


Fig. 5. RDA diagram showing relationship between diatom indices and physical and chemical parameters in the Crnica River at sampling sites (CR1-CR6).

(ecological status was estimated as poor) (Table 5). The ecological status of the investigated parts of both rivers was high (I class) based on phytobenthos as a biological element of ecological status assessment (Table 5). By combining the biological and physical and chemical elements, the final ecological status of the investigated parts of the Radovanska and Crnica rivers was poor, according to the legislation of the Republic of Serbia.

4. Disscusion

4.1. Diatom diversity

No data have been published to date on the diatom flora in the Radovanska River, but there is a few about Crnica river. During the algological investigation of the Crnica River, Ranković et al. (1995) identified a total of 44 diatom taxa, as well as their domination at all sites (84.61% of the total number of identified algal taxa). Our research has shown the presence of 170 diatom taxa. The most likely reason for such a large difference in the number of identified diatom taxa is development of microscopy techniques, which played a major role in detecting very tiny, hardly noticeable taxa (e.g. *Adlafia minuscula*, *Mayamaea permitis*). *Diatoma*, *Cymbella*, *Navicula* and *Nitzschia* were the most species-rich genera in algological analysis of the Crnica River (Ranković et al., 1995), while in our research, besides *Nitzschia* and *Navicula*, genus *Gomphonema* was one of the most species-rich genera.

Floristic composition is one of the most important characteristics of biological communities that reflects evolutionary processes, as well as ecological functions and stability of the ecosystem (Komulaynen, 2009). Although we studied benthic diatoms in the small hilly-mountainous rivers, a very high diversity can be noticed. It can be concluded by comparison with studies of similar rivers in Europe. Thus, diatom studies of four mountain rivers in Spain resulted in the identification a total of 108 taxa (Gomà et al., 2005). Similar diatom diversity was recorded by benthic diatoms investigation of rivers in Turkey and Poland (Solak et al., 2012; Noga et al., 2016). Some studies have shown that minor changes of environmental factors can lead to an increase in diversity (Bellinger et al., 2006) in contrast to others (Jüttner et al., 2003) that showed there was no change in diversity, despite the great anthropogenic effect. Also, often there is a lack of significant correlations between diversity and environmental variables. In general, the relationship between diversity and chemical pollution often is not a simple linear association (positive or negative), but a parabolic relation between diversity and water quality, with the highest diatom diversity at intermediate pollution levels can be noticed (Pandey et al., 2017). What we observed in terms of diversity is consistent with the hump-shaped relationship described from diatom assemblages reflecting this intermediate disturbance hypothesis. Comparing studies of diatom diversity in large and small mountainous rivers, it can be concluded that small rivers are often centers of diversity and should not be ignored (Dembowska, 2014; White and Harrington, 2008). This is also indicated by our research. These are small rivers, but great diversity can be observed, even when compared to the diversity of large rivers. 353 taxa were recorded by studying diatoms of the San River (Noga et al., 2014). It is the longest river of the Carpathians and the second-longest river in south-eastern Poland, with a total length of 443.4 km. Isheva and Ivanov (2016) believe that catchment size doesn’t influence diatom diversity, but local habitat heterogeneity plays a crucial role for the diversity of epilithic diatoms. Observing the total number of taxa by sites in our study, it is noticed that the fish farms affect diatom taxa diversity. Diatom diversity (number of species) in both rivers was significantly higher at site after discharge (Fig. 2). In contrast, according to Camargo and Jiménez (2007), epilithic diatoms were completely absent at site after fish farm, which is built on the Tajuña River (Spain). Unlike the Radovanska River, where the number of species does not change significantly with distance from the fish farm, in the Crnica River there was an increase and then second decrease in the number of species, to the last site where the highest diversity was noticed. In the Tajuña River, there was a clear tendency of diatom decreasing with distance from the fish farm (Camargo and Jiménez, 2007). Strong sedimentation of organic matter on the river bottom, which produced a thick layer of organic sediment, can be caused by a trout farm effluent. As a consequence, epilithic diatom diversity tended to decrease or be completely absent (Camargo and Jiménez, 2007). Although epilithic diatoms can be very sensitive to the sedimentation of suspended solids (Szoszkiewicz et al., 2006), diversity can increase as a consequence of nutrient enrichment (Liess et al., 2009), as indicated by our study.

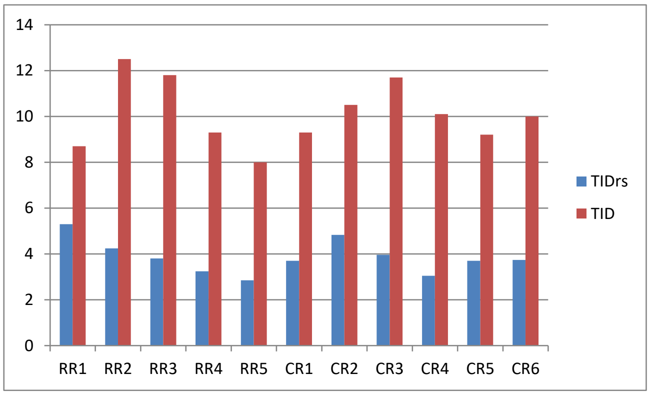


Fig. 6. TID_{RS} and TID values in the Radovanska and Crnica rivers. Bad water quality (<5), poor (≥5–<9), moderate (≥9–>13).

Taxa that are considered rare have been identified in our study, in addition to the cosmopolitan and endangered taxa. One of these taxa is *Stauroneis parathermicola*, which is recorded in both rivers. This is an aerophytic species, but also can be found among mosses in running waters (Lange-Bertalot et al., 2017). *S. parathermicola* is on the red list of algae in Poland (Stanek-Tarkowska et al., 2015). In our research, the highest abundance of this taxon was 3.2% in the Crnica River at site 4 where higher concentrations of “P_u” and “PO_4^{3-}“ were recorded (Table 2), which is following ecological preferences of this taxon. *S. parathermicola* was found with high abundance at sites that were under the influence of anthropogenic factors, where high concentrations of nitrogen and phosphorus were detected, which is also shown by our research. In arable land, it can represent a dominant species with a relative number of over 20% (Stanek-Tarkowska et al., 2015; Antonelli et al., 2017).

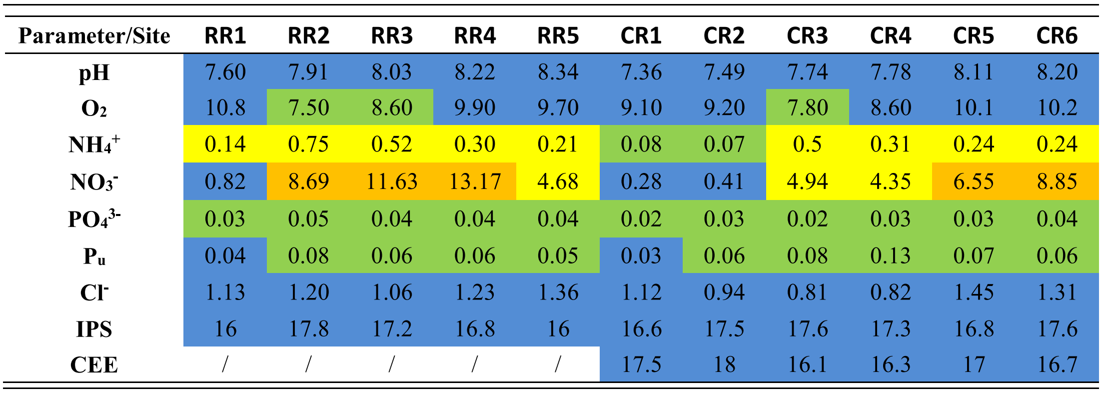
4.2. Diatom-environmental relationships

CCA analysis has shown that fish farms affect the physical and chemical parameters and therefore the composition of the epilithic diatom assemblage (Fig. 3).

The importance of nutrient concentrations on diatom diversity and distribution is evidenced in many studies (Ponader et al., 2007; Danielson, 2012; Tan et al., 2017). The importance of nutrient concentrations on diatom assemblages distribution can also be noticed in our research where “NO_3^-” and “NH_4^+” were single out as one of the most important chemical parameters (Fig. 3). Taxa on the right side of Fig. 3 were positively correlated with the concentration of these parameters. Among them, some taxa are tolerant to moderate and high concentrations of nutrients (eg. *Navicula metareichardtiana*, *Encyonema auerswaldii*, *E. caespitosum*, *Gyrosigma sciotoense*). These species tolerate oligo- to eutrophic conditions but mainly are recorded in eutrophic freshwater habitats (Lange-Bertalot et al., 2017). Fish farms are one of the largest sources of excessive nutrient discharge in rivers (Moraes et al., 2015). Nutrients originate from food that is used to feed fish or are released as metabolic products of fish. This primarily depends on the farm size, biomass amount, farm management practice and food quality used for fish feeding. Due to the total content of phosphorus and nitrogen that is used for fish nutrition, it is considered that this is the basic factor

Table 5

Ecological status assessment of the Radovanska and Crnica rivers according to the legislation of the Republic of Serbia (high ecological status - blue, good - green, moderate - yellow, poor - orange). The Table gives the values of the physical and chemical and biological parameters on the basis of which the ecological status of rivers is determined according to the legislation of the Republic of Serbia*.



Parameter/Site	RR1	RR2	RR3	RR4	RR5	CR1	CR2	CR3	CR4	CR5	CR6
pH	7.60	7.91	8.03	8.22	8.34	7.36	7.49	7.74	7.78	8.11	8.20
O_2	10.8	7.50	8.60	9.90	9.70	9.10	9.20	7.80	8.60	10.1	10.2
NH_4^+	0.14	0.75	0.52	0.30	0.21	0.08	0.07	0.5	0.31	0.24	0.24
NO_3^-	0.82	8.69	11.63	13.17	4.68	0.28	0.41	4.94	4.35	6.55	8.85
PO_4^{3-}	0.03	0.05	0.04	0.04	0.04	0.02	0.03	0.02	0.03	0.03	0.04
P_u	0.04	0.08	0.06	0.06	0.05	0.03	0.06	0.08	0.13	0.07	0.06
Cl^-	1.13	1.20	1.06	1.23	1.36	1.12	0.94	0.81	0.82	1.45	1.31
IPS	16	17.8	17.2	16.8	16	16.6	17.5	17.6	17.3	16.8	17.6
CEE	/	/	/	/	/	17.5	18	16.1	16.3	17	16.7

* The value of the parameters for the annual/multi-year period is determined as C80 (80 percentide) except for O_2 which is determined as C10 (10 percentide) according to the legislation.

affecting the increase of nutrient concentrations in rivers (Lazzari and Baldisserotto, 2008). Approximately 30% of nutrients remain in fish bodies, and the rest goes into the water with the ability to cause eutrophication. In Germany, Norway and the U.S. there are strict laws regarding regulation of the work of trout farms. The content of phosphorus in fish food should not exceed 1% (Moraes et al., 2015). In our research, the effect of fish farms on the concentration of "P_u", as well as "NH_4^+" and "NH_3", is evident, which is confirmed by other studies (Camargo and Gonzalo, 2007; Camargo et al., 2011; Moraes et al., 2015). Their concentrations significantly increased at site after discharge from the trout farm in both rivers (Table 2). However, since the water from the farms is directly released into the rivers, the "P_u" values were less than expected. The reason for this is probably the lower content of phosphorus in fish food (Bureau and Cho, 1999; Živić et al., 2009). This explanation is supported by studies where it is demonstrated that there is a difference in discharge nutrients into water based on the difference in food input into the farm and what fish uses to grow and supply energy requirements (Teodorowicz, 2013). The release of ammonium and orthophosphate is primarily associated with metabolic activity of fish, while the release of phosphorus and nitrogen is associated with unused food. Therefore, if fish food contains more phosphorus, then the amount of secreted and soluble phosphorus in the water will be higher. According to our results, there was a decrease in the concentration of "NH_4^+" with distance from the fish farm, downstream, but they still were higher than concentrations at sites upstream from the fish farms (Fig. 3). In the case of "NO_3^-" values vary in a small range at sites downstream from the farm, but they still were higher than concentrations at sites upstream from the fish farms, same as "NH_4^+". Moraes et al. (2015) were obtained similar results. They believe that phosphorus and nitrogen concentrations from the farm can be reduced by nutritional strategies based on the energy requirements of fish species.

In rivers flowing through sedimentary rocks, especially if the limestone is dominant, the pH value ranges from 7.5 to 8.5 (neutral to slightly basic water reaction) (Giller et al., 1998). Since this was the case with our studied rivers, the measured "pH" values are expected. The slightly basic reaction of water in our research also faithfully reflected the structure of diatom assemblage, since the frequent and dominant taxa were mainly alcalophiles (*Amphora pediculus*, *Cocconeis lineata*, *Denticula tenuis*, *Gomphonema micropus*, *Navicula cryptotenella*, *N. tripunctata*, *Nitzschia dissipata*, *N. fonticola*, *N. linearis*) (Van Dam et al., 1994, Lange-Bertalot et al., 2017). There was an increasing trend in "pH" values going downstream in both rivers. After fish farms, it continued to increase downstream. Thus, it can be assumed that "pH" is under the small influence of fish farms and that the observed trend of "pH" increase is more influenced by natural processes (Stojanović et al., 2019).

One of the important physical factors affecting algae communities on micro-scale in aquatic ecosystems is the type of substrate. The effect of the substrate is less noticeable on habitats where physical and chemical factors reach maximum values, causing stress in living organisms (Lengyel, 2016). This is confirmed by our results where chemical parameters in total explained the highest portion of variability in our data and were followed by physical/river parameters and substrate type (Table 3). The total content of mud was increased at first site downstream from the fish farm in both studied rivers, so the farm's impact is obvious when considering this parameter (Fig. 3). Organic matter accumulation from farms is noticed at sites immediately downstream from farms, so a large representation of this type of substrate at these sites is expected. Jüttner et al. (2011) think the role of substrate, in determining distribution of benthic diatoms, is significant, but the combined role of substrate, light and hydrological regime requires further studies. Szczepocka et al. (2019) showed that type of substrate is very important for development of some dominant taxa, as *Surirella brebissonii* var. *kuetzingii*, *Diatoma vulgaris* and *Achnanthidium minutissimum*. Our analysis indicates the similar results; that the type of substrate has a major influence on the distribution of dominant taxa, such as *Achnanthidium minutissimum* and *A. pyrenaicum* (Fig. 3). *A. minutissimum*, comparing to other species of genus *Achnanthidium*, has a much wider tolerance range to various environmental factors, but some models showed that higher abundances of *A. minutissimum* were mostly associated with low nutrient and ionic content (Ponader and Potapova, 2007). Our study does not support this models. Recent separation of the *Achnanthidium minutissimum* complex showed it consist of many ecotypes or species that are very similar morphologically, but differ in their ecology preferences (Wojtal et al., 2011), which have been insufficiently examined.

4.3. Diatom indices and ecological status assessment of investigated rivers

IPS and GDI indices singled out in our research by using between 93% and 100% of identified taxa in the calculation. Szczepocka et al. (2014) indicated a high degree of correlation between these two indices. Some studies support the view that the efficiency of diatom indices in a particular region depends largely on the degree of overlapping of the taxa list used by a particular index and list of identified taxa (Tan et al., 2017). In many European countries, the IPS has proven to be the most efficient index in water quality assessment, primarily because it involves a large number of taxa (about 2500) (Noga et al., 2016). This index is included in Serbian legislative system, representing one of the obligatory biological parameters in the ecological status assessment (Table 5).

Feio et al. (2009) pointed at weak correlation of GDI with other indices. This is usually explained by the fact that identification to the genus level is sufficient to obtain the value of this index, unlike all other diatom indices for which the calculation requires identification to the species level, and even lower taxonomic categories. IBD and TDI used between 84% and 100% of identified taxa in our study pointing their efficiency in ecological status assessment of the investigated rivers. Epilithic diatom studies of rivers in Europe and Asia also point at the efficiency of these indices based on a similar percentage of identified taxa (Kalyoncu and Şerbetci, 2013; Tan et al., 2013). IBD is one of the most frequently used diatom indices in European countries where its proved to be very useful in running waters monitoring (Ács et al., 2004; Hlúbiková et al., 2007; Kalyoncu and Şerbetci, 2013). It is considered suitable for “EC” concentration assessment (Della Bella et al., 2007). This was also confirmed by our analysis in which the IBD showed a positive correlation with the “EC” (Fig. 5).

According to values of diatom indices, trout fish farms have no significant negative impact on the water quality of the investigated rivers. Along the investigated part of the Radovanska River (although there is natural variability in water quality along the river network), most indices values varied in a narrow range, i.e. there was no noticeable decline in water quality at sites downstream from the farm (Table 4). Although the decline in value of most diatom indices at sites 3 and 4 along the explored part of the Crnica River is evident, these values continued to indicate the same class of water quality (Table 4). IBD, IPS, DESCY and CEE indicated high water quality of the explored rivers. Their values are changed by the influence of different pollutants (oxygen and chloride concentration, nutrients, organic matter) (Della Bella et al., 2007). This is confirmed by our results, showing the negative correlation of these indices with the concentration of “NO_3^-“ in the Radovanska River (Fig. 4). Therefore, a value of this parameter and water quality are inversely proportional. Also, our analysis clearly showed that these indices are very well correlated with each other in the Radovanska River (Fig. 4). However, in this study, positive correlation was observed between IPS and IBD. A strong correlation between these indices was noticed by Almeida (2001) and Feio et al. (2009), who recommended the use of these indices for routine water quality monitoring in Portugal. Also, Triest et al. (2003) proposed IBD and IPS as the most suitable diatom indices for Fleminsh water courses (Belgium). Taking into account the class boundaries based on the two diatom indices given in the legislation of the Republic of Serbia and our results (Table 5), this study indicates the need for revision of legislation for which this type of research is required on as many water bodies as possible.

Diatom indices have been developed to evaluate trophic status, general pollution, biological integrity, saprobity, nutrients (Sgro et al., 2009). TDI and TID were the only two indices indicating the moderate and poor water quality of both investigated rivers during the entire investigated period (Table 4). These indices have been designed to detect the concentration of nutrients in aquatic ecosystems (Kelly and Whitton, 1995; Rott et al., 1999). In addition, TDI calculation provides data on the proportion of taxa tolerant to organic pollution (% PT), indicating proportion of eutrophication associated with organic pollution (Kelly and Whitton, 1995). In our study, % PT values were mostly$<$20%. This tells us that sampling sites were free from significant organic pollution. Tan et al. (2017) indicated that the TID values mostly depend on the concentration of nitrogen compounds and pH, which explains 70% of the variation in the value of this index. This is confirmed by our results. In the Crnica River, TID responded most to changes in three factors (“pH”, “NH_3” and “NH_4^+”) (Fig. 5). In the Radovanska River, the values of this index were also greatly affected by “NH_3” and “NH_4^+” concentrations (Fig. 4). Thus, TID in our research has proven to be a very good indicator of the change in the concentration of nitrogen compounds, for which it was originally designed. Comparing the obtained results for TID_{RS} and TID, it is noticed that, although they take into account the same “stressor”, the water quality is not the same, ie. to vary from moderate to bad. The reason for deviation even for two water quality classes on most sites is a small number of taxa that are used to calculate TID_{RS} (from 17% to 92%). This further indicates that the proposed indicator list should be expanded based on a larger number of samples. Another reason for the deviation is the fact that indicator taxa have different indicator values. This deviation is due to different numbers of samples, different taxonomic approaches and differences in phytogeographical regions. Therefore, it is recommended that each region do ecological calibration, as well as intercalibration with other countries, especially in the region, in order to be able to determine with certainty the ecological status of water bodies (Poulíčková et al., 2004).

Some indices are designed to be used in certain geographical areas and include species specific to that area (Kalyoncu and Şerbetci, 2013). Our results showed a high degree of correlation between the majority of indices (Figs. 4 and 5), implicating that some diatom indices can be applied in different geographic regions, with detailed testing. WAT singled out as an index that is negatively correlated with other indices in the Radovanska River (Fig. 4). This can be explained by the great difference between the geographical region of its origin and our region (Watanabe et al., 1986). This index serve for the assessment of water saprobity (Dalu et al., 2016). Index that include species specific to area of origin is EPI-D, which is distinguished in our research by the percentage of identified taxa used for his calculation (over 80%) in the Crnica River. It originated in central Italy intending to monitor the Mediterranean rivers (DelÍUomo et al., 2004; DelÍUomo & Torrisi, 2011). However, many diatom species are cosmopolitan and this fact facilitates the indices applicability in many areas and not just the area of their origin (Sgro et al., 2009), as indicated by our study. A positive correlation between EPI-D, IPS, GDI and nutrient concentrations (Della Bella et al., 2007) was noticed, which is also shown by our analysis (Figs. 4 and 5).

As mentioned before, IPS involves about 2500 taxa, which is the largest number of taxa used to calculate some diatom index (Descy and Coste, 1991; Tan et al., 2017). For this reason, it is considered a “reference” index, based on which it is possible to assess the applicability of other indices. In our study, IPS showed high correlation with EPI-D, IDSE/5, IBD and CEE, making them a suitable tools for river monitoring in Serbia. This is confirmed by the high number of taxa used for their calculation. Having in mind all the above (principally indices correlation and percentage of identified taxa included in indices calculation), we can conclude that IPS and IBD are the most reliable diatom indices in ecological status assessment of mountainous rivers in Serbia.

5. Conclusion

The present study showed a high degree of inter-correlation of most diatom indices. The most reliable diatom indices for assessing the ecological status of mountainous rivers in Serbia are IPS and IBD. As one of the results of this study a high diversity of diatoms was also observed. Our research indicates that when assessing the ecological status of rivers should be taken into account as many parameters (both biological and physical and chemical). Fish farms built on these rivers affected the physical and chemical properties of the water, but did not have a significant negative impact on water quality based on diatom indices (indices are not enough sensitive). In order to form a list of diatom indicators for Serbia, establish adequate water quality class boundaries and adjust the appropriate trophic diatom index, a comprehensive study is needed that will include sampling at a large number of sites (including all types of running water in Serbia), defining reference habitats and determination of new class boundaries in relation to the values of diatom indices. It also points to the need for constant review of legislation regarding the ecological status assessment of water bodies, which requires this type of research on as many water bodies as possible.

CRediT authorship contribution statement

Olga S. Jakovljević: Conceptualization, Investigation, Writing -

original draft. **Slađana S. Popović:** Formal analysis, Writing - original draft. **Ivana M. Živić:** Writing - review & editing. **Katarina Z. Stojanović:** Resources, Writing - review & editing. **Danijela P. Vidaković:** Methodology. **Zorana Z. Naunovic:** Methodology. **Jelena Ž. Krizmanić:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Ács, É., Szabó, K., Tóth, B., Kiss, K.T., 2004. Investigation of benthic algal communities, especially diatoms of some Hungarian streams in connection with reference conditions of the water framework directives. Acta Bot. Hung. 46 (3–4), 255–278. https://doi.org/10.1556/ABot.46.2004.3-4.1.

Almeida, S.F.P., 2001. Use of diatom for fresh water quality evaluation in Portugal. Limnetica 20 (2), 205–213.

Antonelli, M., Wetzel, C., Ector, L., Teuling, A., Pfister, L., 2017. On the potential for terrestrial diatom communities and diatom indices to identify anthropic disturbance in soils. Ecol. Ind. 75, 73–81. https://doi.org/10.1016/j.ecolind.2016.12.003.

Bellinger, B.J., Cocquyt, C., ÓReilly, C.M., 2006. Benthic diatoms as indicators of eutrophication in tropical streams. Hydrobiologia 573 (1), 75–87. https://doi.org/10.1007/s10750-006-0262-5.

Bere, T., Mangadze, T., Mwedzi, T., 2016. Variation partitioning of diatom species data matrices: Understanding the influence of multiple factors on benthic diatom communities in tropical streams. Sci. Total Environ. 566–567, 1604–1613. https://doi.org/10.1016/j.scitotenv.2016.06.058.

Božanić, M., Todorović, D., Živić, M., Perić-Mataruga, V., Marković, Z., Živić, I., 2018. Influence of a trout farm on antioxidant defense in larvae of *Ephemera danica* (Insecta: Ephemeroptera). Knowl. Manag. Aquat. Ec. 419 (47), 1–11. https://doi.org/10.1051/kmae/2018036.

Bureau, D.P., Cho, C.Y., 1999. Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output. Aquaculture 179 (1–4), 127–140. https://doi.org/10.1016/S0044-8486(99)00157-X.

Camargo, J.A., Jiménez, A., 2007. Ecological responses of epilithic diatoms and aquatic macrophytes to fish farm pollution in a Spanish river. Anales Jard. Bot. Madrid. 64 (2), 213–219. https://doi.org/10.3989/ajbm.2007.v64.i2.177.

Camargo, J.A., Gonzalo, C., 2007. Physicochemical and biological changes downstream from a trout farm outlet: Comparing 1986 and 2006 sampling surveys. Limnetica 26 (2), 405–414. https://doi.org/10.23818/limn.26.34.

Camargo, J.A., Gonzalo, C., Alonso, Á., 2011. Assessing trout farm pollution by biological metrics and indices based on aquatic macrophytes and benthic macroinvertebrates: a case study. Ecol. Ind. 11 (3), 911–917. https://doi.org/10.1016/j.ecolind.2010.10.001.

Carvalho, L., Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K. L., et al., 2019. Protecting and restoring Europe's waters: an analysis of the future development needs of the Water Framework Directive. Sci. Total Environ. 658, 1228–1238. https://doi.org/10.1016/j.scitotenv.2018.12.255.

Coste, M., Boutry, S., Tison-Rosebery, J., Delmas, F., 2009. Improvements of the Biological Diatom Index (BDI): description and efficiency of the new version (BDI-2006). Ecol. Ind. 9 (4), 621–650. https://doi.org/10.1016/j.ecolind.2008.06.003.

Dalu, T., Bere, T., Froneman, W., 2016. Assessment of water quality based on diatom indices in a small temperate river system, Kowie River, South Africa. Water SA 42 (2), 183–193. https://doi.org/10.4314/wsa.v42i2.02.

Danielson, T.J., 2012. Protocol for Calculating DTPI and DTNI for Wadeable Streams and Rivers (DEPLW-0970A). Maine Department of Environmental Protection, Augusta.

DellÍUomo, A., Torrisi, M., 2011. The Eutrophication/Pollution Index-Diatom based (EPI-D) and three new related indices for monitoring rivers: the case study of the river Potenza (the Marches, Italy). Plant Biosyst. 145 (2), 331–341. https://doi.org/10.1080/11263504.2011.569347.

DellÍUomo, A., Torrisi, M., Corsini, A., Cavalieri, S., 2004. L"indice diatomico di eutrofizzazione/polluzione (EPI-D) nel monitoraggio delle acque correnti: linee guida. Agenzia per la protezione dell"ambiente e per i servizi tecnici, Roma (In Italian).

Della Bella, V., Puccinelli, C., Marcheggiani, S., Mancini, L., 2007. Benthic diatom communities and their relationship to water chemistry in wetlands of central Italy. Int. J. Lim. 43 (2), 89–99. https://doi.org/10.1051/limn/2007021.

Dembowska, E., 2014. Diatoms of the lower Vistula River phytoseston. Arch. Polish Fish. 22, 53–67. https://doi.org/10.2478/aopf-2014-0006.

Descy, J.P., Coste, M., 1991. A test of methods for assessing water quality based on diatoms. Verh. Internat. Verein. Limnol. 24, 2112–2116. https://doi.org/10.1080/03680770.1989.11899905.

Feio, M.J., Almeida, S.F.P., Craveiro, S.C., Calado, A.J., 2009. A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. Ecol. Ind. 9 (3), 497–507. https://doi.org/10.1016/j.ecolind.2008.07.001.

Gavrilović, L., Dukić, D., 2002. Reke Srbije. Zavod za udžbenike i nastavna sredstva, Beograd (in Serbian).

Giller, P.S., Giller, P., Malmqvist, B., 1998. The Biology of Streams and Rivers. Oxford University Press, Oxford.

Gomà, J., Rimet, F., Cambra, J., Hoffmann, L., Ector, L., 2005. Diatom communities and water quality assessment in mountain rivers of the upper segre basin (La Cerdanya, Oriental Pyrenees). Hydrobiologia 551 (1), 209–225. https://doi.org/10.1007/s10750-005-4462-1.

Guiry, M.D., Guiry, G.M., 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.

Hlúbiková, D., Hindáková, A., Haviar, M., Miettinen, J., 2007. Application of diatom water quality indices in influenced and non-influenced sites of Slovak rivers (Central Europe). Arch. Hydrobiol. Suppl. 161 (3–4), 443–464. https://doi.org/10.1127/lr/17/2007/443.

Hurlimann, J., Niederhauser, P., 2006. Methoden zur Untersuchung und Beurteilung der Fliessgewasser: Kieselalgen Stufe F (flachendeckend). Bundesamt für Umwelt BAFU, Bern (in German).

Isheva, T., Ivanov, P., 2016. Epilithic diatom flora from sub-Mediterranean intermittent rivers in Bulgaria during two hydrological periods. Bot. Serb. 40 (2), 153–160. https://doi.org/10.5281/zenodo.162213.

Jüttner, I., Chimonides, J., Cox, E.J., 2011. Morphology, ecology and biogeography of diatom species related to *Achnanthidium pyrenaicum* (Hustedt) Kobayasi (Bacillariophyceae) in streams of the Indian and Nepalese Himalaya. Algol. Stud. 136 (137), 45–76. https://doi.org/10.1127/1864-1318/2011/0136-0045.

Jüttner, I., Sharma, S., Dahal, B.M., Ormerod, S.J., Chimonides, P.J., Cox, E.J., 2003. Diatoms as indicators of stream quality in the Kathmandu Valley and Middle Hills of Nepal and India. Freshw. Biol. 48 (11), 2065–2084. https://doi.org/10.1046/j.1365-2427.2003.01138.x.

Kalyoncu, H., Şerbetci, B., 2013. Applicability of diatom-based water quality assessment indices in dari stream. Isparta-Turkey. World Acad. Sci. Eng. Technol. 7, 386–394. https://doi.org/10.5281/zenodo.1056737.

Karacaoğlu, D., Dalkıran, N., 2017. Epilithic diatom assemblages and their relationships with environmental variables in the Nilüfer Stream Basin, Bursa. Turkey. Environ. Monit. Assess. 189, 227. https://doi.org/10.1007/s10661-017-5929-z.

Kelly, M.G., Whitton, B.A., 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. J. Appl. Phycol. 7 (4), 433–444. https://doi.org/10.1007/BF00003802.

Komulaynen, S., 2009. Diatoms of periphyton assemblages in small rivers in Northwestern Russia. Stud. Trent. Sci. Nat. 84, 153–160.

Krammer, K., Lange-Bertalot, H., 1986. Bacillariophyceae, 1 Teil: Naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Süßwasserflora von Mitteleuropa, Band 2/1. G. Fischer Verlag, Jena, Germany, pp. 1–876.

Krammer, K, 2000. The genus Pinnularia, Vol. 1, in: Lange-Bertalot, H. (Ed.), Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. ARG Gantner Verlag K.G., Rugell, pp. 1-703.

Krammer, K., 2002. The genus Cymbella. Vol. 3, in: Lange-Bertalot, H. (Ed.), Diatoms of Europe: Diatoms of European Inland Waters and comparable habitats. Gantner Verlag, Ruggell, pp. 1-584.

Lange-Bertalot, H., 2001. Navicula sensu stricto. 10 Genera Separated from Navicula sensu lato. Frustulia. Vol. 2. In: Lange-Bertalot, H. (Ed.), Diatoms of Europe: Diatoms of European Inland Waters and comparable habitats. Gantner Verlag, Ruggell, pp. 1–526.

Lange-Bertalot, H., Hofmann, G., Werum, M., Cantonati, M., 2017. Freshwater benthic diatoms of central Europe: Over 800 common species used in ecological assessment. In: Cantonati, M., Kelly, M.G., Lange-Bertalot, H. (Eds.), Koeltz Botanical Books. Schmitten-Oberreifenberg, Germany.

Lazzari, R., Baldisserotto, B., 2008. Nitrogen and phosphorus waste in fish farming. Bol. Inst. Pesca 34 (4), 591–600.

Leclercq, L., Maquet, B., 1987. Deux nouveaux indices diatomique et de qualité chimique des eaux courantes. Comparaison avec différents indices existants. Cah. Biol. Mar. 28, 303–310 (in French with English abstract).

Lengyel, E., 2016. Stress and disturbance in benthic diatom assemblages. Examples from lakes and streams and from the field and laboratory studies. Doctoral dissertation. University of Pannonia, Veszprém, Hungary (Unpublished results).

Lenoir, A., Coste, M., 1996. Development of a practical diatom index of overall waterquality applicable to the French National Water Board network. In: Whitton, B. A., Rott, E. (Eds.), Use of Algae for Monitoring Rivers. Universität Innsbruck, II, pp. 29–45.

Levkov, Z., 2009. Amphora sensu lato. In: Lange-Bertalot, H. (Ed.), Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats 5. A.R.G. Gantner Verlag K.G, Ruggell, pp. 1–916.

Levkov, Z., Mitic-Kopanja, D., Reichardt, E., 2016. The diatom genus Gomphonema in the Republic of Macedonia. In: Lange-Bertalot, H. (Ed.), Diatoms of Europe: Diatoms of the European inland waters and comparable habitats 8. Koeltz Botanical Books, Oberreifenberg, pp. 1–552.

Liess, A., Lange, K., Schulz, F., Piggott, J.J., Matthaei, C.D., Townsend, C.R., 2009. Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. J. Ecol. 97, 326–336. https://doi.org/10.1111/j.1365-2745.2008.01463.x.

Lobo, E.A., Callegaro, V.L.M., Hermany, G., Bes, D., Wetzel, C.A., Oliveira, M.A., 2004. Use of epilithic diatoms as bioindicators from lotic systems in southern Brazil, with special emphasis on eutrophication. Acta Limnol. Bras. 16 (1), 25–40.

Milić, G., 2006. Reka Crnica – morfohidrološki prikaz. Zemlja i ljudi 56, 39–50 (in Serbian).

Mogna, M., Cantonati, M., Andreucci, F., Angeli, N., Berta, G., Miserere, L., 2015. Diatom communities and vegetation of springs in the south-western Alps. Acta Bot. Croat. 74 (2), 265–285. https://doi.org/10.1515/botcro-2015-0024.

Moraes, M.A.B., Carmo, C.F., Ishikawa, C.M., Tabata, Y.A., Mercante, C.T.J., 2015. Daily mass balance of phosphorus and nitrogen in effluents of production sectors of trout farming system. Acta Limnol. Bras. 27 (3), 330–340. https://doi.org/10.1590/S2179-975X1415.

Naylor, R.L., Goldburg, R., Primavera, J.H., Kautsky, N., Beveridge, M., Clay, J.W., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of Aquaculture on World Fish Supplies. Nature 405, 1017–1024. https://doi.org/10.1038/35016500.

Noga, T., Stanek-Tarkowska, J., Kloc, U., Kochman-Kędziora, N., Rybak, M., Peszek, Ł., Pajączek, A., 2016. Diatom diversity and water quality of a suburban stream: a case study of the Rzeszów city in SE Poland. Biodiv. Res. Conserv. 41 (1), 19–34. https://doi.org/10.1515/biorc-2016-0004.

Noga, T., Stanek-Tarkowska, J., Pajączek, A., Kochman, N., Peszek, Ł., 2014. Ecological assessment of the San River water quality on the area of the San Valley Landscape Park. J. Ecol. Eng. 15 (4), 12–22. https://doi.org/10.12911/22998993.1125453.

Official Gazette of Republic of Serbia, 2010. Ordinance on the determination of surface and groundwater bodies 96/10.

Official Gazette of Republic of Serbia, 2011. Ordinance on parameters of ecological and chemical status of surface waters and parameters of chemical and quantitative status of groundwater 74/11.

Pander, J., Geist, J., 2013. Ecological indicators for stream restoration success. Ecol. Ind. 30, 106–118. https://doi.org/10.1016/j.ecolind.2013.01.039.

Pandey, L.K., Bergey, E.A., Lyu, J., Park, J., Choi, S., Lee, H., Depuydt, S., Oh, Y., Lee, S., Han, T., 2017. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. Water Res. 118, 39–58. https://doi.org/10.1016/j.watres.2017.01.062.

Poikane, S., Kelly, M., Cantonati, M., 2016. Benthic algal assessment of ecological status in European lakes and rivers: Challenges and opportunities. Sci. Total Environ. 568, 603–613. https://doi.org/10.1016/j.scitotenv.2016.02.027.

Ponader, K.C., Charles, D.F., Belton, T.J., 2007. Diatom-based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecol. Ind. 7 (1), 79–93. https://doi.org/10.1016/j.ecolind.2005.10.003.

Ponader, K.C., Potapova, M., 2007. Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): ecology, distribution and taxonomic notes. Limnologica 37, 227–241. https://doi.org/10.1016/j.limno.2007.01.004.

Poulíčková, A., Duchoslav, M., Dokulil, M., 2004. Littoral diatom assemblages as bioindicators of lake trophic status: A case study from perialpine lakes in Austria. Eur. J. Phycol. 39 (2), 143–152. https://doi.org/10.1080/0967026042000201876.

Prygiel, J., Coste, M., 2000. Guide méthodologique pour la mise en oeuvre de l"Indice Biologique Diatomées NF T 90-354. Agence de l'eau Artois Picardie (in French).

Ranković, B., Čomić, L., Simić, S., Milovanović, D., 1995. Algological and saprobiological analysis of the water of the river Crnica, In: Proceedings of Conference on Current Problems of Water Protection "Water Protection ́95", 7-9 Juny 1995, Tara, Serbia, pp. 134–136.

Rott, E., Pipp, E., Pfister, P., Van Dam, H., Ortler, K., Binder, N., Pall, K., 1999. Indikationslisten für Aufwuchsalgen in österreichischen Fliessgewässern. Teil 2: Trophieindikation und autökologischen Anmerkungen. Bundesministerium für Land - und Forstwirtschaft, Wien (in German).

Sgro, G., Reavie, E., Kireta, A., Angradi, T., 2009. Comparison of diatom-based indices of water quality of mid-continent (USA) great rivers. J. Environ. Indicat. 5 (1), 48–67.

Smol, J.P., Stoermer, E.F., 2010. The Diatoms: Applications for the Environmental and Earth Sciences. Cambridge University Press, Cambridge.

Solak, C.N., Àcs, É., 2011. Water quality monitoring in European and Turkish rivers using diatoms. Turk. J. of Fish. Aquat. Sc. 11 (2), 329–337. https://doi.org/10.4194/trjfas.2011.0218.

Solak, C.N., Barinova, S., Ács, É., Dayioglu, H., 2012. Diversity and ecology of diatoms from Felent creek (Sakarya river basin). Turkey. Turk. J. Bot. 36 (2), 191–203. https://doi.org/10.3906/bot-1102-16.

Stanek-Tarkowska, J., Noga, T., Kochman-Kędziora, N., Peszek, Ł., Pajączek, A., Kozak, E., 2015. The diversity of diatom assemblages developed on fallow soil in Pogórska Wola near Tarnów (southern Poland). Acta Agrobot. 68 (1), 33–42. https://doi.org/10.5586/aa.2015.011.

Stojanović, K., 2017. Uticaj pastrmskih ribnjaka na zajednice makrozoobentosa tekućica sa posebnim osvrtom na larve roda Baetis (Ephemeroptera, Insecta). Doctoral dissertation, University of Belgrade, Faculty of Biology (Unpublished results, in Serbian).

Stojanović, K., Živić, M., Dulić, Z., Marković, Z., Krizmanić, J., Milošević, Dj, Miljanović, B., Jovanović, J., Vidaković, D., Živić, I., 2017. Comparative study of the effects of a small scale trout farm on the macrozoobenthos, potamoplankton, and epilithic diatom communities. Environ. Monit. Assess. 189 (8), 403. https://doi.org/10.1007/s10661-017-6114-0.

Stojanović, K., Živić, M., Marković, Z., Đorđević, J., Jovanović, J., Živić, I., 2019. How changes in water quality under the influence of land-based trout farms shape chemism of the recipient streams—case study from Serbia. Aquacult. Int. 27, 1625–1641. https://doi.org/10.1007/s10499-019-00414-1.

Szczepocka, E., Szulc, B., Szulc, K., Rakowska, B., Żelazna-Wieczorek, J., 2014. Diatom indices in the biological assessment of the water quality based on the example of a small lowland river. Oceanol. Hydrobiol. Stud. 43 (3), 265–273. https://doi.org/10.2478/s13545-014-0141-z.

Szczepocka, E., Żelazna-Wieczorek, J., Nowicka-Krawczyk, P., 2019. Critical approach to diatom-based bioassessment of the regulated sections of urban flowing water ecosystems. Ecol. Ind. 104, 259–267. https://doi.org/10.1016/j.ecolind.2019.04.078.

Szoszkiewicz, K., Ferreira, T., Korte, T., Baattrup-Pedersen, A., Davy-Bowker, J., O'Hare, M., 2006. European river plant communities: the importance of organic pollution and the use-fulness of existing macrophyte metrics. Hydrobiologia 566, 211–234. https://doi.org/10.1007/s10750-006-0094-3.

Tan, X., Sheldon, F., Bunn, S.E., Zhang, Q., 2013. Using diatom indices for water quality assessment in a subtropical river. China. Environ. Sci. Pollut. Res. 20 (6), 4164–4175. https://doi.org/10.1007/s11356-012-1343-9.

Tan, X., Zhang, Q., Burford, M.A., Sheldon, F., Bunn, S.E., 2017. Benthic Diatom Based Indices for Water Quality Assessment in Two Subtropical Streams. Front. Microbiol. 8, 1–10. https://doi.org/10.3389/fmicb.2017.00601.

Taylor, J.C., Prygiel, J., Vosloo, A., Rey, P.A., Rensburg, L., 2007. Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area. Hydrobiologia 592 (1), 455–464. https://doi.org/10.1007/s10750-007-0788-1.

Teodorowicz, M., 2013. Surface water quality and intensive fish culture. Arch. Polish Fish. 21 (2), 65–111. https://doi.org/10.2478/aopf-2013-0007.

Ter Braak, C.J., Šmilauer, P., 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer power, Ithaca, USA.

Triest, L., Peretyako, A., Ndum Foy, T., Kaur, P., 2003. Studie van scores en indices voor het biologisch kwaliteitselement fytobenthos in het licht van de kwaliteitsevaluatie van de Vlaamse oppervlaktewateren (rivieren, meren en overgangswateren) overeenkomstig de Europese kaderrichtlijn water. Vrije Universiteit Brussel, in opdracht van VMM, Brussel (in German).

Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol. 28 (1), 117–133. https://doi.org/10.1007/BF02334251.

Vidaković, D., 2019. Bioindikatorske karakteristike epilitskih silikatnih algi i procena ekološkog statusa odabranih reka zapadne i centralne Srbije. Doctoral dissertation, University of Belgrade, Faculty of Biology (Unpublished results, in Serbian).

Wang, H., Zheng, B., Liu, L., Li, L., 2014. Use of diatoms in River health assessment. Annu. Res. Rev. Biol. 4 (24), 4054–4074. https://doi.org/10.9734/ARRB/2014/11963.

Watanabe, T., Asai, K., Houki, A., 1986. Numerical estimation to organic pollution of flowing water by using the epilithic diatom assemblage - diatom assemblage index (DAIpo). Sci. Total Environ. 55, 209–218. https://doi.org/10.1016/0048-9697(86)90180-4.

WFD, 2000. Water Framework Directive - Directive of European Parliament and of the Council 2000/60/EC - Establishing a Framework for Community Action in the Field of Water Policy. European Union, the European Parliament and Council, Luxembourg.

White, B., Harrington, T., 2008. Benthic diatoms of the River Deel: Diversity and community structure. Biol. Environ. 108 (1), 29–42. https://doi.org/10.3318/BIOE.2008.108.1.29.

Wojtal, A., Ector, L., Van de Vijver, B., Morales, E., Blanko, S., Piatek, J., Smieja, A., 2011. The *Achnanthidium minutissimum* complex (Bacillariophyceae) in southern Poland. Algol. Stud. 136 (137), 211–238. https://doi.org/10.1127/1864-1318/2011/0136-0211.

Xue, H., Zheng, B., Meng, F., Wang, Y., Zhang, L., Cheng, P., 2019. Assessment of Aquatic Ecosystem Health of the Wutong River Based on Benthic Diatoms. Water 11, 727. https://doi.org/10.3390/w11040727.

Živić, I., Marković, Z., Filipović-Rojka, Z., Živić, M., 2009. Influence of a trout farm on water quality and macrozoobenthos communities of the receiving stream (Trešnjica River, Serbia). Int. Rev. Hydrobiol. 94 (6), 673–687. https://doi.org/10.1002/iroh.200811137.