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RESEARCH PAPER

Responses of Epilithic Diatoms to the Construction of Small Hydropower Plant in a Serbian River

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Abstract

Energy of running water accounts for almost 3/4 of the global supply of renewable energy sources and almost 1/5 of total electricity generation. Large and small hydropower plants are associated with a number of environmental problems. The main aim of this study is to investigate the impact of SHP on the diatom community. A total of 149 diatom taxa classified in 47 genera were identified, some of which are considered invasive (*Achnanthidium druartii*), rare (*Gomphonema zellense*, *Frustulia amphipleuroides*) and *Navicula radiosafallax* is first finding for Serbia. The impact of SHP is evident considering diatoms diversity and relative abundance of dominant species in three seasons (summer, autumn and winter). Values of most measured physico-chemical parameters changed immediately downstream of the SHP. Based on most diatom indices, the water of the Ljubovińa River had a good ecological status. TID and TDI indicated a predominantly moderate water quality of the Ljubovińa River. According to Serbian regulations, the Ljubovińa River had a high ecological status.

Introduction

One of the greatest challenges of our time is the generation of sufficient electricity due to the constant growth of the human population. The most important renewable energy source is electricity generated from the energy of running water, which accounts for almost 3/4 of the global supply of renewable energy sources and almost 1/5 of total electricity generation, with the aim of reducing dependence on fossil fuels (Panić et al., 2013). In terms of capacity, power plants are divided into small and large (Marszelewski et al., 2022). The main difference between large and small hydropower plants (SHPs) lies in the capacity of electricity generation. Both types of hydropower plants are associated with a number of environmental problems that do not necessarily differ (Walczak, 2018). However, considering many factors, especially the harmful effects on the environment, SHPs are receiving more attention from the scientific community. According to the study by Ristić et al. (2020), about 400 SHPs have been demolished in Europe due to their harmful effects on the environment. SHPs can affect many environmental issues, such as altering the distribution, composition and abundance of aquatic organisms, river flow, natural flow regime, destruction of surrounding forests, deposition of waste, sediment and nutrient loading of a river, and disruption of ecosystem stability (Wang et al., 2022). The impacts of SHPs can be better illustrated and more clearly recognized if we monitor SHPs globally and collectively capture their impacts (Couto & Olden, 2018). According to data from 2019, 110 small hydropower plants were built in Serbia (Ristić et al., 2020).

At the beginning of the 21st century, the issue of biodiversity loss in the aquatic environment was high on the political agenda in many European countries. The first legal framework in the European Union dealing with the conservation of all water bodies is the Water Framework Directive (WFD) (WFD 2000). The WFD contains a list of organisms that must be taken into account when assessing the ecological status of surface waters, including a group of microalgae, the diatoms. Diatoms have been shown to be good bioindicators of environmental and climate change (Goeyers et al, 2022) as well as good indicators of trophic status, organic water pollution and acidity. In addition diatom indices are important tools for monitoring and managing the health of aquatic ecosystems. In view of this, diatoms can be a useful tool to interpret the ecological impact of hydropower plants on biological communities.

The aim of this study is to analyze the diatom community and assess the ecological status of the Ljuboviđa River in order to investigate the impact of SHP on the diatom community. So far, the diatom community of the Ljuboviđa River has not been studied. A review of the literature data revealed that there are no studies on the effects of SHPs on diatoms in Europe, so this is the first study to gather knowledge on the effects on this group of organisms. Jakovljević et al. (2020, 2022) published preliminary results of a study on the effects of SHP on the Prištavica River on the diatom community in the form of conference abstracts, as well as Milićević et al. (2022) from this study. There are studies on the effects of SHPs on macroinvertebrates and fish in Europe (Česonienė et al., 2021; Vaikasas et al., 2015), while in Serbia there are only a few studies investigating the effects of SHPs on aquatic organisms (Crnobrnja-Isailović et al. 2021, Mitrović et al., 2021). Their effects on amphibian and reptile populations have been studied (Crnobrnja-Isailović et al., 2021), while there is very little data on algal communities (Mitrović et al., 2021). Mitrović et al. (2021) pointed out that the intensive construction of SHPs in recent years has endangered most red algal habitats in Serbia. In addition, some of the individual objectives were to determine the impact of SHPs on physical and chemical parameters of water and its quality.

Material and Methods

Ljuboviđa River and Sampling Sites

The Ljuboviđa River rises near the summit of the Jablanik mountain (western Serbia). It flows into the Drina and is part of the Black Sea drainage system (Marković, 1990). The river is 34 km long. Its absolute gradient is 1180 m, and it carries an average of 2.09 m^3 of water into the Drina every second (Marković, 1990). The Ljuboviđa is a mountain river surrounded by fields and forests. The diatoms were collected at five sites (LJB1–LJB5) along the Ljuboviđa River. Sampling sites LJB1 and LJB2 are located upstream of the SHP, while LJB3-LJB5 are located downstream of the SHP (Figure 1). Table 1 lists the main characteristics of the sampling sites.

Sampling and Measurement of Physical and Chemical Parameters

The studies of diatoms in the Ljuboviđa River were carried out during four seasons in 2019 and 2020 (spring - April, summer - July and autumn - October in 2019 and winter - January in 2020). A total of 20 samples were collected (5 sites, 4 seasons). Epilithic diatom communities were sampled by scraping medium-sized stones with a toothbrush into a plastic bottle (SRPS EN 13946: 2015). The samples were then preserved in 4% formaldehyde before further processing in the laboratory. Physical and chemical parameters (temperature (T), pH, conductivity (EC), oxygen concentration (O_2) and oxygen saturation (O_2)) were measured in the field using Eutech Instruments OaktonR7 and YSI ProODO.

Laboratory Work and Microscopic Analysis

After transferring the samples to the laboratory, the organic content of the diatom cells was removed with HCl and $KMnO_4$. The samples were then dried and fixed in Naphrax® medium to make permanent slides (Taylor et al., 2007). The permanent diatom slides were then analyzed using a Carl Zeiss AxioImagerM.1 light microscope with an AxioCam MRc5 camera. Magnifications of 1600x and 1000x were used as well as

Table 1. Main characteristics of the sampling sites (LJB1-LJB5) along the investigated course of the Ljuboviđa River.

Sampling site	Position in relation to the SHP	Coordinates	Flow depth (m)	Flow width (m)	Coastal vegetation
LJB1	150 m upstream	N 44.24406 E 19.47797	0.2–7.5	3.5–7.5	field farm and forest
LJB2	50 m upstream	N 44.24327 E 19.47801	0.19–0.2	4–7.5	field farm
LJB3	50 m downstream	N 44.24267 E 19.47769	0.15	5.5	field farm
LJB4	1.2 km downstream	N 44.23339 E 19.47104	0.14–0.22	7–8	field farm
LJB5	2.8 km downstream	N 44.22682 E 19.45796	0.18–0.44	4–4.5	field farm and forest

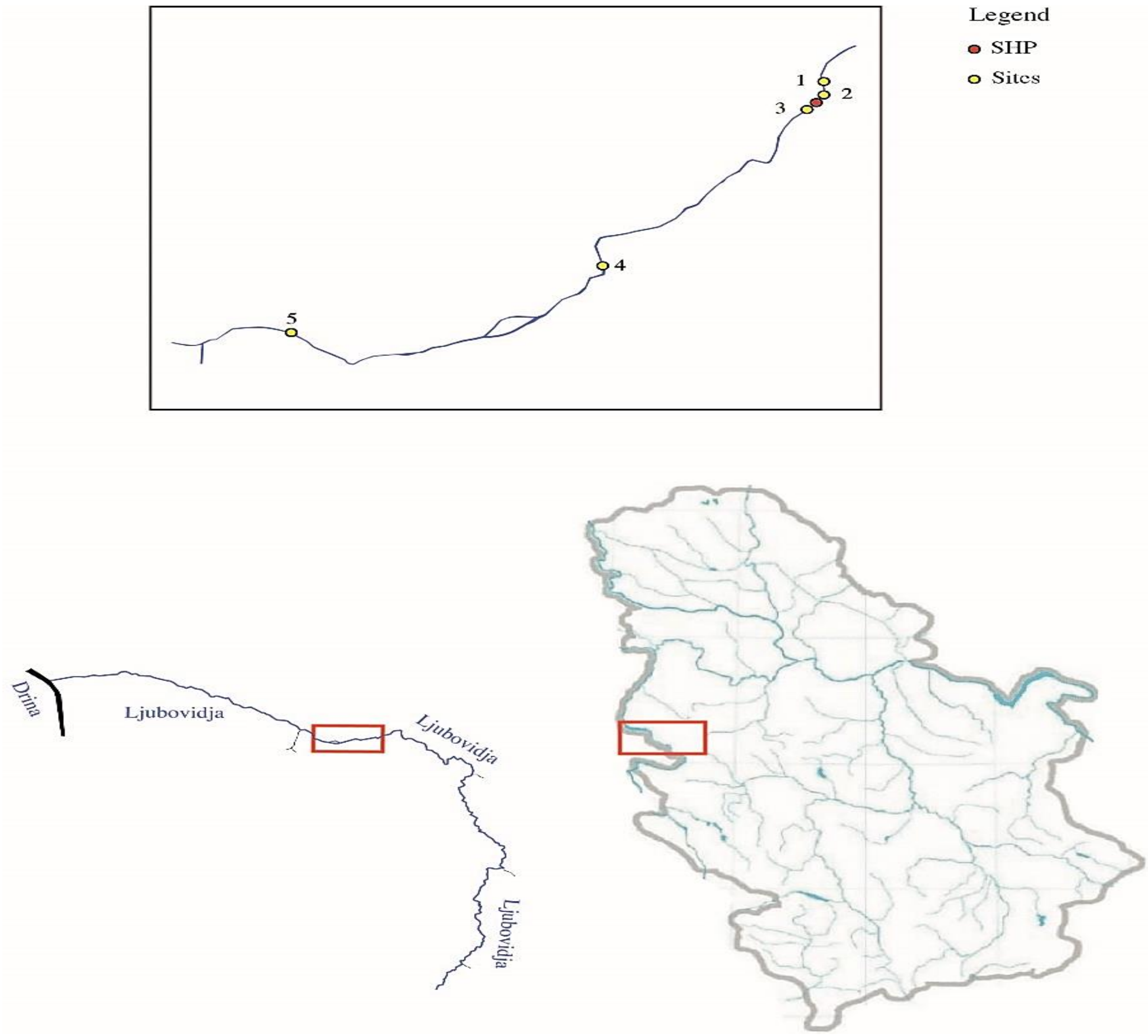

Figure 1. The location of the Ljubovića River, sampling sites (1-5) and SHP along the studied.

differential interference contrast (DIC), which creates a three-dimensional effect on the micrographs captured with Axio Vision 4.9 software. After the qualitative analysis, a quantitative analysis was performed by counting 400 valves on each slide (SRPS EN 14407: 2015).

Ecological Status and Statistical Analyses

Diatom indices were calculated on the basis of qualitative and quantitative analyses to assess the ecological status of the Ljubovića River. The indices were calculated using the OMNIDIA 6.0.8 software package (Lenoir and Coste, 1996). According to the Serbian legislation (Official Gazette of the Republic of Serbia, 2011), the CEE index (Coste index) (Descy and Coste, 1991) and the IPS index (Index of Pollution Sensitivity) (Cemagref, 1982) are two diatom indices used to assess the ecological status based on the benthic diatom community. Depending on the type of aquatic ecosystem, either both indices or only the IPS index are used.

Multivariate analyses (two canonical correspondence analyses (CCAs)) were performed using CANOCO software, version 5.0 (Ter Braak & Schmilauer, 2012). The CCA (unimodal method) was chosen because in both cases the gradient length was greater than 3.5 SD units, which was first checked by performing detrended correspondence analysis (DCA).

First CCA was performed to observe the relationship between the recorded diatom taxa and the measured physico-chemical parameters (EC, pH, O_2, T,). The imported dataset used for CCA contained all identified taxa, but when performing CCA, the option "downweight rare response variables" was used to give less weight to rare species. In addition, only the best fitted taxa were displayed in the final ordination diagram. No data transformations were applied to the response variables, while the explanatory variables were log transformed. The statistical significance of each variable (for the 0.05 level) was assessed using the Monte Carlo unrestricted permutation test with 499 permutations. To test the significance of the explanatory variables, the "Interactive forward selection" option was used, which showed that the following variables were significant (EC (p=0.002), O_2 (p=0.002) and T (p=0.004)).

For four selected taxa, *Achnanthidium druartii* (invasive taxon in Europe), *Frustulia amphipleuroides* and *Gomphonema zellense* (rare taxa) and *Navicula radiosafallax* (observed for the first time in Serbia), an additional constrained analysis (CCA) was performed. The mentioned taxa were related to the measured physico-chemical parameters used as explanatory variables (EC, pH, O_2, T,) and supplementary variable indicating the sampling location in relation to SHP ("before SHP" and "after SHP"). As in the previous analysis, the explanatory variables were log transformed, the statistical significance of each variable was assessed using the Monte Carlo test (499 permutations) and the "Interactive forward selection" option was used to test the explanatory variables for significance [EC ($p=0.002$) and O_2 ($p=0.038$) were significant].

In addition, the Mann-Whitney U test was used to compare two groups of physico-chemical parameters and diatom indices (group of values measured before SHP and after SHP) to see if there were significant differences. This was done separately for each variable and each diatom index.

Results

Diatoms Community

A total of 149 taxa in 47 genera belonging to the Bacillariophyta have been identified. The genera with the largest number of taxa in the Ljuboviđa River were *Gomphonema* (17), *Nitzschia* (15) and *Navicula* (14). The dominant taxa, present in all seasons and at all sites, were *Achnanthidium minutissimum* Kützing (51.55% in winter) and *Achnanthidium pyrenaicum* Hustedt (69.71% in summer). In total, there were 10 species in our study that were represented with more than 5% in at least one site (dominant species) (Figure 2). All recorded dominant species can also be defined as frequent, i.e. they were detected in more than 70% of the samples.

The number of taxa in spring varied between the highest value (61) at LJB1 and the lowest value (50) at LJB5 (Figure 3). In summer, the diversity of taxa decreased after the SHP (41) and then increased at LJB4, where the highest diversity was recorded (51). In the autumn, large changes in the number of taxa were observed at LJB3 and downstream. The highest number of taxa was recorded in the autumn at LJB3 (70) (after the SHP), and the lowest at LJB4 (25). During winter, the number of taxa decreased slightly at LJB3 and then increased at the other sites. The lowest number of taxa was recorded in LJB3 (27) and the highest in LJB5 (65) (Figure 3).

First Finding of Navicula radiosafallax Lange-Bertalot for Serbia

The taxon *Navicula radiosafallax* (Figure 4, 1-3) was observed for the first time in Serbia in epilithic samples from the Ljubovida River. The species was identified at three sites (LJB1, LJB2 and LJB3) in autumn. The valve length ranges from 33.06 µm to 38.65 µm, the valve width from 5.73 µm to 6.48 µm. The number of striae is 15–16 per 10 µm.

Rare and Invasive Species of the Ljubovida River

Gomphonema zellense Reichardt, a rare diatom taxon, was detected at 15 sampling sites (Figure 4, 4-7). The valve length is between 34.2 µm and 67.12 µm, the width between 8.7 µm and 12.08 µm. The number of striae is 9-11 in 10 µm, and the areola 19-26 in 10 µm. It was identified in each season (spring – LJB3, LJB4 and LJB5; summer – LJB1, LJB2 and LJB5; autumn – LJB1, LJB2, LJB3; winter – LJB3, LJB4, LJB5) with a maximum relative abundance of 0.24% in winter immediately after SHP (LJB3). In addition, a rare taxon, *Frustulia amphipleuroides* Grunow, was found at 11 sampling sites, one cell at each of these 11 sites (Figure 4, 11-13). The valve length in our samples ranges from 87.6 µm to 90.28 µm. The valve width varies between 17.69 µm and 18.66 µm. The striae number is 24 in 10 µm in all recorded cells of this species. It was identified in all studied seasons and at all sampling sites depending on the season. The highest abundance (1.24%) was found at LJB1 in winter.

The study of the diatom community of the Ljubovida River revealed the species *Achnanthidium druartii* Rimet & Couté (Figure 4, 8-10), which is considered invasive in Europe. It was identified in more than half of the samples (11), with a maximum relative abundance of 1.74%. This species was found in summer at three sites (LJB2, LJB3 and LJB4), in autumn (LJB2, LJB3 - maximum abundance, and LJB5) and in winter at sites after SHP (LJB4 and LJB5). The valve length is 17-26.44 µm; the width is 3.85-5.33 µm. The number of striae ranges from 16 to 21 in 10 µm, mostly 18.

Seasonal and Temporal Diatoms Distribution and Environmental Variables

The mean values of the physical and chemical water parameters in the individual seasons and at the sampling sites are given in Table 2.

The pH of the water fluctuated between 8.5 and 9, except in spring (LJB5), when it was 7. The highest pH was recorded in winter (9) (LJB5) and the lowest (7.12) in spring (Table 2), also at the same site.

The lowest temperature measured was in winter (LJB1) (4.9°C) and the highest (16.9 °C) in summer (LJB5). Within the seasons, the water temperature in the Ljubovida River fluctuated slightly. Water temperature increased at the site downstream of the SHP (LJB3) in all seasons (except autumn) compared to the sampling sites upstream of the SHP.

Oxygen concentrations ranged from a minimum of 7 mg/l in spring at LJB3 to a maximum of 13.56 mg/l in winter at LJB4 (Table 2). A significant decrease in oxygen saturation can be observed in spring and autumn at the

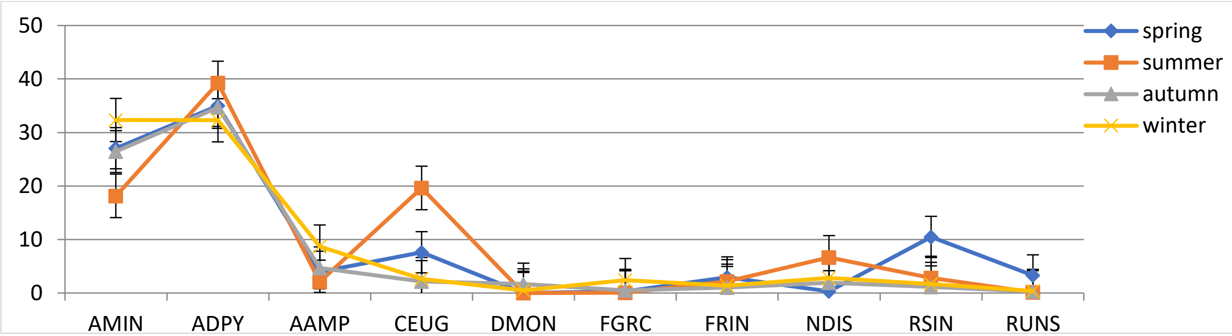

Figure 2. Mean values of relative abundances (%) of dominant species in the Ljuboviđa River in four seasons. ADMI - *Achnanthidium minutissimum,* ADPY **-** *Achnanthidium pyrenaicum,* AAMP - *Achnanthes amphicephala*, CEUG - *Cocconeis euglypta*, DMON - *Diatoma moniliformis*, FGRC - *Fragilaria gracilis*, FRIN - *Fragilaria rinoi*, NDIS - *Nitzschia dissipata*, RSIN - *Reimeria sinuata*, RUNS - *Reimeria uniseriata*.

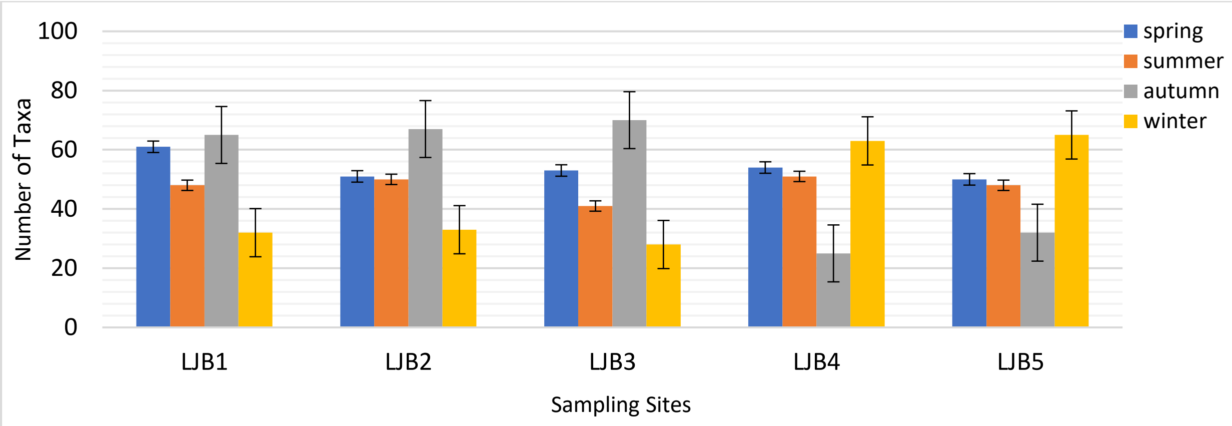

Figure 3. Number of taxa per sampling sites (LJB1-LJB5) in 4 seasons (spring, summer, autumn, winter)

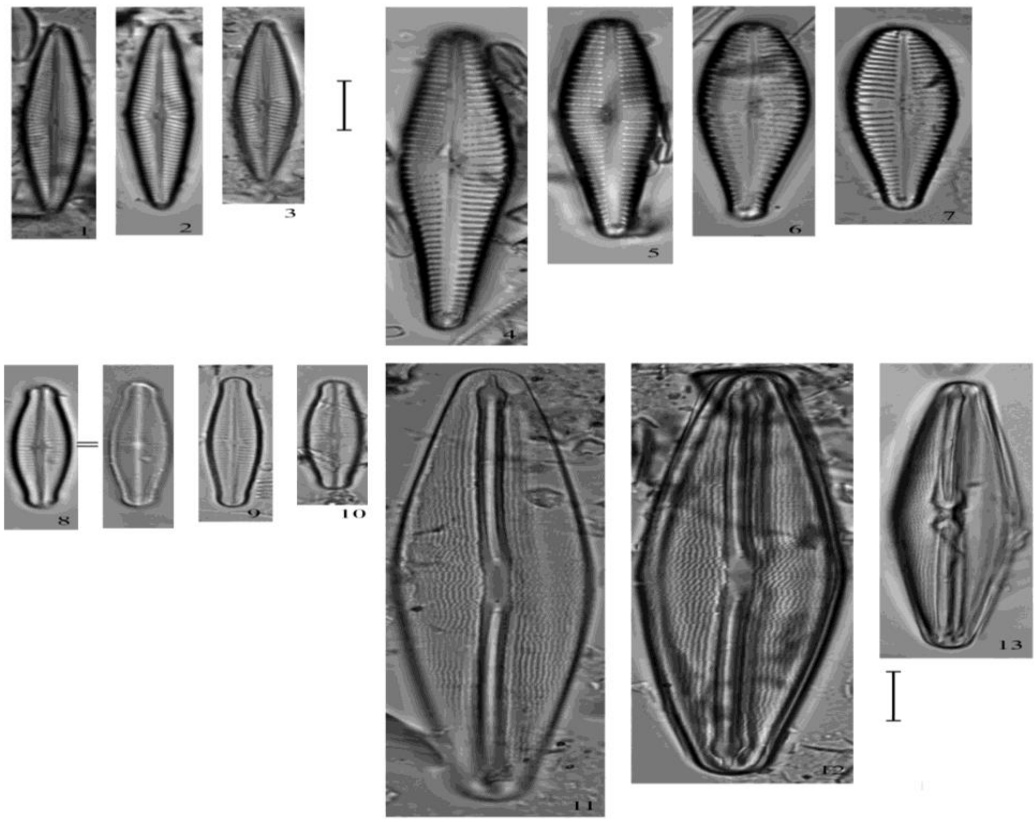

Figure 4. LM micrographs 1-12: 1-3 *Navicula radiosafallax*; 4-7 *Gomphonema zellense*; 8-10 *Achnanthidium druartii*; 11-13 *Frustulia amphipleuroides*. Scale bar = 10 µm.

Table 2. Mean values of physical and chemical water parameters in the seasons and at the sampling sites (LJB1-LJB5) of the Ljubović River.

Season	pH	T (°C)	EC (µS/cm²)	DO (mg/l)	O_2 (%)
Spring	8.27±0.64	13.56±0.29	316.2±3.03	8.84±1.46	86.72±10.21
Summer	8.81±0.05	15.7±0.75	318.4±3.29	10.75±0.19	106.8±2.49
Autumn	8.9±0.06	9.94±0.27	353.6±3.29	12.10±0.23	106.28±1.85
Winter	8.94±0.03	5.16±0.18	298±4.00	13.34±0.15	103.95±1.08
Site	**pH**	**T (°C)**	**EC (µS/cm²)**	**DO (mg/l)**	**O_2 (%)**
LJB1	8.76±0.18	10.82±4.38	323±7.21	11.18±1.93	100.25±8.55
LJB2	8.78±0.16	10.9±4.46	321.25±23.8	11.34±1.85	101±9.26
LJB3	8.78±0.15	11.2±4.60	319±22.78	10.76±2.77	96.8±15.56
LJB4	8.83±0.17	11.15±4.57	320.5±22.09	11.31±0.90	105.62±5.29
LJB5	8.48±0.91	11.37±5.08	324±20.67	11.28±1.00	106.65±1.34

site downstream of the SHP (LJB3). Oxygen saturation recovered at the next site and reached the previous value, after which it increased at the last site (LJB5). At the site downstream of the SHP (LJB3), a decrease in conductivity was observed in all seasons except winter.

When comparing the variable values measured at the pre-SHP sites with the variable values measured at the post-SHP sites using the Mann-Whitney U test (for each variable separately), a significant difference ($p<0.05$) was found only for T.

The CCA diagram shows the relationship between the physico-chemical parameters and the identified diatom taxa (Figure 5). The analysis showed significance ($F=1.6$, $P=0.002$); in addition, the total variation is 1.12735, the explanatory variables account for 36.05%, while the explained variation (cumulative) of the first and second axis is 12.35% and 19.93% respectively. Although the analysis included all 149 identified diatom taxa, only the 35 best-fitted are shown in the ordination diagram. O_2 correlated positively with the taxa in the lower left part of the diagram (i.e. UACU, CHIB, CLAN, ESUM, but also NRFA); pH and EC with NRFA, ESUM, GEXL, SBIR, SVTL, SHEL, and T with PTLA and DMES, FPRU, NIOG. A large number of taxa were located in the middle of the ordination diagram (they correlated slightly positively or negatively with a particular factor).

By using CCA with option of adding supplementary variables, *Achnanthidium druartii*, *Frustulia amphipleuroides*, *Gomphonema zellense* and *Navicula radiosafallax* were observed in relation to physico-chemical parameters and a supplementary variable indicating the sampling location in relation to the SHP (Figure 6). This analysis was significant ($F=3.3$, $P=0.002$), the total variation is 0.60530, the explanatory variables account for 48.30% and the explained variation (cumulative) of the first and second axis is 31.12% and 42.29% respectively. CCA showed that NRFA was most strongly positively correlated with EC, but also with pH and O_2 (as can also be seen in Figure 5). ADRU showed a positive correlation with pH and O_2, while FAPP and GZEL correlated positively with T. Looking at the sampling location in relation to the SHP, no clear conclusion can be drawn for FAPP, GZEL and NRFA, but ADRU correlates positively with sites located downstream of the SHP.

Ecological Status Assessment

The values of 5 diatom indices are given in Table 3. Based on the values of most diatom indices in spring, it can be concluded that the ecological status was good or high at most sampling sites in the studied part of the Ljubovića River. The values of diatom indices were within a narrow range, without major fluctuations depending on the sampling site. In summer, the values of the diatom indices were similar to those in spring (Table 3). The decrease in the IPS index can be observed between the sites upstream and downstream of the SHP (LJB2 and LJB3). TID and TDI are two indices that indicate a predominantly to moderate ecological status in both seasons, spring and summer. In the summer, the values of the TID index after the SHP decreased, but the ecological status did not change (moderate). IPS, IBD and CEE indicated a high ecological status of the river at all sampling sites in autumn. The impact of SHP is clearly visible in autumn when looking at the TDI values. The ecological status based on its values can be characterized as good at all sites, except for the site immediately after SHP (LJB3), where it can be characterized as moderate (sudden drop in value). TID values were also indicative of moderate status in the autumn. In winter, the IBD, IPS and CEE values indicated a high ecological status, similar to the autumn. The TID values indicated good and moderate ecological status (after SHP in winter, the value of the index increased (LJB3) and then decreased again at LJB4).

When comparing the values of the indices (for each index separately) measured at the pre-SHP sites with the variable values measured at the post-SHP sites using the Mann-Whitney U test, a significant difference ($p<0.05$) was found only for TID.

The water quality of the Ljubovića River was assessed as having a high ecological status in accordance with the legislation of the Republic of Serbia (Official Gazette of the Republic of Serbia, 2011) taking into account the IPS and CEE indices.

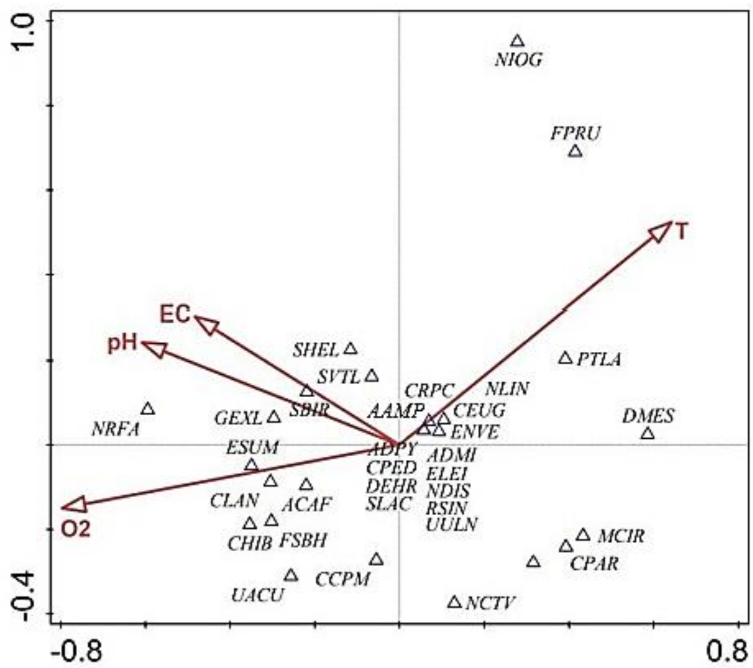

Figure 5. CCA analysis: the relationship between physico-chemical parameters and identified diatom taxa. ACAF - *Achnanthidium affine,* AAMP - *Achnanthes amphicephala,* ADMI - *Achnanthidium minutissimum,* ADPY - *Achnanthidium pyrenaicum,* CHIB - *Campylodiscus hibernicus*, CEUG - *Cocconeis euglypta*, CPED - *Cocconeis pediculus*, CCPM - *Cymbella compacta*, CLAN - *Cymbella lanceolata*, CPAR - *Cymbella parva,* CRPC - *Cymbopleura rupicola,* DEHR - *Diatoma ehrenbergii,* DMES - *Odonthidium mesodon*, ELEI - *Encyonema leibleinii*, ENVE - *Encyonema ventricosum,* ESUM - *Encyonopsis subminuta*, FSBH - *Fallacia subhamulata*, FPRU - *Fragilaria pararumpes*, GEXL - *Gomphonema exilissimum*, MCIR - *Meridion circulare*, NCTV - *Navicula caterva,* NRFA - *Navicula radiosafallax,* NDIS - *Nitzschia dissipata*, NLIN - *Nitzschia linearis,* NIOG - *Nitzschia oligotraphenta*, PTLA - *Planothidium lanceolatum*, RSIN - *Reimeria sinuata,* SVTL - *Sellaphora ventraloides*, SHEL - *Surirella helvetica*, SLAC - *Surirella lacrimula*, UACU - *Ulnaria acus,* UULN - *Ulnaria ulna*.

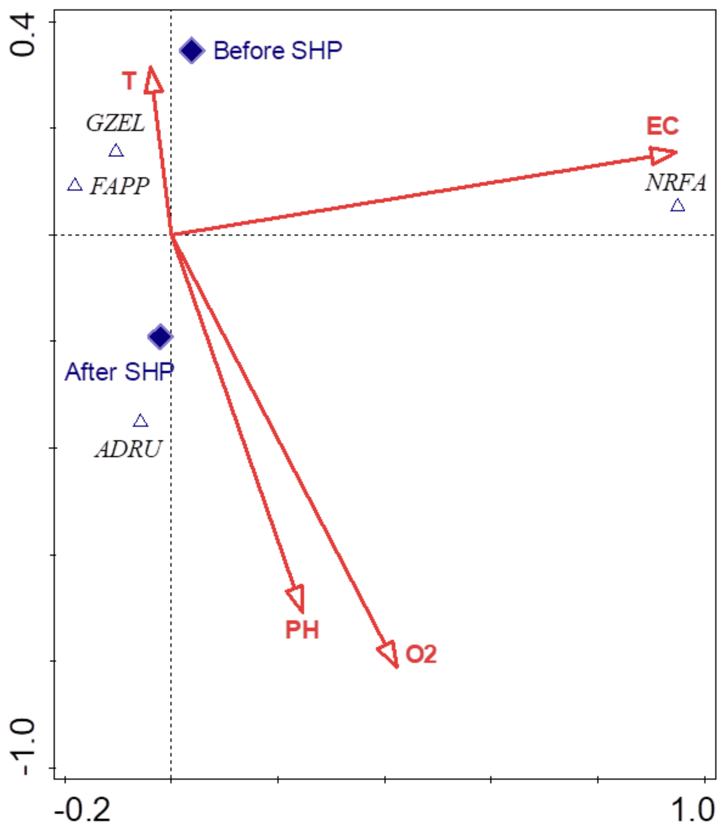

Figure 6. The relationship between physico-chemical parameters and new, rare and invasive diatom taxa. ADRU - *Achnanthidium druartii,* FAPP - *Frustulia amphipleuroides,* GZEL - *Gomphonema zellense*, NRFA - *Navicula radiosafallax.*

Discussion

Diatoms Composition

So far, there is no data on the study of the diatoms epilithic community of the Ljuboviđa River. The analysis revealed the presence of 149 taxa distributed among 47 genera. This high diversity supports the hypothesis of some authors that river length and diversity are not always positively correlated (Wojtal 2003; Jakovljević et al., 2021).

The dominant taxa occurring in the Ljuboviđa River in all seasons and at all sites belong to the genus *Achnanthidium*: *A. minutissimum* and *A. pyrenaicum*. One of the most widespread and earliest colonizing taxon is *A. minutissimum* (Lange-Bertalot et al., 2017). It has a wide ecological range. This taxon is absent in environments that are moderately or highly acidic and/or very low in electrolytes, which is consistent with our results. The impact of SHP on the number of this dominant and common taxon is remarkable. In the Ljuboviđa River, it is most abundant after SHP in LJB3 with a pH of 8.92. It requires an oxygen-rich environment (Van Dam et al., 1994). The percentage of oxygen saturation at the sites with the highest abundance of this species ranged from 104% to 105.6%. The abundance of *A. minutissimum* after SHP increases in every season except summer. *A. pyrenaicum* is more sensitive than *A. minutissimum* to organic pollution, metals and other pollutants (Cantonati et al., 2014). The abundance of *A. pyrenaicum* changed drastically in spring and autumn after the SHP (decrease, especially in autumn), while the abundance of this taxon increased in summer and winter. It was also observed that oxygen concentration decreased in spring and autumn after the SHP, which is consistent with the ecological preferences of *A. pyrenaicum* in terms of oxygen concentration (Lange-Bertalot et al., 2017).

The results show that SHP can affect the number of both dominant and some frequent diatoms. One of the frequent taxa in our study (identified in more than 70% of the samples) is *Amphipleura pellucida* Kützing – a taxon that is more common in stagnant freshwater habitats than in flowing waters (Lange-Bertalot et al., 2017). A decline in the abundance of this taxon can be observed in summer at LJB3, downstream of the SHP. *Cocconeis euglypta* Ehrenberg is more common in mesotrophic waters and tolerates different levels of pollution (Monnier et al., 2007). In the Ljuboviđa River, it is found at all sites and in all seasons. Immediately after the SHP in summer at LJB3, its abundance increased from 6.73% to 54%. The physico-chemical parameters are more or less the same at the two sites (before and after the SHP), with the exception of increasing temperature. *Ulnaria ulna* Compère (frequency of more than 70% in Ljuboviđa) prefers a slightly alkaline environment and moderate conductivity. It occurs in oligosaprobic, moderately eutrophic habitats (Lange-Bertalot et al., 2017). The abundance of this taxon was found to decrease in winter at the site downstream of the SHP. At the same site, there was an increase in temperature and oxygen saturation, which is consistent with literature data on the ecological preferences of this taxon.

In the epilithic diatom community of the Ljuboviđa River, the largest number of taxa was found in the autumn and the smallest in the winter. Autumn and spring differed by 3 taxa and were the seasons with the highest number of taxa (Figure 3). Various studies from Europe and North America on the seasonal dynamics of

Table 3. Ecological status assessment of the Ljuboviđa River at the sampling sites (LJB1-LJB5) according to the values of diatom indices in 4 seasons (high - blue, good - green, moderate - yellow).

Season	Sites	IBD	IPS	TID	CEE	TDI
Spring	LJB1	19.2	17.5	11.4	16.5	10.7
	LJB2	20	18.7	13	16.9	12.9
	LJB3	20	18.9	13.8	17.1	13.3
	LJB4	20	18.5	12.5	17.1	12
	LJB5	20	18.9	13.7	17.3	13.5
Summer	LJB1	19.4	16.8	10.5	17.2	11.8
	LJB2	20	17.8	11.8	17.5	12.8
	LJB3	18.4	16	10.7	17.7	13.2
	LJB4	20	18	12.6	17.7	13.8
	LJB5	20	17.9	12.5	17.6	13.1
Autumn	LJB1	20	17.6	12.2	17.2	13
	LJB2	20	17.7	12.1	17.4	13.5
	LJB3	20	18	12.6	17.4	12.6
	LJB4	20	18.1	13.1	17.3	14.6
	LJB5	20	17.5	13.5	17.6	14.3
Winter	LJB1	20	18	12.2	17.1	13.8
	LJB2	20	18	12.4	17.5	13.3
	LJB3	20	18.6	14.2	17.7	14.4
	LJB4	20	18.2	12	17.3	13.8
	LJB5	20	18.1	14.3	18.1	14.7

algae indicate regular seasonal patterns in temperate rivers and streams (Hausmann & Pienitz, 2007; Kirilova et al., 2008). The diversity and biomass of diatoms in the temperate zone is highest in spring, while the second maximum usually occurs in autumn, which is consistent with our results. In temperate rivers, the seasonal changes are mainly due to the different availability of light and nitrogen concentration (Allan & Castillo, 2007). Regarding the seasonal dynamics of diatoms from the Ljubovińa River, the effects of SHP in three seasons (summer, autumn and winter) are evident when considering the diversity and abundance of dominant species. The number of taxa and the abundance of dominant taxa have decreased at the site after the SHP. An exception is spring, where the number of taxa increased slightly (from 51 to 53) after LJB2 (where the SHP is located) (Figure 3). The number of dominant species of the genus *Achnanthidium* also increased. Changes in the hydraulic regime can cause significant changes in the composition of benthic algae upstream and downstream of the SHP (Growns & Growns, 2001). Water flow, which depends on both the anthropogenic factor (in this case – SHP) and natural factors (rainfall, season), undoubtedly has a major impact on the communities of all aquatic organisms (algae, aquatic plants, invertebrates, fish) (Bunn & Arthington, 2002). Regarding the seasonal dynamics of diatoms from the Ljubovińa River, the influence of the SHP can be seen in the total number of taxa over the seasons and the relative abundance of the dominant taxa.

Navicula radiosafallax was recorded in the Ljubovińa River and is the first finding of this taxon in Serbia. Due to the difficulty of identifying this taxon and distinguishing it from other similar taxa, the first information on the distribution of this taxon in Europe dates back to 2010 (Guiry & Guiry, 2018). As for invasive and rare taxa occurring in the Ljubovińa River, *A. druartii* can be easily distinguished (morphologically) from other *Achnanthidium* taxa. This taxon was found for the first time in France (River Rona, 2004), initially in low numbers, but later in greater relative abundance in many rivers and canals of this country (Ivanov, 2008; Wojtal et al., 2011). This taxon has been described as a new invasive species in rivers in France and Spain (Rimet et al., 2010). In Serbia, it has so far been found in the Đetinja River and the Jovac stream (Vidaković et al., 2018). In the Ljubovińa River, it was identified in more than half of the samples (11), with a maximum relative abundance of 1.74%. The rare taxon *G. zellense*, which in Serbia has so far only been detected in the Rača River (Vidaković et al., 2018), was discovered during the study of the Ljubovińa River. In Macedonia, it was only discovered in the Vardar River and Lake Debar (Levkov et al., 2016). Both locations have a high carbonate content and are mesotrophic. During our investigations, we identified individuals at 15 samples. Prior to our research, the taxon *F. amphipleuroides* had only been discovered in Lake Đeravičko, in 1996, before our finding (Lange-Bertalot, 2001). During our investigations, we identified the taxa at 11 samples. Unlike most taxa of this genus, *F. amphipleuroides* has a broad ecological range (Lange-Bertalot et al., 2017).

Physical and Chemical Parameters of Water

Rivers and streams are dynamic and complex ecosystems in which physical and chemical factors can change and influence the composition of diatom communities. Temperature, dissolved oxygen, pH, substrate composition, flow regime and light are just some of the most important factors that influence the diversity of organisms (Behrenfeld et al., 2021). Similar compositions of diatoms in regions with largely similar ecological conditions can be explained by the fact that most of these factors depend on climate, geology, topography and other features of the relief (Bere & Tundisi, 2009). Physical and chemical changes in the water lead to fluctuations in the seasonal and spatial dynamics of diatoms (Nascimento et al., 2021).

The pH value is an important factor that influences the composition of algae communities in rivers. Many diatoms can only tolerate a small range of pH fluctuations. Changes in pH can have a direct or indirect effect on diatom communities. Their direct influence is reflected in the direct physiological stress on the cells of diatoms, and their indirect influence is reflected in other chemical factors (Bere & Tundisi, 2009). Changes in pH affect the rate of CO_2 uptake, metabolic processes in the algal cells and membrane transport processes (Taraldsvik & Myklestad, 2010). In the Ljubovińa River, the change in water pH from alkaline to neutral (spring, LJB5 – 7.12 pH) can be explained by the large amounts of precipitation during this period. In spring and summer, the pH values increased after the SHP.

Diatoms, which prefer different temperature ranges, can react differently to increasing and changing temperatures (Zhang et al., 2018). This factor plays a major role in many biological processes (e.g. enzymatic reactions, photosynthesis, etc.). Both very low and high temperatures can cause serious damage to these processes (Jakovljević, 2019). The water temperature of the Ljubovińa River fluctuated depending on the season, from a minimum (4.9°C) in winter to a maximum (16.9°C) in summer. It was found that the temperature at the sites after SHP increased in all seasons except autumn. *A. minutissimum*, a dominant and frequent taxon in our study, was most abundant in winter (LJB3), where the highest temperature was also recorded. At this site, immediately downstream of the SHP, the temperature is higher than at the upstream sites, suggesting the influence of the SHP on this important physical parameter. The CCA diagram (Figure 5) also proves that temperature is an important factor influencing the distribution of this taxon, as a slight positive correlation is observed. *A. pyrenaicum* is the most abundant species at LJB5 in summer, where the highest temperature was measured in this season (and generally the highest temperature measured during this study).

Dissolved oxygen, pH values and substrate type have been shown in many studies to have a major influence on the dynamics and distribution of diatoms (Schneider et al., 2013). One of the most important environmental factors in aquatic ecosystems that is necessary for the survival of microorganisms is dissolved oxygen. It is an important indicator of pollution and eutrophication of rivers. The solubility of oxygen depends mainly on temperature and salt concentration. However, other factors such as the change in flow velocity, oxygen consumption by microorganisms and eutrophication also influence this factor (Jakovljević, 2019). According to our data, the Ljubovića River is rich in oxygen. There are fluctuations that indicate that in some cases there was more oxygen than in others, which we can associate with primary production. In winter, the cold water absorbs oxygen better, so the oxygen concentration is the highest. A decrease in oxygen concentration was observed in all seasons except in winter immediately after the SHP (Table 2). SHP can affect the flow regime of water, and often ecosystems can become hypoxic (Benchimol & Peres, 2015). In the Ljubovića River, there were no drastic changes, but the influence of SHP is noticeable when it comes to oxygen concentration.

Conductivity increases as the amount of minerals in the water increases (Bere & Tundisi, 2011). In the autumn, the water conductivity of the Ljubovića River is the highest compared to other seasons, which can be explained by precipitation. When it rains, the substrate (soil) rises, so the water is richer in minerals and the conductivity increases. In winter, when there is less precipitation, conductivity decreases (Bere & Tundisi, 2011).

Ecological Status of the Ljubovića River Based on Diatom Community

Epilithic diatom communities have been used in routine monitoring in many European countries for more than a decade (Jakovljević, 2019). The IPS index is characterized in our research by the fact that it includes between 89% and 100% of the identified taxa in its calculation, unlike the SLA index, for example, which uses only 50%. In many European countries, the IPS index has proven to be the most effective index for assessing water quality, as it includes a large number of taxa (Noga et al., 2016). If we look at the values of the IPS index, we can see that the value changed in the summer when indicated good ecological status at two sites (in other seasons at all sites, it indicated a high ecological status).

Based on the TDI, which indicated good and moderate ecological status of the studied river, our results showed the influence of SHP on the ecological status of the water of the Ljubovića River. In the autumn, the influence of SHP is clearly visible when observing TDI values. After the SHP, the TDI index values decreased and the ecological status changed from good to moderate. TDI and TID (Rott TI) are indices that show changes in water quality due to increased phosphorus and nitrogen concentrations. TDI describe the distribution of taxa in relation to "dissolved"/"total" phosphorus, which is generally closely correlated with the nitrogen concentration in the water (Pavluk & Bij de Vaate, 2017). The location of the SHP and nutrient concentration can be related, at least in the autumn, as TDI values showed a decrease in ecological status class at the site immediately after the SHP. The TID values (Rott TI) stood out the most in our study, indicating a moderate ecological status at most sites (Table 3). Also, it is noticed that on LJB4 in two seasons (spring and winter), the ecological status changed from good to moderate if we look at the values of the TID index. It should be pointed out that although both of these indices indicate the amount of nutrients, in summer downstream the SHP in our study (at LJB3), the TID value decreased (although still indicating moderate ecological status) and the TDI value increased (from moderate to good). In autumn, however, the situation is reversed: the TID index value increased (although still moderate ecological status), while the TDI value decreased (from good to moderate). Another index value that stood out in this study is the LOBO index (Lobo et al., 2002). The deterioration of water quality at the site immediately after the SHP in summer (LJB3) was shown regarding the values of LOBO index. It should be noted that although the LOBO index clearly showed the impact of SHP during the summer, only 20% to 50% of the current taxa were used for its calculation in our study, which is not surprising as it was developed in South America (Lobo et al., 2002), which is why its values are not shown in Table 3.

Ecological status assessment of the Ljubovića River based on the legislation of the Republic of Serbia indicated that the water quality classes have a wide range and our results indicate that their limits should be revised.

Conclusion

The analysis of diatom community of the Ljubovića River revealed a high diversity. The taxon *Achnanthidium druartii*, which is considered invasive in Europe, was detected as well as the rare taxa *Gomphonema zellense*, *Frustulia amphipleuroides* and *Navicula radiosafallax* (first finding for Serbia). When studying the seasonal dynamics of diatoms in the Ljubovića River, the impact of SHP in three seasons (summer, autumn and winter) becomes evident when considering the overall diversity, but also the relative abundance of dominant species. Most of the physico-chemical parameters measured changed immediately downstream of the SHP. The CCA diagram shows a positive correlation between physico-chemical parameters (pH, electrical conductivity, temperature and oxygen) and certain diatom taxa. Based on most diatom indices, the water of the Ljubovića River had a good ecological status. TID and TDI, which provide information on the nutrient load of aquatic ecosystems, indicated a predominantly moderate water quality of the Ljubovića River. The TDI values indicated a deterioration of the ecological status class at the site immediately downstream the SHP in autumn (LJB3) and TID value decreased in summer although the ecological status class did not changed, so that the position of the SHP and the concentration of nutrients can be linked, at least during these two seasons. According to Serbian regulations, the Ljubovića River had a high ecological status (Class I). The LOBO and TDI index values differed the most at the site downstream of the SHP (LJB3) compared to the values at the sites upstream. According to our results, as many parameters (biological, physical and chemical) as possible should be considered when assessing the ecological status of rivers containing SHPs. This study should be an incentive to further monitor the impact of SHPs on river biodiversity. The study also points to the need to constantly review legislation on the assessment of the ecological status of water bodies.

Ethical Statement

There is no ethical statement

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Author Contribution

Ana Milićević, Slađana Popović and Olga Jakovljević conceived the ideas and designed the methodology; all authors contributed to the collection of data; Ana Milićević analysed the data; Ana Milićević, Olga Jakovljević, and Jelena Krizmanić led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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