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PROOF
RESEARCH PAPER

The Potential Performance of Benthic Algal Community-based Biotic Indices for Assessing the Ecological Status: A Case Study of the Kamenica River (Serbia)

Kristina Markeljić[1,4,*], Jelena Krizmanić[2], Aleksandra Rakonjac[3,4], Nevena Đorđević[4], Elma Bašović[4], Snežana B. Simić[4]

[1]University of Kragujevac, Faculty of Agronomy in Čačak. Cara Dušana 34. 32102 Čačak. Serbia.
[2]University of Belgrade, Faculty of Biology, Department of Algology and Mycology. Studentski trg 16. 11000 Belgrade. Serbia.
[3]Institute for Vegetable Crops Smederevska Palanka. Karađorđeva 71. 11420 Smederevska Palanka. Serbia.
[4]University of Kragujevac, Faculty of Science, Department of Biology and Ecology. Radoja Domanovića 12. 34000 Kragujevac 34000. Serbia.

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Corresponding Author
E-mail: kristina.markeljic@kg.ac.rs

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Abstract

In most European countries, ecological status assessment of hilly-mountain rivers following the Water Framework Directive relies on diatom indices as parameters of phytobenthos. In contrast, only five of them use non-diatom algal-based indices. This study aimed to evaluate the performance of indices based on epilithic diatoms (IBD, IPS, IDG, SLAD, EPID, TID, TDI), non-diatom benthic algae (PIT, BI, NeD, RAPPER), and both groups (TI) for ecological status assessment compared to assessments based on the physical and chemical parameters of the water. The study was conducted in four seasons at six sites on the Kamenica River (Serbia). A total of 142 algal taxa were detected, including 85 diatoms and 57 non-diatoms, with 24 forming macroscopic aggregations. Among tested diatom indices, TDI and TID were most consistent with physical and chemical parameters-based assessment. Non-diatom indices PIT, BI and RAPPER method showed potential for broader application, although the indicator lists and the boundaries of the ecological status classes need to be adapted. The TI index provided more accurate results compared to the same index that only considers diatoms (TID), underlining the importance of including non-diatom algae as bioindicators in the ecological status assessment.

Introduction

The Water Framework Directive (WFD, 2000) prescribes algae and cyanobacteria for ecological status assessment of water bodies, divided into two subgroups–phytoplankton and phytobenthos. Within the analysis of phytobenthos, the focus is on the use of epilithic diatoms as bioindicators. The most commonly used diatom indices in the European Union (EU) member States are the IPS (*Specific Pollutant Sensitivity Index*) (Coste, 1982) and the SID (*Rott Saprobic Diatom Index*) (Rott et al., 1997) mainly for the assessment of organic pollution as well as trophic indices TDI (*Trophic Diatom Index*) (Kelly & Whitton, 1995), EPID (*Eutrophication/Pollution Index*) (Dell'Uomo, 2004) and TID (*Rott Trophic Diatom Index*) (Rott et al., 1999) (Masouras et al., 2021). In Serbia, the national regulations (Official Gazette of the Republic of Serbia, 2011, 2023) prescribe the method for assessing ecological status and the use of two diatom indices–IPS and CEE (*European Economic Community Index*) (Descy & Coste, 1991)–as parameters of phytobenthos. In addition, the Serbian Environmental Protection Agency (SEPA) used the EPID diatom index in its reports (Čađo et al., 2021). Additionally, numerous studies have tested the performance of diatom indices in different regions, demonstrated their correlation with environmental gradients and supported their widespread use (e.g. Bere

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et al., 2014; Martin et al., 2020; Tokatlı et al., 2020; Çetin et al., 2021; Ongun Sevindik et al., 2023).

While diatom-based indices are well established, the role of other algal groups (non-diatoms) in ecological assessment has been largely overlooked. Macroalgae are often included in macrophyte indicator lists and used to calculate macrophyte indices (Poikane et al., 2016). Only a few countries have specific metrics based on non-diatom benthic algae and/or macroalgae. In Bulgaria, the ecological status is assessed through *Cladophora* coverage (Cheshmedjiev et al., 2010). The NeD index (*Non-Diatom Index*), based on Chlorophyta and Cyanobacteria coverage, is used in Croatia (Mihaljević et al., 2020). In Germany, the BI index (*Biotic Index*) (Gutowski et al., 2004) and in Norway, the PIT index (*Periphyton Index of Trophic Status*) (Schneider & Lindstrøm, 2011) are used, both relying on non-diatom benthic algae. The Czech Republic, on the other hand, uses the Czech saprobic-trophic index (Marvan et al., 2011), incorporating data on all algal groups. Although only epilithic diatoms are often used in the calculation of the indices according to Rott et al. (1997, 1999) (TID, SID), they were originally created for the assessment based on all algal groups (TI, SI) in Austrian waters. The authors themselves claim that the inclusion of only diatoms reduces the accuracy of the assessment. The main reasons for the infrequent use of non-diatoms are challenges related to species identification and quantification (Fetscher et al., 2014; Poikane et al., 2016), as well as their uneven distribution in rivers (Vis, 2016). To address this, Kelly et al. (2016) developed RAPPER (*Rapid Assessment of PeriPhyton Ecology in Rivers*), a method that does not require species-level identification.

In contrast to diatom indices, existing indices based on non-diatom benthic algae or integrative approaches remain limited to the regions where they were developed (Gutowski et al., 2004; Schneider & Lindstrøm, 2011; Fetscher et al., 2014), which restricts their broader application despite their potential to improve assessment accuracy. Developing and refining methods to assess water quality using non-diatom benthic algae is important, as they are often the dominant group in rivers and reflect long-term physical and chemical conditions (Gutowski et al., 2004). Therefore, it is essential to evaluate the performance of both diatom and non-diatom indices in new geographical and ecological settings to improve bioassessment practices. To address this gap, the aim of this study was to test the performance of available biotic indices—based on epilithic diatoms (IBD, IPS, IDG, SLAD, EPID, TID, TDI), non-diatom benthic algae (PIT, BI, NeD, RAPPER) and both groups (TI)—in assessing the ecological status of hilly-mountain rivers. Obtained results were compared with assessments based on physical and chemical parameters of water. Additionally, the study aimed to monitor the occurrence of benthic macroscopic aggregations and the influence of environmental factors on benthic algal communities.

Materials and Methods

Study Area and Investigated Localities

The Kamenica River, located in western Serbia, is part of the Black Sea catchment area. It has a length of 38.03 km and a drainage basin of 216 km² (Marković, 1990). The river is formed by the confluence of the Bela Kamenica and Crna Kamenica rivers. Since they merge to form the Kamenica River, all three were studied as one unit.

Bela Kamenica and Crna Kamenica rivers are relatively small, especially in their upper reaches, where they often dry up completely. They flow through the tourist area of Divčibare Mountain, where they collect wastewater from hotels and residential facilities. Since the Kamenica River is formed by the confluence of these two rivers, their ecological status also affects the ecological status of the Kamenica River. Therefore, one representative sampling site was selected on the Bela Kamenica River (L1–N 44.05566, E 20.03944) (Figure 1) and one on the Crna Kamenica River (L2–N 44.05991, E 20.0485) (Figure 1), located approximately 1-2 km upstream of their confluence. Four sampling sites were selected on the Kamenica River (L3–N 44.05258, E 20.04266; L4–N 44.02863, E 20.07686; L5–N 43.95433, E 20.17919; L6–N 43.90911, E 20.26816) (Figure 1). Sampling site L3 was situated in the river's upper reach, after the merge of the Bela Kamenica and Crna Kamenica rivers. Since the Kamenica River flows through populated areas with extensive agricultural activities and, therefore, a high risk of pollutants (fertilizers, pesticides, sediments, and organic waste from both domestic and agricultural sources), sampling sites L4 and L5 were situated in these areas. Sampling site L6 was in the river's lower reach, before its confluence into the Zapadna Morava River. Basic hydromorphological characteristics of the investigated sites (river width and water depth) are summarized in Table 1.

Measurement of Physical and Chemical Parameters

At each locality, measurements of physical and chemical parameters were carried out in accordance with American Public Health Association (2005) using "AQUALITIC AL450" instruments. On-site measurements included physical parameters – temperature (Temp), electrical conductivity (EC), water depth (Depth) and river width (Width), as well as chemical parameters – pH (0–14) and water hardness (WH). The concentrations of nitrate (NO_3), total nitrogen (TN), orthophosphate (PO_4), total phosphorus (TP), ammonium ions (NH_4) and ammonia (NH_3) were measured in the laboratory.

Algal Sampling and Analysis

Epilithic diatoms were sampled following SRPS EN 13946:2015 standard and non-diatom benthic algae according to SRPS EN 15708:2011 standard. All samples

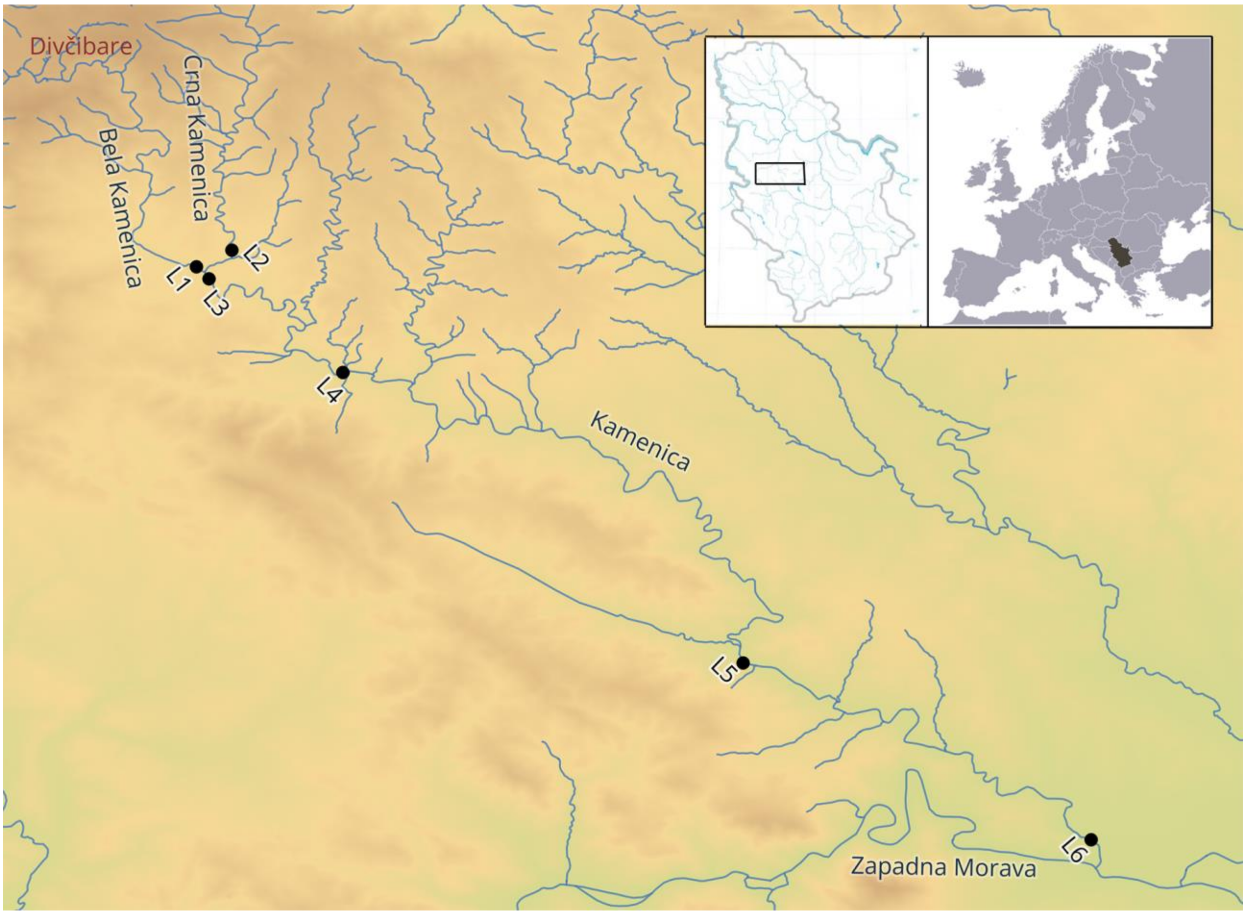

Figure 1. The position of Serbia and investigated localities on the Bela Kamenica River (L1), Crna Kamenica River (L2), and Kamenica River (L3, L4, L5, L6).

Table 1. Altitudes of the investigated sites and mean values (±standard deviation) of measured physical and chemical parameters

Parameter	L1	L2	L3	L4	L5	L6
Altitude (m)	575	576	569	508	359	258
Temp (°C)	8.63±5.46	10.65±7.6	9.63±6.60	11.25±7.1	9.25±5.87	9.28±5.90
pH	8.43±0.25	8.17±0.18	8.29±0.02	8.24±0.12	8.16±0.16	8.33±0.22
EC (µs/cm)	340±128.1	245±70.5	342.5±124	347.5±124	435±76.8	440±84.9
WH (mg L^{-1})	167.5±62	120±35.6	167.5±67	167.5±61	207.5±49	215±43.6
NO_3 (mg L^{-1})	9.75±3.30	11.86±9.4	10±4.40	12.5±10.6	9.38±6.21	10±6.06
TN (mg L^{-1})	2.28±0.91	2.71±2.09	2.34±1.01	2.88±2.18	2.18±1.42	2.31±1.28
PO_4 (mg L^{-1})	0.40±0.27	0.18±0.20	0.15±0.25	0.03±0.04	0.26±0.09	0.12±0.13
TP (mg L^{-1})	0.13±0.09	0.06±0.07	0.05±0.09	0.01±0.01	0.08±0.03	0.04±0.04
Depth (m)	0.24±0.08	0.19±0.05	0.40±0.12	0.24±0.05	0.29±0.05	0.36±0.08
Width (m)	5.38±0.25	3.38±0.25	17.25±0.5	3.63±0.25	10±0.25	16.5±0.25

were preserved with 4% formaldehyde solution and stored in wet collection of the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac. The epilithic diatoms were further processed in accordance with the SRPS EN 13946:2015 standard. The microscopic examination was conducted with a Motic BA310 microscope, BRESSER (9MP) camera and the MicroCamLab software package and with a Carl Zeiss AxioImager M1 microscope, AxioCam MRc5 camera and AxioVision 4.9 software. The quantitative analysis of epilithic diatoms was carried out following the SRPS EN 14407:2015 standard. Semi-quantitative analysis of non-diatom benthic algae was conducted by recording coverage in the field and, if necessary, converting to appropriate abundance scales according to Rott et al. (1999), Gutowski et al. (2004), Schneider & Lindstrom (2011), HR EN 15708:2011 and Kelly et al. (2016).

Calculation of Biotic Indices

The diatom indices were calculated using OMNIDIA 6.1.8 software (Lecointe et al., 1993). For further analysis, indices that included more than 50% of the identified epilithic diatoms at all localities and seasons were used. These indices were: IBD (*Biological Diatom Index*) (Prygiel & Coste, 2000), IDG (*Generic Diatom Index*) (Coste & Ayphassorho, 1991), SLAD (*Sladechek's Pollution Index*) (Sládecek, 1986), IPS (*Specific Pollutant Sensitivity Index*) (Coste, 1982), EPID (*Eutrophication/Pollution Index*) (Dell'Uomo, 2004), TID (*Rott Trophic Diatom Index*) (Rott et al., 1999) and TDI (*Trophic Diatom Index*) (Kelly & Whitton, 1995). In addition, the TI (*Rott Trophic Index*) (Rott et al., 1999), which includes all algal groups, was calculated to compare the results with those obtained using the diatom-based TID. The PIT (*Periphyton Index of Trophic Status*) (Schneider & Lindstrøm, 2011), NeD (*Non-Diatom Index*) (HRN EN 15708:2010; Mihaljević et al., 2020) and BI (*Biotic Index*) (Gutowski et al., 2004) indices were calculated manually, if sufficient indicator species were found.

Assessment of Ecological Status

The assessments of the ecological status based on physical and chemical parameters and IPS diatom index (IPS_{RSLEG} in Table 2) were conducted in accordance with the Serbian national regulations (Official Gazette of the Republic of Serbia, 2011, 2023). The ecological status classes' boundaries for the diatom indices were defined following Prygiel & Coste (2000).

The Trophic Index (TI) (Rott et al., 1999), which ranges from 0 to 4, was recalculated using a modified EQR (*Ecological Quality Ratio*) formula to enable comparison with diatom indices and evaluate ecological status according to Prygiel & Coste (2000). The reference and worst values applied were taken from Rott et al. (1999) and reflect conditions in Austrian rivers, as specific values for Serbian rivers are not yet established. When calculating the PIT index, the threshold values from the Norwegian regulations (Direktoratsgruppen for Vanndirektivet, 2018) were used for testing purposes. The ecological status was also assessed using the NeD index with a modified EQR formula (Mihaljević et al., 2020) and the BI index with the scale of Gutowski et al. (2004). The ecological status assessed can be high (class I, blue), good (class II, green), moderate (class III, yellow), poor (class IV, orange), and bad (class V, red). The macroalgal genus data were also used to assess the probable ecological status using the RAPPER (*Rapid Assessment of PeriPhyton Ecology in Rivers*) method (Kelly et al., 2016), which is based on the presence of sensitive taxa (S) and the abundance of competitive taxa (C). The assessed ecological status can be high/good, moderate, or poor/bad (Kelly et al., 2016).

Statistical Analysis

The mean values and standard deviations of environmental parameters were calculated using IBM SPSS 19.0 (IBM Corp., 2010). Pearson or Spearman correlation coefficients ($P<0.05$), depending on the normality of data distribution, were also computed in SPSS to assess relationships between biotic indices. Canonical Correspondence Analysis (CCA) was performed using the "vegan" package (Oksanen et al., 2019) in R-Studio software (R Core Team, 2024) to evaluate the influence of environmental factors on the benthic algal community. To reduce the effect of rare species, only taxa with an abundance of ≥ 10% were included in the analysis. When strong positive correlations were detected among environmental variables, only one variable from each highly correlated pair was retained. Variables that did not meet the assumption of normality were log-transformed before performing the CCA analysis. The significance of the model was tested using a Monte Carlo unrestricted permutation test with 499 permutations.

Results

Physical and Chemical Parameters

Altitudes of the investigated sites and the values of measured physical and chemical parameters are listed in Table 1. Ammonium ion (NH_4) and ammonia (NH_3) concentrations were below the detection limit at all studied sites during all seasons.

Qualitative and Quantitative Composition of Algal Species

A total of 142 algal taxa were identified, including 85 diatom (Bacillariophyceae) and 57 non-diatom benthic algae: Cyanobacteria (14 taxa), Chlorophyta (34 taxa), Charophyta (3 taxa), Euglenophyta (3 taxa), Rhodophyta (2 species) and Chrysophyceae (1 species).

In autumn, the dominant diatom species were *Nitzschia denticula* Grunow (L1–31.5%, L2–74.75%, L3–56.5%), *Ulnaria biceps* (Kütz.) Compère (L4–30%), *Cymbella perparva* Krammer (L5–42.75%) and *Cocconeis pediculus* Ehrenb. (L6–29.5%). The situation differed in winter when the dominant species were *Achnanthidium minutissimum* (Kütz.) Czarnecki (L1–33.25%; L4–37%), *Cymbella affinis* Kütz. (L2–49%), *Gomphonella olivacea* (Horn.) Rabenh. (L3–17.25%; L5–28%; L6–28%), while in summer *Nitzschia palea* (Kütz.) Smith (L1–29.25%), *N. denticula* (L2–53.5%; L3–48.75%; L4–36.5%), *C. pediculus* (L5–19.75%) and *Cymbella tumida* (Bréb.) Van Heurck (L6–23.25%) were dominant. In autumn, *C. perparva* produced large amounts of mucilage stalks, forming thick mats that covered large portions of the riverbed on L5. By winter, these were replaced by thinner, brownish mats of *G. olivacea*. In spring, the dominant species included *Encyonema ventricosum* (Agardh) Grunow (L1–41.25%; L3–35.25%), *Cymbella subhelvetica* Krammer (L2–82.5%), *A. minutissimum* (L3–24%, L4 – 39.25%, L5–26%, L6–27%), *C. affinis* (L4–24.75%), *C. perparva* (L4–18.75%), *D. moniliformis* (L5–26.25%) and *C. pediculus* (L6–42.5%).

Table 2. Ecological status assessment based on biotic indices at investigated sites over four seasons

Indices / Localities	L1	L2	L3	L4	L5	L6
			Autumn 2022			
IBD	17.9	19.9	18.9	17.3	17.2	15.7
IPS	13.8	15	14.6	14.9	17.5	14.6
IPS$_{RSLEG}$*						
IDG	10.7	4.8	6.3	11.7	15.7	12.6
SLAD	12.4	13	12.7	11.9	12.4	11.4
EPI-D	14.5	16.1	15.5	14.8	14.5	12.7
TID	13.2	15.6	13.2	11.1	12.3	8.5
TDI	10.6	8.4	7.5	10.5	12.6	6.8
TI	9.41	10.92	7.84	8.16	10	7.68
PIT	12.32	10.45	15.33	13.73	17.9	12.97
BI	-	-	-	-	-	-11.7
NeD	0.1	0.1	0.1	0.1	0.1	0.1
			Winter 2023			
IBD	17	19.8	17.2	18.7	18.1	17.5
IPS	15.7	15.6	15.3	17.3	17.5	16.4
IPS$_{RSLEG}$*						
IDG	13.3	14.5	14.4	17.1	16.7	15.2
SLAD	14	13.8	12.3	14.3	12.7	12.2
EPI-D	15.5	15.4	15	16.3	15.2	15.1
TID	10.3	16	9.6	14.5	11	9.2
TDI	10.8	16.8	9	14.3	7.8	8.1
TI	10.4	14.22	8.27	13.78	10.54	7.68
PIT	21.04	5.71	-	-	-	33.09
BI	-	-	-	-	-	-
NeD	0.1	0.1	-	0.3	-	0.1
			Summer 2023			
IBD	13.9	20	19.1	17.7	15.9	14
IPS	8.7	15.5	14.4	14.9	14.1	12.5
IPS$_{RSLEG}$*						
IDG	9.9	7	7	8.6	10.3	14.2
SLAD	11.5	13.1	13.1	13	11.6	11
EPI-D	12.1	16.1	15.7	16.5	12.6	10.8
TID	8.9	14.5	13.1	13	6.7	6.8
TDI	10.2	9	8.8	8.1	5.3	5.3
TI	7.89	10.32	8.97	7.24	4.22	7.24
PIT	19.25	12.05	16.85	14.98	27.68	19.76
BI	-28	-0.20	-	-	-	-
NeD	0.1	0.1	0.1	0.1	0.1	0.1
			Spring 2024			
IBD	16.4	20	18.1	19.9	19.4	16.3
IPS	14.5	19.8	16.9	18	16.4	16.1
IPS$_{RSLEG}$*						
IDG	13.8	18	15.9	17.4	14.8	14.4
SLAD	13.3	16.7	14.3	14.6	13.6	12.8
EPI-D	13.3	16.2	14.8	16	14.8	14
TID	6	16.6	9	16	10.6	9.3
TDI	6.4	14.5	8.9	16	10.2	8.9
TI	10.1	16.6	9.9	8.9	10.6	10
PIT	39.27	4.77	27.82	-	31.04	39.27
BI	10.98	-	21.95	34.48	9.02	-30
NeD	0.1	0.1	0.1	0.1	0.3	0.1

*threshold values from the Regulation (Official Gazette of the Republic of Serbia 74/2011)

Macroscopic aggregations were formed by twenty-four species of benthic non-diatom algae. The following species occurred with medium to high coverages in autumn: *Cladophora glomerata* (L.) Kütz. (L1 and L3–80–90%, L2–1–5%, L3–30%, L5 and L6–10%), *Spirogyra* sp. (L3–40%), *S. borgeana* Transeau (L2–10%, L4–5–10%, L5–20%, L6–30%), and *Zygnema* sp. (L6–10%). The coverage of other visible non-diatom benthic algae (*Leptolyngbya tenius* (Gomont) Anagnostidis & Komárek, *Phormidium interruptum* (Gomont) Kütz., *Porphyrosiphon versicolor* (Gomont) Anagnostidis & Komárek, *Bulbochaete* sp., *Dichothrix* sp., *Rivularia* sp., *Microspora amoena* (Kütz.) Rabenh., *Mougeotia* sp., *Oedogonium* sp., *Ulothrix tenuissima* Kütz., *U. zonata* (Weber & Mohr) Kütz.) ranged from 1 to 5%. In winter, macroscopic aggregations of species *C. glomerata*, *Dichothrix* sp., *Rivularia* sp., *Oedogonium* sp., *Spirogyra* sp. and *U. zonata* were present only in traces (1–5%). In summer, macroscopic aggregations of the cyanobacterium *Phormidium lividum* (Hansg.) Forti (L5–20%) and macroalgae *C. glomerata* (L1–95%, L6–90%, L5–80%, L3 and L4–60%), *Spirogyra* sp. (L2–80%, L3 and L4–40%) covered large percentages of the riverbed surface, while others had a coverage of 1–5% (*Oedogonium* sp., *Stigeoclonium tenue* (Agardh) Kütz., *M. amoena*, *U. zonata*, *Mougeotia* sp., *Zygnema* sp., *Klebsormidium* sp., *P. versicolor*, *Audouinella pygmaea* (Kütz.) Weber Bosse). In spring, at L2, *Rivularia* sp. and *Dichothrix* sp. were present with high coverage (40% and 20%, respectively). Additionally, some sites showed notable coverage of *C. glomerata* (L3–5%, L4–1%, L5–80%, L6–65%) and *Microcoleus autumnalis* (Gomont) Strunecky, Komárek & Johansen (L1–1%, L3–5%, L4–5%, L5–10%, L6–2%). Other macroalgae appeared in low coverages (1–5%), including *Oedogonium* sp., *P. lividum*, *Tetraspora gelatinosa* (Vaucher) Desvaux, *Haematococcus pluvialis* Flotow, *Phormidium uncinatum* Gomont, *Riverina rivularis* (Liebmann) Vieira & Saunders and *Spirogyra* sp.

The Relationship Between Benthic Algae and Physical and Chemical Parameters

The results of the CCA analysis between physical and chemical parameters and 31 algal taxa are shown in Figure 2. CCA model explained 37.61% of the data variability and showed significance (F= 1.378; P=0.012). The greatest contribution to explaining the variation comes from CCA1 (12.44%) and CCA2 (7.99%), while the remaining components contribute less. Electrical conductivity (EC, P=0.002) and temperature (Temp, P=0.030) were identified as statistically significant factors in explaining the distribution of the algal taxa. Temp correlated positively with the taxa in the lower right part of the diagram (SPBO, ZYG, UBIC), indicating their preference for higher temperatures. EC was associated with the taxa in the lower left part of the diagram (UCON, CGLO, CPLA), reflecting their tolerance to elevated concentrations of dissolved substances. pH, NO_3, PO_4 and hydromorphological characteristics (Depth and Width) correlated with the taxa in the upper left part of the diagram (CPED, PHLI, MVAR), suggesting an affinity for higher nutrient concentrations, pH, greater water depth and a wider riverbed. Negatively correlated with these factors are the species located in the lower right corner (RISP, DISP, CYSU) (Figure 2).

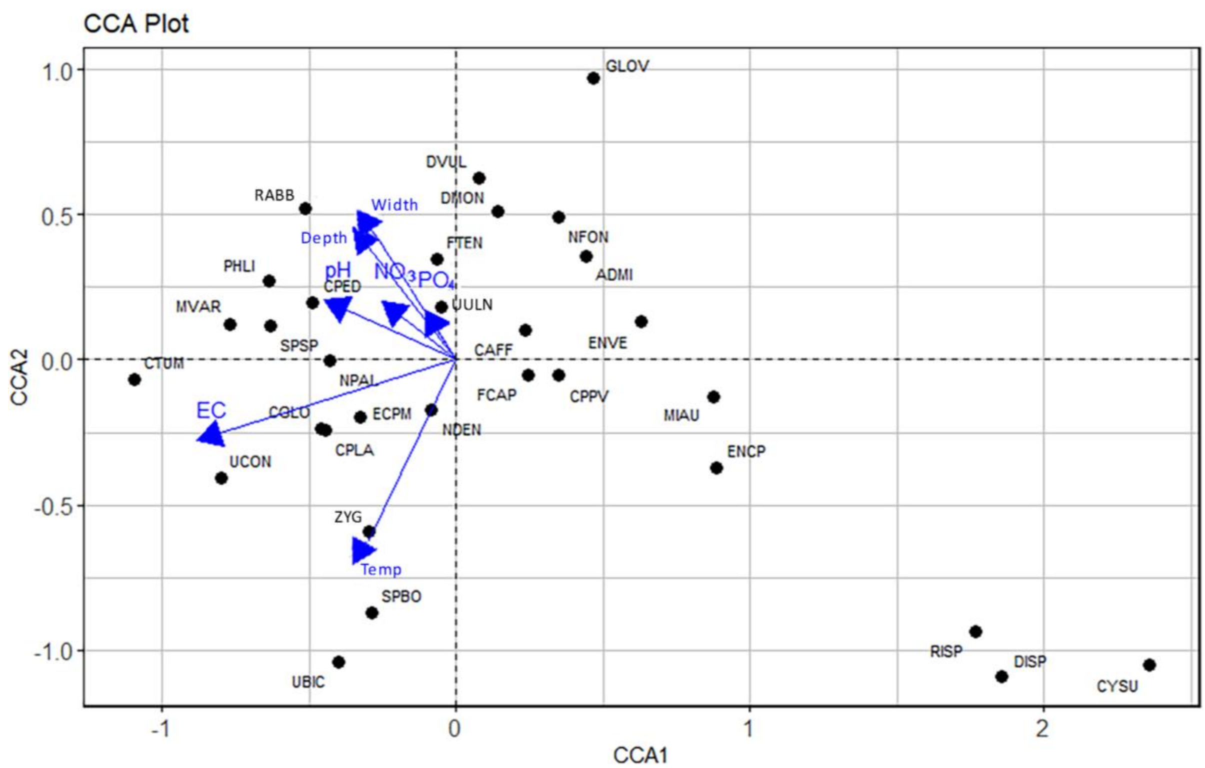

Figure 2. CCA analysis of the relationship between the algae found at six researched sites and the measured physical and chemical parameters (ADMI–*Achnanthidium minutissimum*; CGLO–*Cladophora glomerata*; CPED–*Cocconeis pediculus*; CAFF–*Cymbella affinis*; CPPV–*C. perparva*; CTUM–*C. tumida*; CYSU–*C. subhelvetica*; DISP–*Dichothrix* sp.; DMON–*Diatoma moniliformis*; DVUL–*D. vulgaris*; ECPM–*Encyonopsis minuta*; ENCP–*Encyonopsis* sp.; ENCM–*Encyonema minutum*; ENVE–*E. ventricosum*; FCAP–*Fragilaria capucina*; FTEN–*F. tenera*; GLOV–*Gomphonella olivacea*; MIAU–*Microcoleus autumnalis*; MVAR–*Melosira varians*; NDEN–*Nitzschia denticula*; NFON–*N. fonticola*; NPAL–*N. palea*; PHLI–*Phormidium lividum*; RABB–*Rhoicosphenia abbreviata*; RISP–*Rivularia* sp.; SPBO–*Spirogyra borgeana*; SPSP–*Spirogyra* sp.; UBIC–*Ulnaria biceps*; UCON–*U. contracta*; UULN– *U. ulna*; ZYG–*Zygnema* sp.).

Ecological Status Assessment Based on Physical and Chemical Parameters

Table 3 shows the assessment of the ecological status based on physical and chemical parameters at six investigated sites in four seasons. At most sites, nitrate concentrations (NO_3) were the primary indicator of the worst ecological status, reflecting classes II–V. Phosphate concentrations (PO_4) also contributed to determining the ecological status at some sites. On the other hand, ammonium ions (NH_4) consistently indicated a high ecological status (class I) at all sites (Table 3).

Ecological Status Assessment Based on Biotic Indices

The obtained values of biotic indices and the corresponding assessment of the ecological status are listed in Table 2. Among the diatom indices, the highest values and ecological status assessments were obtained with the IBD, EPID and IPS, while the lowest values and the worst ecological status were assessed by TDI (Table 2). Among non-diatom indices, the NeD indicated mostly bad ecological status (V class) except on two sites where it was poor (IV class). The assessment based on the TI and BI index ranged from good to bad (II–V class), while based on the PIT index it was high to poor (I–IV class) (Table 2). The TI index generally had lower values than the TID index and indicated a higher ecological status class (Table 2). In autumn and summer, the RAPPER method assessed high/good ecological status at L2, while other sites had moderate ecological status (L1, L3, L4, L5, L6). In winter, high/good ecological status was determined at all sites examined. At L1, L3, and L4 a moderate ecological status was determined by this method in spring, while at L2 it was high/good, and at L5 and L6 it was poor/bad.

The Correlation Between the Biotic Indices

The results of the Pearson and Spearman correlation tests between the calculated biotic indices are shown in Table 4. All indices, except for IDG and NeD, exhibited significant correlations with at least one other index. Among the non-diatom indices, BI was positively correlated with SLAD. For the PIT index, high values indicate a poorer ecological status, resulting in a strong negative correlation with TID. Additionally, TI exhibited significant positive correlations with IBD, IPS, SLAD, EPID, TID and TDI (Table 4).

Discussion

The use of diatom indices for assessing the ecological status of inland waters is widespread in EU countries, with various studies comparing their

Table 3. Ecological status assessment based on physical and chemical parameters at investigated sites over four seasons

	Autumn 2022						Winter 2023					
Parameter / Sites	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
NO_3												
PO_4												
TP												
NH_4												
Final assessment												
	Summer 2023						**Spring 2024**					
Parameter / Sites	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
NO_3												
PO_4												
TP												
NH_4												
Final assessment												

Table 4. Pearson's[a]/Spearman's[b] correlation coefficients between the calculated biotic indices

Indices	IPS	IDG	SLAD	EPID	TID	TDI	TI	PIT	NeD	BI
IBD	$.66^{a,*}$	$-.04^{a}$	$.70^{a,*}$	$.79^{b,*}$	$.83^{a,*}$	$.44^{b}$	$.59^{b,*}$	$-.40^{a}$	$.24^{b}$	$.72^{a}$
IPS		$.56^{a,*}$	$.71^{a,*}$	$.48^{b}$	$.45^{a}$	$.52^{b,*}$	$.57^{b,*}$	$-.03^{a}$	$.23^{b}$	$.66^{a}$
IDG			$.38^{a}$	$.04^{b}$	$-.10^{a}$	$.43^{b}$	$.37^{b}$	$.30^{a}$	$.21^{b}$	$.54^{a}$
SLAD				$.66^{*}$	$.60^{a,*}$	$.54^{b,*}$	$.71^{b,*}$	$-.18^{a}$	$.34^{b}$	$.85^{a,*}$
EPID					$.80^{b,*}$	$.38^{b}$	$.43^{b}$	$-.49^{a}$	$.15^{b}$	$.53^{a}$
TID						$.65^{b,*}$	$.54^{b,*}$	$-.75^{a,*}$	$.15^{b}$	$.40^{a}$
TDI							$.57^{b,*}$	$-.39^{b}$	$.18^{b}$	$.18^{b}$
TI								$-.27^{b}$	$.35^{b}$	$.14^{b}$
PIT									$.26^{b}$	$.13^{a}$
NeD										$.08^{b}$

*correlation is significant at the 0.05 level (two-tailed performance and correlations with physical and chemical parameters (e.g. Bere et al., 2014; Martin et al., 2020; Tokatlı et al., 2020; Çetin et al., 2021; Ongun Sevindik et al., 2023). In our study, the TDI index was the most aligned with ecological status assessment based on physical and chemical parameters, showing concordance in 83.3% of cases. Following TDI index, the TID index showed the closest agreement, corresponding in 58.3% of cases. Previous studies have shown that TDI (Rimet et al., 2005; Atazadeh et al., 2007; Çelekli et al., 2023; Ongun Sevindik et al., 2023) and TID (Rimet et al., 2005; Bere et al., 2014) are sensitive to changes in environmental conditions, especially to fluctuations in phosphorus and nitrogen concentrations (Milićević et al., 2024). Moreover, these indices exhibited strong correlations and indicated similar conditions (Besse-Lototskaya et al., 2011), which was also confirmed in our research (Table 4). TDI has previously demonstrated good performance in the assessment of hilly-mountin streams in Serbia (Vasiljević et al., 2014). In contrast, in a study conducted in Turkey, Çetin et al. (2021) noted that TDI exhibited the weakest correlation with general habitat degradation. Such differences in index performance across studies suggest that regional or habitat-specific factors influence their effectiveness, underscoring the need for local validation prior to application.

In Serbia, the IPS and CEE indices are used according to national regulation (Official Gazette of the Republic of Serbia, 2011), while the EPID index is applied by the Environmental Protection Agency in official reports (Čađo et al., 2021). Although some previous studies have shown that the IPS, CEE and EPID indices were strongly correlated and aligned with ecological status assessments based on physico-chemical parameters (Jakovljević et al., 2016, 2021), our results did not confirm such consistency. In our study, both the IPS and EPID indices showed notable discrepancies compared to the assessment based on physico-chemical parameters, and they were not mutually correlated. Moreover, the IPS index, when applied according to the national regulation, showed greater disagreement than when using the classification based on the Prygiel & Coste (2000) scale (Table 2). Similar inconsistencies between physico-chemical and biological (phytobenthos-based) assessments, as observed in our study, have been reported in previous studies conducted in Serbia (Vidaković et al., 2018; Ćirić et al., 2018), suggesting insufficient sensitivity of the IPS index. One of the underlying reasons may be that diatom species can exhibit varying ecological preferences across different regions (Tapolczai et al., 2016), while a national list of bioindicator species for Serbia has not yet been developed. These findings further emphasize the necessity of index intercalibration and regional adaptation, which has already been implemented in most EU member states (Masouras et al., 2021). The CEE index, although prescribed by national regulation, did not account for more than 50% of the identified diatom species in our study—a limitation also highlighted in earlier studies (e.g. Jakovljević et al., 2016; Simić et al., 2021; Đukić et al., 2020). This further questions its reliability and suitability for comprehensive ecological status assessment in Serbian rivers.

As climate change affects temperature, precipitation and other factors, adapting ecological status assessments is crucial, as these changes affect diatom communities and their indicative values (Anderson, 2000). Our research has provided new data on the ecology of certain bioindicator species that differ from those previously described in the literature. *Cymbella perparva* occurred in an atypical habitat in terms of nutrient concentrations. In our study, it was found in water with 4–26 mg L^{-1} NO_3 and 0.06–0.38 mg L^{-1} PO_4, while previously described as species that prefers oligotrophic waters (Krammer, 2002; Le Cohu & Azamar, 2011). In the Kamenica River, *C. perparva* also formed dense mucilage mats, which had not been observed before. Although this species is widespread in Europe (Krammer, 2002), including Serbia, it has so far only been recorded in small quantities (e.g. Andrejić et al., 2012; Krizmanić et al., 2015; Đukić et al., 2020). The mats resembled those of the invasive *Didymosphenia germinata* (Lyngbye) Schmidt, known for its harmful impacts on ecosystems (Ejaz et al., 2021). It is therefore necessary to conduct further in-depth research into this occurrence. Also, dominant species *Cymbella affinis* was found in water with 4–26 mg L^{-1} NO_3 and 0.06–0.8 mg L^{-1} PO_4. In contrast to the results obtained, this species is known in most literature sources as an indicator of oligo- to β-mesosaprobic water that is sensitive to inorganic pollution (Krammer, 2002; Pourheydar Khoshkrudi et al., 2014; Çelekli & Bilgi, 2019). The other dominant species were found in an environment typical for them. The CCA analysis revealed a negative correlation between *Gomphonella olivacea*, which formed mats in winter, and temperature. This observation is consistent with the findings of Aykut et al. (2021) and Vidaković et al. (2020). In contrast, *Ulnaria biceps* showed a positive correlation with higher water temperatures, aligning with the results of Seu-Anoï et al. (2017). *Cocconeis pediculus* and *Melosira varians* were positioned along environmental gradients associated with higher nutrient availability (nitrate and phosphate), elevated pH, greater water depth and riverbed width, as suggested by the ordination pattern in the CCA analysis. This ecological preference is consistent with previous findings, as the abundance of *C. pediculus* was positively correlated with nitrate and phosphate concentrations (Riouchi et al., 2022), and both *C. pediculus* and *M. varians* have been identified as indicators of elevated phosphorus levels (Rott et al., 1999). Conversely, *Cymbella subhelvetica* appeared to prefer nutrient-poor environments, occurring under conditions characterized by low nitrate and phosphate concentrations. The same was found by Krammer (2002), who described this species as typically inhabiting oligotrophic mountain water ecosystems.

Non-diatom algae respond to changes in aquatic ecosystems, especially in water chemistry, which is why they are considered good bioindicators (Stancheva & Sheath, 2016). The Kamenica River is rich in non-diatom species, including those forming macroscopic aggregations. *Cladophora glomerata*, a species often found in nutrient-rich waters (Michalak & Messyasz, 2021) and used as an indicator of inorganic pollution (Cheshmedjiev et al., 2010), was dominant at most sites. The CCA analysis showed its correlation with electroconductivity. High coverages of *Spirogyra* sp. and *S. borgeana* were also observed. In Californian streams, *S. borgeana* thrived in waters with low TP (< 0.01 mg L^{-1}) (Stancheva & Sheath, 2016), while in our study it was found in a wider range of TP concentrations (< 0.02–0.13 mg L^{-1}). The coverage of *S. borgeana* and *Zygnema* sp. increased with river temperature. Studies have shown that species of the genus *Zygnema* can tolerate a wide range of temperatures, but optimal growth and photosynthetic activity are generally achieved at higher temperatures (20–30°C) (Singh & Singh, 2015). Positive correlations with pH, NO_3, PO_4, river width and depth were determined for *Phormidium lividum* through CCA analysis. In contrast, *Rivularia* sp. and *Dichothrix* sp. exhibited negative associations with these parameters, which is consistent with literature data, as species of these genera are usually found in clean rivers (Livingstone & Whitton, 1984; Casamatta & Hashler, 2016).

The usage and testing of indices that consider non-diatoms and macroalgae or all algal groups is largely overlooked (Fetscher et al., 2014). Due to the limited development of non-diatoms, especially macroalgae, during winter, this season offers unfavorable conditions for the evaluation of these indices. Only the PIT and NeD indices could be calculated at certain sites during this season, as they do not take macroalgal coverage into account. Excluding winter data, the PIT index provided an ecological status assessment similar or identical to that based on physico-chemical parameters in 70.59% of cases. Furthermore, the PIT index correlated with the diatom-based TID index, which performed well in our study. As the PIT index was originally developed for Norwegian rivers, its current class boundaries are tailored to the environmental conditions of these ecosystems. Adapting the index to Serbian rivers — by recalibrating the class boundaries and including region-specific indicator species — would likely improve its accuracy and ecological relevance. The RAPPER method yielded slightly better results, with 78.77% of cases showing complete agreement with the physico-chemical parameters assessment. In the remaining cases, discrepancies were minimal, with only one instance showing a notable deviation, while the others varied by just one class. As a result, the RAPPER method proved effective for rapid assessment of ecological status and can complement diatom-based indices. The BI index, calculated in eight cases, showed no notable differences from the assessment based on physico-chemical parameters. A limitation of this index is its relatively narrow list of indicator species, and expanding this list could enhance its applicability and allow for broader use in ecological assessments. The NeD index showed no correlation with other indices and indicated the worst ecological status at most sites, suggesting that it may be unsuitable for accurate assessment, as noted by Mihaljević et al. (2020).

The TI index, based on all algal groups, has shown a significant positive correlation with the TDI and TID diatom indices. Additionally, in the same number of cases as the TDI (83.3%), its assessment was close to or aligned with physico-chemical parameters. When comparing the results of the TID index and the TI index, the TI index indicated an ecological status that was at least one class worse than the TID index in half of the cases. Additionally, the assessment provided by the TI index was closer to the values determined from physico-chemical parameters. This observation underlines the importance of including non-diatom benthic algae as bioindicators for a more accurate assessment of ecological status.

Conclusion

The results of this study showed that the diatom indices TDI and TID provide the most accurate assessment of ecological status in the studied region, as their evaluations were most consistent with those based on physico-chemical parameters. In contrast, the IPS index, currently applied under Serbian national regulations, proved inadequate. We therefore recommend adjusting its class boundaries to better align with the specific environmental conditions of the rivers in this area. Non-diatom-based indices such as RAPPER, PIT and BI also showed potential for ecological status assessment, either independently or in combination with diatom-based methods. Furthermore, the TI index, which incorporates both diatom and non-diatom algae, offers a more reliable assessment than its diatom-only counterpart (TID), highlighting the role of non-diatom taxa in evaluating ecological status.

Ethical Statement

There is no ethical statement.

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Author Contribution

Conceptualization: KM, SBS. Formal Analysis: KM. Investigation: KM, EB, JK, SBS. Project Administration:

SBS, KM. Supervision: SBS. Validation: SBS, JK, AR, NĐ. Visualization: KM. Writing -original draft: KM. Writing -review and editing: KM, JK, SBS, AR, NĐ.

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 personal conflicts that could have appeared to influence the work reported in this paper.

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