DOI: 10.46793/SBT30.32KM

ALGAL-BASED ECOLOGICAL STATUS ASSESSMENT: A CASE STUDY OF THE DESPOTOVICA RIVER

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Abstract: Diatoms, unlike other algal groups (non-diatoms), are routinely used for ecological status assessment under the Water Framework Directive. The ecological status of the Despotovica River was assessed using epilithic diatoms (IPS, CEE index), non-diatoms (BI index) and physico-chemical parameters. At four sites in autumn 2023 and spring 2024, 99 algal taxa (76 diatoms, 23 non-diatoms) were identified. The ecological status class ranged from I to V based on the physico-chemical parameters, while it ranged from I to IV based on the diatom and BI indices. The BI index results aligned more closely with the physico-chemical data than the diatom indices, suggesting potential for further application.

Keywords: water quality, macroalgae, bioindicators

Introduction

Algae are widely recognized as bioindicators for assessing the ecological status of aquatic ecosystems, as they respond effectively to changes in water quality (Stevenson and Smol, 2003). Under the Water Framework Directive (WFD, 2000) and national regulations, most European Union countries rely primarily on diatoms as the key algal group for river ecological status assessment. Other algal groups (non-diatoms) remain relatively underutilized as bioindicators, despite their potential to provide valuable insights into the long-term health of aquatic ecosystems. Their limited application is largely due to the lack of standardized monitoring protocols and the need for greater taxonomic expertise (Poikane et al., 2016; Schaumburg et al., 2004).

The focus of this study was to assess the ecological status of the Despotovica River by analyzing epilithic diatoms and physico-chemical water quality parameters in accordance with the WFD (2000) guidelines and Serbian national regulations (Official Gazette of the Republic of Serbia 2011, 2023). In

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addition, the indicative properties of the detected non-diatom macroalgae and non-diatom-based Biological Index (BI) (Gutowski et al., 2004; Schaumburg et al., 2004) were analyzed to assess their potential application as water quality indicators for Serbian rivers in the future.

Materials and methods

The Despotovica River, located in central Serbia, is 24 km long and originates from the southern slopes of Rudnik Mountain. Flowing between the Vujan and Ilijak mountains, it forms a gorge stretching between Gornji Milanovac and Brđani. Upon exiting the gorge, the Despotovica River merges with the Di ina River, which eventually connects to the Zapadna Morava River through the emernica River (Drinjakovi et al., 2015). Due to the inflow of wastewater from lead, copper, and zinc mining in the Rudnik mountain, as well as the technological and municipal wastewater from Gornji Milanovac, the Despotovica River is classified as highly polluted (Drinjakovi et al., 2015; Simić et al., 2018).

The study was conducted in autumn 2023 and spring 2024 at four sites along the Despotovica River: S1 – Majdan (44.094500, 20.491556, alt. 426.7 m), S2 – Gornji Milanovac (44.044722, 20.489944, alt. 335.8 m), S3 – Mlakovac (44.000250, 20.434639, alt. 289.1 m) and S4 – Brđani (43.969778, 20.418444, alt. 259.9 m).

Benthic algal samples were collected, followed by qualitative and quantitative analysis of epilithic diatoms, measurement of physico-chemical parameters and ecological status assessment based on diatoms and physico-chemical data. These procedures were conducted using the equipment, standard protocols, and guidelines described in Simić et al. (2018). The qualitative analysis and ecological status assessment using non-diatom benthic algae (BI index) were conducted according to Gutowski et al. (2004). The assessed ecological status can be I class (high), II class (good), III class (moderate), IV class (poor) and V class (bad) (WFD, 2000).

Results and discussion

The analysis of benthic algae in the Despotovica River at four sites in two seasons revealed 99 taxa, with most species belonging to the phyla Bacillariophyta (76), followed by Chlorophyta (11), Cyanophyta (6), Charophyta (Zygnematophyceae) (3), Rhodophyta (2) and Heterocontophyta (1).

The dominant diatom species (abundance ≥10% at least at one site) are presented in Table 1. Teratological forms of *Nitzschia* sp. were found at S1 in both investigated seasons. Compared to the previous algological study of this river by Simić et al. (2018), a higher number of diatom species was observed. Of the 17 previously detected diatoms, 15 were found again in this study, together with 61 species that had not been previously recorded. Also, in contrast with our finding, teratological forms were found at all sites except S1.

Table 1. Dominant diatom species (abundance ≥10% at least at one site) at the studied sites of the Despotovica River

Season	Autumn 2023				Spring 2024			
Taxa	S1	S2	S3	S4	S1	S2	S3	S4
Achnanthidium microcephalum Kütz.	**46**							
Achnanthidium minutissimum (Kütz.) Czarnecki	**15**	5.5	3.6	1.8	1.3	2	**12.8**	
Cocconeis pediculus Ehrenb.		0.5	**12.5**	**43.3**			6	**20**
Cocconeis placentula Ehrenb.		3.6	**21**	**46**		0.8	3	0.3
Encyonema ventricosum (C. Agardh) Grunow					**16.8**	**33.3**	1.3	**16.8**
Eolimna minima (Grunow) Lange-Bertalot		1.6	**11.8**		**11**	3.8	5.8	
Gomphonema elegantissimum Reichardt & Lange-Bertalot					**10**	0.5		
Gomphonema parvulum (Kütz.) Kütz.	4.5		**13**	0.5	**15.5**	2	0.8	2.3
Melosira varians C. Agardh		**62.5**	4			0.5	0.8	5.3
Navicula lanceolata Ehrenb.							2	**17.5**
Nitzschia linearis Smith	**10**	7.5	1.5		2	**24.8**	3.3	4.3
Nitzschia palea (Kütz.) Smith			12	3			2.8	1.8
Surirella brebissonii Krammer & Lange-Bertalot			0.5		1		**11.5**	4.3
Surirella minuta Brébisson ex Kütz.	3.3				**12.5**	**9.3**		
Ulnaria ulna (Nitzsch) Compère	**8.6**	1.5	0.5	1	**30.8**	**14.8**		**27.8**

Of all the detected non-diatom species, 6 formed macroscopic aggregations with varying coverages of the riverbed in the autumn of 2023: *Audouinella pygmaea* (Kütz.) Weber Bosse (S1-30%), *Cladophora glomerata* (L.) Kütz. (S2-10%;

S3-35%), *Cladophora rivularis* (L.) Kuntze (S3-55%; S4-95%), *Hildenbrandia rivularis* (Liebmann) J.Agardh (S1-1%), *Oedogonium* sp. (S2-1%), *Spirogyra* sp. (S4-1%) and *Tetraspora* sp. (S4-1%), while 8 formed macroscopic aggregations in the spring of 2024: *A. pygmaea* (S1-10%), *C. glomerata* (S2-60%), *C. rivularis* (S3-70%; S4-90%), *Homoeothrix janthina* (Bornet & Flahault) Starmach (S1-10%), *Microcoleus autumnalis* (Gomont) Strunecky, Komárek & J.R.Johansen (S4-1%), *Stigeoclonium tenue* (C.Agardh) Kütz. (S1-25%; S2-5%) and *Vaucheria* sp. (S2-5%; S3-25%; S4-10%). This study also recorded a higher number of non-diatom algae, including macroalgae, compared to the study by Simi et al. (2018). Red algae, *H. rivularis* and *A. pygmaea*, are typically found in rivers with low to moderate nutrient concentrations (Sheath and Vis, 2015), which is consistent with our findings. However, recent research suggests that *H.rivularis* can be found in a broader range of environmental conditions and is a poor indicator of good ecological status (Jakubas-Krzak et al., 2023). The species *S. tenue* is known as tolerant to heavy metal pollution (Pradhan et al., 2024). Its presence at S1 in both seasons, along with the teratological form of *Nitzschia*, may suggest their presence. Other detected macroalgae—*M. autumnalis*, *Oedogonium* sp., *Spirogyra* sp., and *Cladophora* species (*C. glomerata* and *C. rivularis*)—have been previously reported in eutrophic conditions (Carmona et al., 2022; Schneider and Lindstrøm, 2011; Pikosz and Messyasz, 2016), which aligns with our findings.

The obtained values of the physico-chemical parameters, the diatom indices (IPS, CEE) and the non-diatom index (BI) were used to assess the ecological status, which is shown in Table 2.

Table 2. The values of the obtained physico-chemical parameters, diatom indices (IPS, CEE) and non-diatom index (BI) with the ecological status assessment of the investigated sites of the Despotovica River

Season	Autumn 2023				Spring 2024			
Parameter Site	S1	S2	S3	S4	S1	S2	S3	S4
Physico-chemical parameters								
Oxygen conc. (mg L^{-1})	9.5	9.36	8.44	8.3	9.77	10.78	8.12	7.91
PO_4-P (mg L^{-1})	<0.06	0.06	0.06	0.31	0.07	<0.06	0.51	1.37
TP (mg L^{-1})	<0.02	0.02	0.02	0.11	0.02	<0.02	0.17	0.45
NO_3-N (mg L^{-1})	<4	7	5	5	<4	5	6	7
NH_4-N (mg L^{-1})	<0.03	<0.03	1.35	0.76	<0.03	<0.03	0.05	0.06
Ecological status class	**I**	**IV**	**V**	**IV**	**II**	**III**	**V**	**V**
Biological parameters – epilithic diatoms								
IPS index	17.2	14.8	10	14.4	13.3	13.4	14	12.7
CEE index	15.3	11.9	8.9	11.4	10.6	12.4	13.1	11.1

Ecological status class	**I**	**II**	**IV**	**II**	**III**	**III**	**III**	**III**
Biological parameters – non-diatom algae								
BI index	50	-40.9	-50	-	16.7	-36.1	-50	-7.1
Ecological status class	**I**	**IV**	**V**	**-**	**III**	**IV**	**V**	**IV**

Based on physico-chemical parameters, the ecological status at S1 was classified as I-II class (high-good), with no significant increase in nutrient concentrations. In contrast, other sites (S2-S4) exhibited elevated concentrations of at least one nutrient in both seasons, with ecological status class ranging from III to V (moderate-bad) (Table 2). On the other hand, according to the diatom indices and the BI index, the ecological status class at all sites varied from I to IV (high-poor) (Table 2). The ecological status obtained using non-diatom benthic algae and physico-chemical parameters differed by no more than one class, whereas comparisons with diatom-based assessments showed deviations of up to two classes (Table 2). Compared to the Despotovica River study by Simić et al. (2018), the ecological status based on diatom indices has improved by one to two classes at most sites, except at S3 in autumn 2023 and S1 in spring 2024.

The use of the non-diatom BI index in our study proved to be relevant, although further research on a larger number of river sites is needed to confirm that this method accurately reflects the ecological status. One of the main challenges of its application is that it was originally designed for German rivers, so some of the abundant non-diatom species found in our study, for example *Cladophora rivularis*, are not included on the indicator list. To improve its applicability, the method should be adapted to our region and a specific indicator list for Serbian rivers should be developed.

Conclusion

The ecological status of the Despotovica River, based on physico-chemical parameters, ranged from I to V (high-bad), while diatom indices and the non-diatom BI index indicated a status ranging from I to IV (high-poor). Some identified non-diatom macroalgae species have been recognized as reliable indicators of inorganic pollution. Additionally, the non-diatom BI index proved useful for ecological assessment; however, further research is needed to validate its applicability to Serbian rivers. Adjustments and the development of a region-specific indicator list are recommended for more accurate assessments.

Acknowledgement

Work on this paper was supported by The Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-136/2025-03/200088, 451-03-137/2025-03/200088, 451-03-136/2025-03/ 200122, 451-03-66/2024-03/200216).

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