

**BENTHIC DIATOMS AS BIOINDICATORS:  
AN ECOLOGICAL STATUS EVALUATION OF THE  
GRAČANKA RIVER (KOSOVO AND METOHIJA)**

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**Abstract**

This research employs benthic diatom communities as bioindicators of the water quality of aquatic ecosystems. The study was conducted on a watercourse of the Gračanka River in Kosovo and Metohija that is exposed to different levels of anthropogenic pressure, including organic pollution, nutrient loading, and industrial mining impacts. Benthic diatom samples were collected from 10 locations. We assessed water quality using combined chemical and biological monitoring, including physicochemical parameters and diatom indices such as the IPS, CEE, and TDI. The diatom assemblage was composed of 93 species, dominated by the genera *Navicula* Bory, 1822 (14 spp.), *Nitzschia* Hassall, 1845 (13 spp.), and *Gomphonema* Ehrenberg, 1832 (9 spp.). Our study revealed that the combined effect of multiple pollutants significantly impacted diatom assemblages, as shown by spatial shifts in community structure that corresponded to anthropogenic water quality pressures at most investigated sites. Although diatom indices are useful for assessing ecological status, their reliability can be compromised in multi-stress environments. Consequently, integrating physicochemical data is essential for accurately interpreting the results of diatom-based bioassessments.

**Key words:** benthic diatoms, biomonitoring, freshwater assessment, anthropogenic pressure, river ecosystem health

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**Introduction.** Accelerated industrialization and urbanization contribute significantly to the degradation of freshwater ecosystems worldwide through multiple forms of pollution. Increased nutrient inflow resulting in the eutrophication of water, as well as the intake of various pollutants (metals, pesticides) – separately or simultaneously, leads to changes in aquatic ecosystems. Initially, this has a strong negative effect on the biotic component of these systems in terms of changes in species composition, then it leads to an increase in the number and abundance of species tolerant to pollution [1].

Physicochemical methods quantify only pollution concentration, whereas biological assessment and monitoring reveal its ecological effects on biota and ecosystems [1]. Aquatic organisms such as diatoms reflect anthropogenic environmental changes, thereby offering a reliable representation of the overall condition of aquatic ecosystems [2].

Consequently, diatoms represent effective bioindicators for assessing water quality in aquatic ecosystems [3]. The role of diatoms as an environmental indicator is aligned with the EU Water Framework Directive (WFD) 2000/60/EC, the foundational legislation for protecting all water bodies in the European Union [2]. It recommends diatom-based bioassessment for evaluating the biological integrity of aquatic ecosystems under various types of stressors [4]. Under the WFD, a clear conceptual distinction is made between ecological status and chemical status. Ecological status is assessed based on biological quality elements (e.g., diatom assemblages) supported by physicochemical parameters (e.g., Zn, Fe, Cr), which are classified into five classes (high, good, moderate, poor, bad). In contrast, chemical status is evaluated separately based on compliance with Environmental Quality Standards (EQS) for priority substances (e.g., Pb, Ni), assessed against Maximum Allowable Concentrations (MACs) [4,5]. In Serbia, the ecological status classification of aquatic ecosystems is assessed using benthic diatoms as a mandatory biological quality element, with evaluation based on two diatom indices, Specific Pollution Sensitivity Index (IPS) and Index of European Economic Community (CEE) [5]. This study aimed to combine chemical monitoring with diatom-based bioassessment using IPS, CEE, and Trophic Diatom Index (TDI) indices to assess the ecological status of the Gračanka River.

**Materials and methods. Study area.** The Kišnica district (Kosovo and Metohija, Serbia) hosts intensive mining of Pb-Zn ores, resulting in severe environmental impact from metal contamination and substantial flotation tailings [6]. Mining activities in the Kišnica district have seriously impacted the Gračanka River, an aquatic ecosystem in its immediate vicinity. Several large villages, including Badovac, Kišnica, Gračanica, Ajvalija, Laplje Selo, Preoci, Lepina, Gornje and Donje Dobrevo, are located within the densely populated composite valley of the river. The Gračanka River receives discharges of both industrial flotation effluent and untreated municipal wastewater [6]. Flotation tailings (total area from 40 ha) near the village of Kišnica, which contain toxic heavy metals (Pb, Zn, Cr,

Ni, Fe), are located in close proximity to the Gračanka River [7].

**Field sampling, physico-chemical analysis and diatom based assessment.** An algological investigation of the Gračanka River was carried out concurrently at 10 sampling sites in September 2024 (Fig. 1). Water temperature ( $t/^\circ\text{C}$ ),

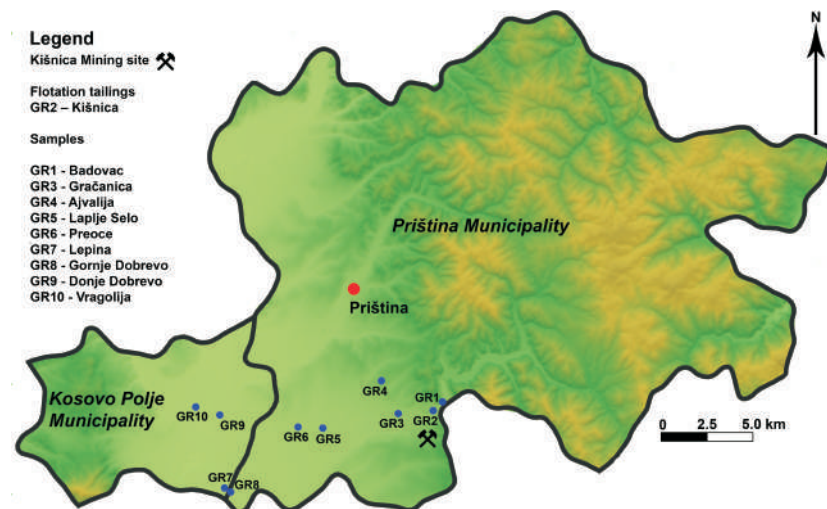


Fig. 1. Location of the phytobenthos sampling sites on the Gračanka River

pH value and electrical conductivity (EC;  $\mu\text{S}/\text{cm}$ ) were analysed and measured with a multiparameter device (PCSTester 35K). The concentrations of nitrates ( $\text{NO}_3\text{-N}$ ), dissolved oxygen (DO) and orthophosphates ( $\text{PO}_4\text{-P}$ ) were measured using a Lovibond photometer PC Multidirect. Concentrations of Fe, Pb, Zn, Cr and Ni (reported in  $\text{mg l}^{-1}$ ) were analyzed with an Agilent 7700x ICP-MS. Ecological status was assessed using type-specific thresholds for the IPS and CEE indices, together with all measured physico-chemical parameters except water temperature and electrical conductivity, according to the national regulation (Official Gazette of the Republic of Serbia, 74/2011) [5]. The same regulation was applied for heavy metals, defining two distinct approaches: (i) for zinc (Zn), chromium (Cr), and iron (Fe), assessment was based on prescribed water quality classes (I–V); (ii) for nickel (Ni) and lead (Pb), which lack I–V classification, assessment was based on compliance with Maximum Allowable Concentrations (MACs).

Sample collection and processing for diatom slide preparation followed the methodology outlined by TAYLOR et al. [8]. Diatoms were classified according to HOFMANN et al. [9].

After determining the composition and abundance of the diatom community, 17 diatom indices were calculated using the OMNIDIA 5.3 software package [10] from which three indices (IPS, CEE and TDI) were taken into consideration for the water quality assessment. IPS and CEE are the most sensitive to eutroph-

ication and organic pollution [11]. TDI index [12] indicates the ecosystem load on nutrients (nitrogen and phosphorus), independent of organic load. The class boundaries for IPS and CEE diatom indices range from 1 to 20, except for TDI whose values range from 1 to 100 [11], where 1 indicates a bad and 20 – a high water ecological status, and, in the case of TDI, 1 indicates a high and 100 – a bad water ecological status [12]. In addition, the percentage contribution of species characteristic of organic pollution (% PT) with a role in validating TDI, was calculated. Where the percentage of these species is less than 20%, the TDI values in a given sample are reliable and indicate good quality water, i.e. low nutrient uptake, while % PT > 20 values indicate that organic pollution influences the eutrophication of the aquatic ecosystem under study [12].

**Results and discussion.** A total of 93 diatom taxa were identified in the Gračanka River. The benthic diatom assemblage of the Gračanka River comprised 33 genera. The most species-rich were *Navicula* Bory (14 taxa), followed by *Nitzschia* Hassall (13 taxa), and *Gomphonema* Ehrenberg (9 taxa). Both the number of species and community structure varied among the sampling sites along the Gračanka River. The lowest number of taxa was recorded at site GR2 (12), followed by sites GR3 and GR5 (14 each). The highest number of taxa was identified at site GR1 (47). Differences in the dominant diatom taxa in the samples were also observed. *Achnantheidium minutissimum* (Kützing) Czarnecki was the most numerous taxon along the Gračanka River; it was joined by *Cymbella affinis* Kützing and *Denticula tenuis* Kützing in the upper reach (GR1), and by *Nitzschia palea* (Kützing) W. Smith and *Fragilaria capucina* Desmazières var. *capucina* in parts of the upper and middle stretches – specifically at sites near the Kišnica mine receiving industrial mining discharges and flotation tailings (GR2, GR3 and GR5). At the sampling locations in the lower stretch of the Gračanka River, taxa of the genus *Nitzschia* (*N. palea*, *N. capitellata* Hustedt, *N. fonticola* (Grunow) Grunow and *N. recta* Hantzsch ex Rabenhorst) and *F. capucina* were subdominant.

In addition to the marked increase in anthropogenic nutrient and organic loading (Table 1), the assessment of the ecological status of the Gračanka River – based on physico-chemical parameters and concentrations of Zn, Cr, Fe, Ni, and Pb – reveals substantial degradation caused by flotation effluents and flotation tailings from industrial mining (Table 1). Zinc (Zn) concentrations generally corresponded to class III (moderate status), while chromium (Cr) and iron (Fe) predominantly fell within class II (good) to class III (moderate) status. In contrast, the assessment of chemical status, based on priority substances such as nickel (Ni) and lead (Pb), revealed that concentrations of both metals surpassed the prescribed Maximum Allowable Concentrations (MACs) at several monitoring stations (GR2, GR3, GR5, GR8 for Ni; GR2, GR3, GR5, GR8, GR10 for Pb), as indicated by asterisks in Table 1. This suggests a potential failure to achieve good chemical status at these sites, as defined by the Official Gazette of the Republic of Serbia (74/2011) [4, 5].

T a b l e 1

Physicochemical parameters and biological indicators at sampling sites along the Gračanka River

Parameter	Unit	GR1	GR2	GR3	GR4	GR5	GR6	GR7	GR8	GR9	GR10
Water temperature (t)	°C	20.8	23.3	18.6	19.8	20.1	22.3	19.5	18	18.9	19.6
pH/Class	/	7.36/I	6.4/V	7.75/I	7.13/I	7.55/I	7.3/I	7.12/I	7.85/I	7.63/I	7.95/I
Electrical conductivity (EC)	µS/cm	515	3475	2205	984	1050	813	969	1035	1009	1050
Dissolved oxygen (DO)/Class	mg l <sup>-1</sup> /Class	8.2/II	6.7/III	3.85/IV	5.5/III	4.2/IV	5.9/III	6.17/III	5.19/III	6.14/III	6.9/III
Nitrate (NO <sub>3</sub> -N)/Class	mg l <sup>-1</sup> /Class	0.2/I	1.8/II	5.5/III	3.7/III	7/IV	4.4/III	5.3/III	6.4/IV	6.1/IV	7.6/IV
Orthophosphate (PO <sub>4</sub> -P)/Class	mg l <sup>-1</sup> /Class	0.029/I	0.087/II	2.376/V	0.731/V	2.487/V	1.253/V	0.979/V	1.369/V	1.547/V	1.642/V
Nickel (Ni)	mg l <sup>-1</sup>	0.015	1.832*	0.788*	0.221	1.656*	0.295	0.361	0.530*	0.407	0.382
Zinc (Zn)/Class	mg l <sup>-1</sup> /Class	0.445/I	1.862/III	1.715/III	0.662/II	1.593/III	1.796/III	1.615/III	1.442/III	1.225/III	0.804/III
Lead (Pb)	mg l <sup>-1</sup>	0.029	0.128*	0.095*	0.017	0.084*	0.027	0.033	0.063*	0.054	0.097*
Chromium (Cr)/Class	mg l <sup>-1</sup> /Class	0.025/II	0.255/V	0.076/III	0.028/II	0.084/III	0.052/III	0.061/III	0.077/III	0.059/III	0.063/III
Iron (Fe)/Class	mg l <sup>-1</sup> /Class	0.3/II	0.404/II	0.501/III	0.363/II	0.786/III	0.435/II	0.520/III	0.449/II	0.415/II	0.570/III
IPS index/Class	/	16/I	14.1/I	17.1/I	16.6/I	9.1/III	10.1/II	9.6/III	7.6/IV	6.5/IV	5.7/V
CEE index/Class	/	16.4/I	15.6/I	16/I	15.1/I	12.4/I	14/I	14.1/I	13/I	11.9/II	11.6/II
TDI index/Class	/	38.1/II	35.3/II	54.9/III	40.8/III	59.4/III	69.9/IV	54.2/III	65.9/IV	61.3/IV	73.1/IV
% PT	/	10.4	7.3	14.6	8.5	5	16.8	12.6	38.7	23.8	64.1
Number of taxa	/	47	12	14	27	14	25	26	30	34	31

Water quality classes (I-V) for pH, DO, NO<sub>3</sub>-N, PO<sub>4</sub>-P, Zn, Cr, Fe, IPS, and CEE are defined according to the Official Gazette of the Republic of Serbia (74/2011) [5], where: I – high, II – good, III – moderate, IV – poor, V – bad. For nickel (Ni) and lead (Pb), an asterisk (\*) indicates that the measured concentration exceeds the Maximum Allowable Concentration (MAC) prescribed by the same regulation [5]. TDI indicates nutrient enrichment; % PT – pollution-tolerant taxa; number of taxa – species richness.

Metal stress in periphyton communities significantly shifts taxonomic composition, depleting sensitive taxa while enriching tolerant ones [13]. In almost all locations under metals exposure, species *A. minutissimum*, *F. capucina* var. *capucina* and *N. palea* were central taxa of the diatom community. *A. minutissimum*, *F. capucina* var. *capucina* and *N. palea* are all described as metal tolerant in a number of studies [13, 14]. These results are in agreement with our data on metal-tolerant diatom communities at locations under the influence of the Kišnica mine and flotation tailings.

Most of the IPS values for the investigated locations (Table 1) indicate a first and second class of ecological status in the upper and middle reaches of the Gračanka River. Water quality deteriorated to classes III-V in the lower Gračanka River (sites GR7-GR10), downstream of Lepina, Gornje/D. Dobrevo, and Vragolija (Table 1). The CEE index shows high ecological status (Class I) at most sites (Table 1). However, downstream of the Kišnica mine (GR2, GR3, GR5), despite good IPS/CEE scores (IPS at GR5 indicated only Class III), a disturbed ecosystem is evidenced by altered diatom composition, low species richness, and high conductivity (Table 1). This discrepancy between the ecological status (which suggests good to high status based on diatom indices and supporting parameters) and the evident metal contamination (which indicates a potential failure of chemical status for Pb and Ni) highlights the limited sensitivity of IPS and CEE to metal stress. Consequently, it is crucial to acknowledge the limitations of diatom indices under multiple stressor conditions. IPS, CEE, and TDI are primarily sensitive to nutrient enrichment and organic pollution and are not specifically calibrated to assess metal contamination [11, 12]. Consequently, these indices may provide a distorted picture of ecological status in mining-affected waters, where metal stress coexists with organic pollution. As documented in the literature, diatom communities respond to multiple stressors in complex ways, and the interpretation of diatom indices should account for potential confounding effects of metal contamination [15, 16]. Interpretation of the IPS and CEE values in this context must consider the dominance of *A. minutissimum* [17], which accounted for over 70% of the assemblages at sites GR2, GR3, and GR5. At site GR5, located downstream of the Gračanica and Laplje Selo municipalities, the subdominant taxon *N. palea* tolerates elevated nutrient levels [18], explaining the lower IPS and CEE values recorded at this location (Table 1).

Most TDI values from the investigated locations (Table 1) indicate moderate to increased nutrient concentrations (Classes III and IV), except in the upper reach of the Gračanka River where nutrient levels are low (Class II). % PT values > 20% at lower stretch sites (GR8-GR10) indicate organic pollution contributes to eutrophication. Elevated nitrogen compounds suggest municipal or agricultural sources [19], with a peak of 64% at GR10.

Diatom indices alone would classify most sites on the Gračanka River as having high or good ecological status (Class I and II). The exceptions are sites

GR5 and GR7 (moderate/III), GR8 and GR9 (poor/IV), and GR10 (bad/V) (Table 1). Orthophosphate, as a physicochemical parameter, influenced the ecological status assessment at nearly all investigated sites on the Gračanka River and demonstrated greater sensitivity compared to the diatom indices (Table 1). Together with the observed exceedances of MACs for Ni and Pb, these results are consistent with the previously discussed limitations of diatom indices under metal stress conditions. Possible reasons for this unreliable assessment include: (i) diatom indices were developed and calibrated for the hydrological conditions of specific countries [20]; and (ii) the broad class boundaries of these indices may mask significant ecological degradation. The latter issue could be addressed by implementing stricter water quality class thresholds, while the former highlights the need for regionally adapted indices.

**Conclusions.** Our study demonstrated that the Gračanka River is subject to varying levels of organic pollution, nutrient loads from municipal wastewater and agriculture, and pressures from industrial mining activities. Pollution is especially pronounced downstream of the industrial area, with its middle and lower reaches being particularly affected by anthropogenic activities. As anticipated, benthic diatom communities near the Kišnica mine and flotation tailings (GR2, GR3, GR5) exhibited a reduction in species richness and a concurrent increase in metal-tolerant taxa, while nutrient-tolerant taxa dominated locations with elevated nutrient levels (GR8, GR9, GR10). A key finding is the striking discrepancy between diatom indices (Class I-II at GR1-GR4 and GR6, notably including mining-affected sites GR2 and GR3) versus orthophosphates (Class V at GR3-GR5, GR7-GR10) and heavy metals (MACs exceedances for Pb and Ni at GR2, GR3, GR5, GR8, GR10). The application of diatom indices for bioassessment is limited in mining-affected waters, where they fail to provide a reliable representation of the actual ecological status. As noted above, these indices were not calibrated for metal contamination, which explains why they fail to detect the ecological disturbance caused by mining effluents. This study underscores that interpreting diatom-based water quality assessments requires the integration of biological, physico-chemical data, and specific assemblage features, such as the abundance of tolerant taxa and community species richness.

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