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# AQUATIC MACROINVERTEBRATE COMMUNITIES AS INDICATORS OF WATER QUALITY IN THE RAŠKA RIVER

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**ABSTRACT.** This study was conducted to determine the water quality of the Raška River based on physical and chemical parameters and the community of aquatic macroinvertebrates as bioindicators. The research was conducted in August 2023 at six sites. A total of 61 taxa of aquatic macroinvertebrates were identified, with Diptera being the most diverse (20 taxa). The indicative ecological status of the source of the Raška River and the site above the trout farm was assessed as good (Class II), while the site below the farm corresponds to a moderate ecological status (Class III). In the three studied sites, located in populated areas with intensive industrialization, we detected high levels of nitrates, orthophosphates, and ammonia, along with organisms that can tolerate high organic pollution. Consequently, all sites downstream from the center of Novi Pazar exhibited poor water quality (Class IV). This underscores the critical necessity for action to improve and sustain water quality.

**Keywords:** ecological status assessment, ecological potential assessment, water physical and chemical parameters, pollution

## INTRODUCTION

Aquatic ecosystems face increasing threats from numerous factors that lead to water depletion, largely driven by anthropogenic activities such as industrialization, urbanization, intensive agriculture, and inadequate waste management. These activities substantially strain aquatic systems, resulting in significant pollution and water quality degradation (AKHTAR *et al*., 2021). Recognizing the scale of these challenges, the European Union implemented the

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Water Framework Directive (DIRECTIVE 2000/60/EC) alongside several related directives, creating a comprehensive legal framework for water management across Europe (DENIĆ *et al*., 2015). Three sets of quality elements have been proposed to assess the ecological status (or potential) of various surface water body categories: physical and chemical, hydro morphological, and biological (DIRECTIVE 2000/60/EC). Although physical and chemical parameters are valuable indicators, they cannot provide a comprehensive understanding of the impact of various human activities on the functioning of aquatic ecosystems over time (CAMARGO *et al*., 2011; SUMUDUMALI and JAYAWARDANA, 2021). Therefore, they should be complemented by biological monitoring. According to the Water Framework Directive (DIRECTIVE 2000/60/EC), aquatic macroinvertebrate fauna is considered the most relevant group for ecological assessments of aquatic ecosystems. Aquatic macroinvertebrates are essential for maintaining environmental balance, contributing to crucial processes such as organic matter decomposition, nutrient cycling, and energy transfer within freshwater ecosystems (SIMAIKA *et al*., 2024). Their sensitivity to environmental changes makes them effective bioindicators for monitoring water quality and ecosystem health (PARMAR *et al*., 2016). They are considered effective indicators due to their widespread presence in aquatic habitats, sedentary nature, and ease of sampling (ERASMUS *et al*., 2021).

The EU Water Framework Directive provides a robust foundation for managing and protecting aquatic ecosystems on both national and international levels, intending to achieve “good status” for all waters. Consequently, numerous studies have been conducted to assess water quality in rivers throughout Serbia (LEŠČEŠEN *et al*., 2018; OBRADOVIĆ, 2021; SIMIĆ *et al*., 2023). The Raška River is a left tributary of the Ibar River, located in the southwestern part of Serbia (VIDAKOVIĆ *et al*., 2018). Most of its flow passes through areas that are highly industrialized and urbanized, which raises concerns about its water quality. Accordingly, the aim of this study was to assess the ecological status and potential of the Raška River by analyzing physical, chemical, and biological parameters, with particular emphasis on macroinvertebrate communities.

## MATERIALS AND METHODS

### *Study area*

The Raška River is 39 km long, located in the southwestern part of Serbia, and is a left tributary of the Ibar River. Its catchment area covers 1.040 km². The river has an average width of 5-6 m at medium water level, reaching up to 10 m in some places in the lower reaches. The river's average depth is approximately 0.7 m (VIDAKOVIĆ *et al*., 2018). The Raška River originates beneath the limestone formation of Brva Golača, 15 km west of Novi Pazar. Its source area is a notable geomorphological phenomenon. The Kraško Koštam, a shortened source located at 950 m above sea level, channels water from the Koštam fields through underground passages, which resurface as a powerful spring emerging from a cave near the Sopoćani Monastery (STOJANOVIĆ, 2017).

According to national regulations (OFFICIAL GAZETTE of RS 72/2023-31 and 74/2011), surface waters in Serbia are categorized into six types based on geology, basin, altitude, and substrate type. In this classification, the Raška River belongs to Type 3, which includes small and medium-sized watercourses at altitudes up to 500 m with predominantly coarse substrate, and Type 4, encompassing small and medium-sized watercourses at altitudes above 500 m, also characterized by a predominantly coarse substrate. The section of the Raška River that passes through Novi Pazar is classified as a significantly changed water body.

A sampling of aquatic macroinvertebrates was conducted on August 23, 2023, at six selected sites along the Raška River (Table 1, Figure 1) following the AQEM protocol (AQEM, 2002). This standardized methodology ensures consistent and reliable assessment of biological quality elements in European rivers.

Table 1. Sampling sites in the Raška River

| Sites* | R1 | R2 | R3 | R4 | R5 | R6 |
|---|---|---|---|---|---|---|
| **Latitude (N)** | 43°06'57.1" | 43°07'51.3" | 43°08'02.4" | 43°08'48.4" | 43°14'04.6" | 43°17'08.3" |
| **Longitude (E)** | 20°22'13.5" | 20°24'45.9" | 20°26'09.1" | 20°31'31.6" | 20°34'50.7" | 20°37'09.2" |
| **Altitude (m)** | 731 | 581 | 543 | 484 | 445 | 401 |

*R1- at the source of the Raška River, below the Sopoćani Monastery, R2 - above the trout farm, R3 - below the trout farm, R4 - the center of Novi Pazar, R5 - the village of Milatkoviće, R6 - at the confluence of the Raška and Ibar Rivers.


Figure 1. Sampling sites on the Raška River

### *Sampling methodology*

#### *Physical and chemical parameters*

Physical and chemical parameters of the water, including temperature (WT, °C), conductivity (EC, µS/cm³), and water hardness (H, mg/L), were measured in the field using combined digital HANNA instruments (thermometer, conductometer, and pH meter), following the EN 5667 1-19 standard (EN 5667 1-19: 2017). Dissolved oxygen concentration (DO, mg/L) and oxygen saturation ($O_{SAT}$, %) were measured with a Mettler Toledo oximeter (APHA, 2012). Additionally, 1-liter water samples were collected in polyethylene bottles for further laboratory analysis conducted on the same day. In the laboratory, concentrations of ammonium-nitrogen ($NH_4$-N, mg/L), ammonia-nitrogen ($NH_3$-N, mg/L), nitrate-nitrogen ($NO_3$-N, mg/L), total nitrogen (N, mg/L), orthophosphate ($PO_4$-P, mg/L), phosphorus pentoxide ($P_2O_5$, mg/L), and total phosphorus (P, mg/L) were analyzed using an Aqualytic AL400 photometer with the appropriate reagents.

### *Aquatic macroinvertebrates*

At each sampling site, macrozoobenthos were collected using a 0.0625 m² Surber sampler with a 250 µm mesh, following the standards outlined in EN ISO 10870:2012 and EN ISO 27828:1994. Samples were preserved in 96% ethanol and subsequently analyzed in the laboratory of the Center for Fishery and Biodiversity Conservation of Inland Waters – Aquarium, at the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Republic of Serbia. The collected material was examined using a NIKON SMZ 800 stereomicroscope equipped with a LEICA camera and a Nikon Eclipse E100 microscope. Identification was carried out to the lowest possible taxonomic level (mostly species), based on reference literature (CONCI and NIELSEN, 1956; AUBERT, 1959; OLMI, 1976; ROZKOŠNÝ, 1980; NILSON, 1997; ELLIOTT, 19888; EISELER, 2005; ZWICK, 2004; TIMM, 2009; WARINGER and GRAF, 2011; ANDERSEN *et al*., 2013).

### *Ecological status/potential assessment*

According to the Rulebook (OFFICIAL GAZETTE of RS 72/2023-31 and 33/2023-3), sites R1, R2, and R3 are classified as Type 4, while sites R5 and R6 are classified as Type 3. Site R4 is classified as a significantly changed water body of Type 3.

The analysis of aquatic macroinvertebrate communities in rivers of Types 3 and 4, as per the Rulebook (OFFICIAL GAZETTE of RS No. 74/2011), involves the following metrics: saprobic index (ZELINKA and MARVAN method), BMWP score, ASPT, Shannon-Weaver diversity index (SHANNON, 1948), total number of taxa, BNBI index, percentage of Oligochaeta-Tubificidae, EPT index, number of families for Type 3, and number of sensitive taxa for Type 4.

The metrics calculation was performed using ASTERICS software (AQEM, 2002). The ecological status class boundaries for the selected metrics, by national legislation (OFFICIAL GAZETTE of the RS No. 74/2011), are presented in Table 2 for Types 3 and 4 water bodies. Ecological potential was assessed for Site R4.

Table 2. Ecological status class boundaries of selected metrics (Types 3 and 4)

| **Metric status** | **high/good** | **good/moderate** | **moderate/poor** | **poor/bad** |
|---|---|---|---|---|
| **Zelinka & Marvan SI** | 1.70 | 2.20/**2.10** | 2.80/**2.70** | 3.20/**3.10** |
| **BMWP Score** | 90.00 | 70.00 | 50.00 | 30.00 |
| **ASPT Score** | 7.00 | 5.00 | 4.00 | 3.00 |
| **Shannon-Weaver** | 2.20 | 1.50 | 1.20 | 0.50 |
| **Total no. of taxa** | 20.00 | 15.00 | 10.00 | 5.00 |
| **BNBI index** | 5.00 | 4.00 | 3.00 | 2.00 |
| **Oligochaeta /Tubificidae (%)** | | 5.00 | | |
| **EPT taxa** | 16.00/**18.00** | 12.00/**14.00** | 8.00/**10.00** | 4.00/**6.00** |
| **No. of families*** | 13.00* | 10.00* | 5.00* | 2.00* |
| **No. of sensitive taxa** | **5.00** | **4.00** | **3.00** | **2.00** |

Normal – values for Types 3 and 4; bold – values for only Type 4; * values for only Type 3;

## RESULTS AND DISCUSSION

### *Ecological status/potential assessment of the Raška River based on physical and chemical parameters*

The results of the physical and chemical parameters of water at the investigated sites along the Raška River are presented in Table 3. Significant deviations from the values prescribed by the Rulebook (OFFICIAL GAZETTE of RS, 74/2011) were observed at sites R3, R4, R5, and R6, where increased levels of pH, conductivity, and ammonium ions (ranging from 1.52 to 0.86 mg/L) were recorded, as well as nitrate (ranging from 18 to 7 mg/L). Concentrations of total dissolved phosphorus and orthophosphate also increased from sites R3 to R6. A decrease in oxygen concentration and saturation was recorded at these same sites (Table 3).

Table 3. Physical and chemical parameters of water of the Raška River in August 2023

| **Prameter / sites** | **R1** | **R2** | **R3** | **R4** | **R5** | **R6** |
|---|---|---|---|---|---|---|
| *pH (0-14) | 7.3 | 8.1 | 8.21 | 9.30 | 9.17 | 9.08 |
| Temperature (°C) | 11.4 | 13.7 | 14.7 | 22.8 | 18.7 | 16.9 |
| Conductivity (µS/cm$^3$) | 360 | 350 | 340 | 620 | 450 | 460 |
| *Dissolved oxygen (mg/l) | 9.28 | 8.89 | 8.57 | 4.2 | 6.4 | 6.04 |
| Oxygen saturation (%) | 88 | 89.4 | 88.5 | 40.2 | 68.9 | 68.5 |
| Water hardness (mg/l) | 180 | 170 | 170 | 310 | 220 | 230 |
| Ammonia ($NH_3$) (mg/l) | < min. | 0.04 | < min | 1.43 | 0.45 | 0.81 |
| *Ammonium ion ($NH_4$) (mg/l) | < min. | 0.04 | < min | 1.52 | 0.48 | 0.86 |
| *Total dissolved phosphorus (P) (mg/l) | < min. | < min | 0.05 | 0.16 | 0.16 | 0.17 |
| *Orthophosphates ($PO_4$) (mg/l) | < min. | < min | 0.14 | 0.50 | 0.50 | 0.53 |
| Phosphorus pentoxide ($P_2O_5$) (mg/l) | < min. | < min | 0.11 | 0.37 | 0.38 | 0.40 |
| *Nitrates ($NO_{3)}$ (mg/l) | < min | 6 | 18 | 15 | 13 | 7 |
| Total nitrogen (N) (mg/l) | < min | 1.4 | 4.1 | 3.3 | 3 | 1.6 |
| Class status/potential | II | II | III | IV | IV | IV |
| **Assessment of ecological status/potential** | **good** | **good** | **moderate** | **poor** | **poor** | **poor** |

*Physical and chemical parameters of water are monitored according to Serbian national regulation on ecological and chemical status parameters of surface waters and chemical and quantitative status parameters of ground waters (OFFICIAL GAZETTE of RS No. 74/2011)
**< min-below the level of detection

Based on the measured physical and chemical parameters and in accordance with the Rulebook (OFFICIAL GAZETTE of RS, 74/2011), the ecological status of the Raška River can be assessed as good (Class II) at sites R1 and R2, moderate (Class III) at site R3, and poor (Class IV) at sites R5 and R6. The ecological potential of the significantly changed water body (site R4), based on physical and chemical parameters, was also assessed as poor (Class IV) (Table 3).

Previous data on water quality based on the physical and chemical parameters of the Raška River were collected by BJELANOVIĆ *et al.* (2013). Their findings indicated a significant decrease in the mean dissolved oxygen concentration at the channel outlet associated with the trout farm. Consistent with their results, our data also recorded a significant decrease in oxygen concentration at sites R4, R5, and R6, along with increased concentrations of ammonium, orthophosphate, and nitrate, indicating organic pollution of the water.

***Taxonomic composition of the aquatic macroinvertebrate community***

A total of 61 aquatic macroinvertebrate taxa were recorded across all investigated sites (Table 4). The community was dominated by Diptera, with 20 identified taxa, including the family Chironomidae. Trichoptera and Oligochaeta were the next most diverse groups, each represented by 10 taxa. Other groups showed significantly lower diversity, with Ephemeroptera recorded with 6 taxa, followed by Plecoptera and Coleoptera, each represented by 4 taxa. Hirudinea and Amphipoda were represented by 2 taxa each, while the remaining groups were represented by a single taxon.

Table 4. Qualitative and quantitative analysis of aquatic macroinvertebrates from the Raška River

| **Taxa/ sites** | **R1** | **R2** | **R3** | **R4** | **R5** | **R6** |
|---|---|---|---|---|---|---|
| **Turbellaria** | | | | | | |
| *Dugesia gonocephala* (Duges, 1830) | - | - | 3 | - | - | - |
| **Oligochaeta** | | | | | | |
| *Eiseniella tetraedra* (Savigny, 1826) | - | - | 5 | - | - | - |
| *Enchytraeus* sp. Henle, 1837 | - | - | 5 | - | - | - |
| *Limnodrilus hoffmeisteri* Claparède, 1862 | - | - | - | 8 | 21 | 5 |
| *Potamothrix hammoniensis* (Michaelsen, 1901) | - | 3 | - | - | - | - |
| *Psammoryctides albicola* (Michaelsen, 1901) | - | - | 8 | - | - | - |
| *Stylodrilus heringianus* Claparède, 1862 | - | 5 | 5 | - | - | - |
| *Tubifex tubifex* Müller, 1774 | - | - | - | 43 | 5 | 32 |
| fam. Lumbricidae | 3 | - | - | - | - | - |
| fam. Lumbriculidae | - | - | 13 | - | - | - |
| fam. Naididae | - | - | - | - | 27 | 64 |
| **Hirudinea** | | | | | | |
| *Erpobdella octoculata* (Linnaeus 1758) | - | - | 13 | 3 | 21 | 16 |
| *Erpobdella* sp. Lamarck, 1818 | - | - | - | - | 3 | 3 |
| **Crustacea-Isopoda** | | | | | | |
| *Asellus aquaticus* (Linnaeus, 1758) | - | - | - | - | 27 | 8 |
| **Crustacea-Amphipoda** | | | | | | |
| *Gammarus balcanicus* Schäferna, 1923 | 37 | - | 91 | - | - | - |
| *Gammarus* sp. Fabricius, 1775 | - | - | - | 93 | 3 | 19 |
| **Ephemeroptera** | | | | | | |
| *Baetis muticus* (Linnaeus, 1758) | - | 13 | 77 | - | - | - |
| *Baetis rhodani* (Pictet, 1843) | 8 | 77 | 171 | 56 | 67 | 19 |
| *Baetis* sp. Leach, 1815 | 11 | - | 5 | - | - | 8 |
| *Ecdyonurus* sp. Eaton, 1868 | 3 | 37 | 35 | - | 3 | - |
| *Ephemera danica* Müller, 1764 | - | 3 | - | - | - | - |
| *Ephemerella ignita* Poda, 1761 | - | 5 | 16 | - | 43 | 19 |

*Table 4. continued*

| Taxa/ sites | R1 | R2 | R3 | R4 | R5 | R6 |
|---|---|---|---|---|---|---|
| **Plecoptera** | | | | | | |
| *Dinocras megacephala* (Klapálek, 1907) | - | - | 3 | - | - | - |
| *Leuctra* sp. Stephens, 1836 | - | 24 | 117 | - | 13 | 3 |
| *Protonemura praecox* (Morton, 1894) | 72 | - | - | - | - | - |
| *Protonemura* sp. Kempny, 1898 | 3 | 5 | - | - | - | - |
| **Trichoptera** | | | | | | |
| *Glossosoma* sp. Curtis, 1834 | 5 | - | - | - | - | - |
| *Hydropsyche incognita* Pitsch, 1993 | - | - | - | - | - | 16 |
| *Hydropsyche* sp. Pictet 1834 | - | - | - | - | 11 | 3 |
| *Oligoplectrum maculatum* (Fourcroy, 1785) | - | - | 5 | - | - | 3 |
| *Philopotamus montanus* (Donovan, 1813) | - | - | 3 | - | - | - |
| *Potamophylax* sp. Wallengren, 1891 | - | 3 | - | - | - | - |
| *Rhyacophila tristis* Pictet, 1834 | - | 11 | 3 | - | - | - |
| *Rhyacophila* sp. Pictet, 1834 | 3 | 3 | 19 | - | 3 | - |
| *Sericostoma flavicorne* Schneider 1845 | - | 27 | 11 | - | - | - |
| *Thremma anomalum* McLachlan, 1876 | 3 | - | - | - | - | - |
| **Odonata** | | | | | | |
| *Onychogomphus forcipatus* (Linnaeus, 1758) | - | 3 | - | - | - | - |
| **Diptera** | | | | | | |
| *Antocha vitripennis* (Meigen, 1830) | - | 3 | - | - | - | - |
| *Bezzia* sp. Kieffer, 1899. | - | - | 5 | - | - | - |
| *Ibisia marginata* (Fabricius, 1781) | 5 | 13 | - | - | - | - |
| *Pericoma* sp. Walker, 1856 | 5 | - | - | - | - | - |
| *Psychoda alternata* Say, 1824 | - | - | - | 24 | - | - |
| *Simulium* sp. Latreille, 1802 | - | 43 | - | - | 11 | 8 |
| *Tipula* sp. Linnaeus, 1758. | 5 | | | | | |
| *Wiedemannia* sp. Zetterstedt, 1838 | - | - | 3 | - | - | - |
| fam. Limoniidae | - | - | - | - | 3 | - |
| **Diptera-Chironomidae** | | | | | | |
| *Chironomus thummi* (Kieffer, 1911) | - | - | - | 245 | 5 | - |
| *Conchapelopia* sp. Fittkau, 1957 | - | 16 | 27 | - | - | - |
| *Corynoneura* sp. Winnertz, 1846 | - | 5 | 19 | - | - | - |
| *Cricotopus bicinctus* (Meigen, 1818) | - | - | - | - | 11 | 24 |
| *Orthocladius thienemanni* Kieffer & Thienemann, 1906 | - | 8 | 21 | - | - | - |
| subfam. Orthocladiinae | - | - | 8 | 130 | - | - |
| *Micropsectra* sp. Kieffer, 1908 | 3 | 19 | 51 | - | 5 | 8 |
| *Paratrichocladius rufiventris* (Meigen, 1830) | - | - | - | - | 24 | 43 |
| *Prodiamesa olivacea* (Meigen, 1818) | - | - | - | - | - | 5 |
| *Rheocricotopus chalybeatus* (Edwards, 1929) | - | - | - | - | 29 | 56 |
| *Tvetenia calvescens* (Edwards, 1929) | - | - | - | - | 3 | 16 |

*Table 4. continued*

| Taxa/ sites | R1 | R2 | R3 | R4 | R5 | R6 |
|---|---|---|---|---|---|---|
| **Coleoptera** | | | | | | |
| *Elmis* sp. Latreille, 1802 | 24 | 3 | - | - | - | - |
| *Hydraena gracilis* Germar, 1823 | - | 5 | 16 | - | - | - |
| *Hydraena sp.* Kugelann, 1794 | - | - | - | - | 3 | - |
| *Riolus subviolaceus* (Müller, 1817) | - | 11 | - | - | - | - |
| **Number of individuals per m$^2$** | **190** | **345** | **758** | **602** | **341** | **378** |
| **Number of taxa** | **15** | **24** | **28** | **8** | **22** | **21** |

It is important to note that some taxa have not been identified at the species level due to the complexity of the identification process and associated uncertainty. Therefore, the actual taxon richness is likely to be higher. Quantitatively, the dominant groups were Diptera and Ephemeroptera, with 40.2% and 25.9%, respectively. Chironomidae contributed most to the richness and abundance of benthic communities, especially at sites downstream from the farm. This was particularly evident at site R4, where *Chironomus thummi* (Kieffer, 1911) was highly represented, with a density of 245 ind/m$^2$. Species within the family Chironomidae are generally tolerant of organic pollutants, and their dominance in water is typically attributed to poor water quality (MOLINERI *et al.*, 2020). These species are well adapted to low dissolved oxygen concentrations and can survive complete anoxia. Additionally, their abundant presence in polluted streams is attributed to the relatively low levels of competition and predation in such environments (MOLINERI *et al.*, 2020).

At the first three sites, we recorded several species sensitive to changes in water conditions, including *Protonemura praecox* (Morton, 1894), *Dinocras megacephala* (Klapálek, 1907), *Rhyacophila tristis* (Pictet, 1834), *Philopotamus montanus* (Donovan, 1813), *Sericostoma flavicorne* (Schneider, 1845), *Thremma anomalum* (McLachlan, 1876), *Baetis muticus* (Linnaeus, 1758), and *Ephemera danica* (Poda, 1761) (Table 4). Most of these taxa are included in the Austrian list of sensitive taxa (MOOG et al., 2017).

***Ecological status/potential assessment of the Raška River based on aquatic macroinvertebrate communities***

The assessment of the ecological status and potential of the Raška River, based on the analysis of aquatic macroinvertebrate communities, is presented in Table 5.

The recorded values of the Shannon-Weaver Diversity Index were highest at sites R2 (2.338) and R3 (2.476), indicating high ecological status (Class I). The index values at other sites ranged from 1.78 to 2.19, indicating good ecological status (Class II), while site R4 was characterized by the lowest index value (1.43) and moderate water quality (Class III) (Table 5). The Zelinka & Marvan Saprobic Index values ranged from 0.95 (indicating oligomesosaprobity, recorded at R1) to 2.68 (suggesting α-mesosaprobity, recorded at R6), reflecting a moderate level of organic pollution across sites. The highest percentages of Oligochaeta were recorded at R5 and R6, at 17.273% and 25.85%, respectively, indicating bad water quality. High populations of Oligochaeta often suggest enriched nutrient conditions, typically associated with organic pollution in freshwater environments (BEHREND *et al.*, 2012). Conversely, the increase in Oligochaeta taxa at these sites is accompanied by a decrease in the abundance of Ephemeroptera, Plecoptera, and Trichoptera taxa (Table 5). The BMWP Score values ranged from 16 at R4 to 96 and 91 at R2 and R3, respectively. At all investigated sites except R4 and R6, the ASPT scores indicated good ecological status and Class II water quality. The number of recorded taxa at sites R1 (15), R5 (22), and R6 (21) indicated a good ecological status (Class II), while the number of taxa at sites R2 (24) and R3 (28) suggested a high ecological status (Class I). At site R4, the lowest number of taxa (8) was recorded, reflecting poor ecological potential (Class IV) (Table 5). Finally, a gradient was observed in the decline of the BNBI index values downstream from the first two sites. Thus, the values of this index indicate good ecological status at sites R1 and R2, moderate status at sites R3 and R5, and poor ecological status at R6 (Table 5).

Table 5. Ecological status/potential assessment of the Raška River based on aquatic macroinvertebrate community

| **Prameter / sites** | **R1** | **R2** | **R3** | **R4** | **R5** | **R6** |
|---|---|---|---|---|---|---|
| Zelinka & Marvan SI | 0.951 | 1.924 | 1.951 | 2.72 | 2.649 | 2.681 |
| BMWP | 70 | 96 | 91 | 16 | 66 | 49 |
| ASPT | 5.222 | 6.4 | 6.5 | 3 | 5.7 | 4.9 |
| Shannon-Weaver | 1.787 | 2.338 | 2.476 | 1.43 | 2.192 | 2.105 |
| Total No. of Taxa | 15 | 24 | 28 | 8 | 22 | 21 |
| BNBI | 4.25 | 4 | 3.875 | 2 | 3.16 | 2.86 |
| Oligochaeta / Tubificidae [%] | 1.562 | 1.765 | 3.86 | 9.34 | 17.273 | 25.85 |
| EPT taxa | 6 | 11 | 12 | | 6 | 7 |
| Number of sensitive taxa | 4 | 7 | 3 | * | * | * |
| Number of families | * | * | * | 6 | 15 | 12 |
| Class status/potential | II | II | II | IV | III | IV |
| **Assessment of ecological status / potential** | **good** | **good** | **good** | **poor** | **moderate** | **poor** |

Based on the analysis of aquatic macroinvertebrate communities, a good ecological status (Class II) was observed at sites R1, R2, and R3. A moderate ecological status (Class III) was recorded at site R5, while a poor ecological status (Class IV) was determined at site R6. The ecological potential of the significantly changed water body (Site R4), based on aquatic macroinvertebrates, was assessed as poor (Class IV) (Table 5).

The ultimate ecological status assessment, based on macroinvertebrates and supporting physical and chemical quality elements, indicates a good ecological status (Class II) at sites R1 and R2, a moderate status (Class III) at site R3, and a poor status (Class IV) at sites R5 and R6. The ecological potential at site R4 was also assessed as poor (Class IV) (Table 6).

Table 6. The ultimate assessment of the ecological status/potential of the Raška River in August 2023

| **Prameter / Sites** | **R1** | **R2** | **R3** | **R4** | **R5** | **R6** |
|---|---|---|---|---|---|---|
| Class of ecological status/potential based physical and chemical parameters | **II** | **II** | **III** | **IV** | **IV** | **IV** |
| Class of ecological status/potential based on the macroinvertebrate community | **II** | **II** | **II** | **IV** | **III** | **IV** |
| **Ultimate ecological status/potential assessment** | **II** | **II** | **III** | **IV** | **IV** | **IV** |
| **Ultimate ecological status/potential class** | **good** | **good** | **moderate** | **poor** | **poor** | **poor** |

The research on the quality of the Raška River, conducted by BJELANOVIĆ *et al.* (2013), utilized zoobenthos and zooplankton organisms as bioindicators. Based on saprobiological analyses, it was determined that the water quality in the Raška River is primarily classified as second class or at the transition between the first and second classes, particularly at sites upstream of the farms. As expected, high water quality in the upper course of the Raška River, including its source, is attributed to its location outside urban settlements and minimal anthropogenic influence. Several studies have recognized these sites as hotspots of high biodiversity, with a dominance of insect larvae from the orders Ephemeroptera, Plecoptera, and Trichoptera, along with the presence of salmonid fish species, which indicate good water quality (STOJANOVIĆ, 2017; SIMOVIĆ *et al*., 2022; 2024). In addition, the upper reaches of the Raška River (R2 site) feature a natural phenomenon of calcium carbonate deposition, forming tufa barriers that indicate "healthy" but vulnerable natural environments (SIMOVIĆ *et al*., 2024). Moreover, at the source of the Raška River, we recorded two rare and endangered species of macroinvertebrates, *Thremma anomalum* and *Protonemura praecox*. Both species are protected by national regulations in Serbia and are listed as strictly protected and protected species according to the Rulebook on the Proclamation and Protection of Strictly Protected and Protected Wild Species of Plants, Animals, and Mushrooms (OFFICIAL GAZETTE of RS, No. 5/2010, 47/2011, 32/2016, and 98/2016).

Physical and chemical parameters downstream from fish farms can be altered by residual food and fecal materials, leading to increased inorganic nutrients and suspended solids, as well as decreased dissolved oxygen in the river (RADOJEVIĆ *et al*., 2018; CAMARGO *et al.,* 2021). This is confirmed by our research, particularly in terms of elevated nitrate levels, whose values, according to the Rulebook (OFFICIAL GAZETTE of RS, 74/2011), indicate Class V water quality. On the other hand, the values of various parameters based on the aquatic macroinvertebrate community at the site downstream from the trout farm still indicated good water quality (Class II), the same as the site upstream from the trout farm.

In 2013, the Serbian Republic Hydrometeorological Institute monitored the Raška River and classified its water quality as class III. While the pH and chloride levels met class I standards, the dissolved oxygen levels and biological oxygen demand were consistent with class II standards. However, deviations in the concentrations of ammonium ions, orthophosphates, and total dissolved phosphorus impacted the overall assessment of water quality (GROUP OF AUTHORS, 2013).

The data from the Serbian Environmental Protection Agency (SEPA) show that none of the rivers passing through the city achieved good ecological status or potential, and some did not meet the criteria for good chemical status during the research period (GROUP OF AUTHORS, 2018, 2019, 2021). The Raška River, which flows through Novi Pazar, reflects a similar situation, as do the rivers that flow through larger cities in Serbia, such as the Lepenica in Kragujevac (SIMIĆ *et al*., 2023) and the Despotovica in Gornji Milanovac (SIMIĆ et al., 2018). A concerning aspect of the Raška River is the intense urbanization of its banks and the high level of pollution, particularly in the section from Novi Pazar to the city of Raška. There is significant pollution along the banks of the Raška River, including plastic, metal, and organic waste, untreated wastewater from industrial and household facilities, and runoff from quarries, which we also observed during our field research. (ANONYMOUS, 2013; OBRADOVIĆ, 2021). Moreover, such problems, which negatively affect the ecological value of the river, are often reported in journalistic circles (OBRADOVIĆ, 2021).

According to the SEPA, the ecological status, based on physical and chemical parameters and biological elements, primarily aquatic macroinvertebrates, was classified as Class III (moderate status) for the periods 2012-2016 and 2017-2019 (GROUP OF AUTHORS, 2018; 2021). However, in 2015 and 2016, a decline in water quality was recorded, with both physical and chemical parameters and aquatic macroinvertebrates indicating a Class IV (poor ecological status). Compared to the previous ecological assessments, our results show a similar situation: R1 and R2 are characterized by a good ecological status, while the estimated status for the other locations ranged from Class III to Class IV.

The assessment of water quality based on physical and chemical parameters aligns with findings related to biological elements, primarily aquatic macroinvertebrates. A slight increase in the concentrations of total phosphorus, orthophosphate, and nitrate from the third to the sixth site, along with a gradual decrease in oxygen levels, is accompanied by a shift in the composition of the aquatic macroinvertebrate community. This is further reflected in a decrease in the saprobity index, a decline in the BNBI index value, an increase in the number of Oligochaeta (Tubificidae), and other parameters indicating a change in water quality.

This study provides a preliminary, one-time assessment of the ecological status and potential of the Raška River, based on physical and chemical parameters and macroinvertebrate communities. We have noted the degradation of the Raška River ecosystem, particularly in areas near populated places, which are characterized by high levels of industrialization. Therefore, these results indicate that proactive measures are necessary to mitigate further ecological damage, ensuring the protection and restoration of the aquatic ecosystem and safeguarding the health of the surrounding environment. However, a comprehensive ecological evaluation requires the analysis of additional biological communities, such as fish, diatoms, and macrophytes, which would provide a deeper understanding of the ecological health of the water ecosystem. Establishing a systematic monitoring program is essential for both ecological balance and the health of local populations.

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