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INTEGRATED PRELIMINARY ASSESSMENT OF WATER QUALITY AND ECOLOGICAL FEATURES OF THE KRUPA RIVER (REPUBLIC OF SRPSKA, BiH)

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Abstract

The Krupa River, a left tributary of the Vrbas, is exposed to various anthropogenic pressures, but due to its hydromorphological specificities it represents a suitable model for ecological features' assessment. The aim of this study was to determine water quality and ecological features by analyzing physicochemical parameters, macrozoobenthos, and ichthyofauna. Sampling was conducted in three sites in autumn 2023 using standard field and laboratory methods. Saprobic status was assessed using the Pantle–Buck index (ichthyofauna), while water quality based on macrozoobenthos was evaluated using the Trent Biotic Index. Most physicochemical parameters indicated water of quality classes I–II. Analysis of macrozoobenthos revealed 14 taxa at the spring, 12 above the waterfalls, and 11 near the confluence. The dominance of the genus *Gammarus* confirmed stable microhabitats, while the higher abundance of Chironomidae near the confluence indicated increased organic load. Values of the Trent Biotic Index were VIII at the spring and VII at the other two sites. The ichthyofauna comprised six fish species, dominated by brown trout (*Salmo trutta*), a reliable indicator of clean, well-oxygenated waters. The Pantle–Buck saprobic index (0.91) confirmed oligosaprobic conditions, corresponding to class I water quality. The results showed that the Krupa River, despite anthropogenic pressures, retains the characteristics of very good water quality. At the same time, they emphasize the importance of continuous monitoring in safeguarding water quality and protecting that aquatic ecosystem.

Key words: benthic invertebrates, fish assemblages, running waters, saprobic status, biotic indices, anthropogenic pressure

INTRODUCTION

For assessing water quality in the Republic of Srpska, the Regulation on Water Classification and Categorization of Watercourses (Official Gazette of the Republic of Srpska, 42/01) is applied. According to this regulation, surface waters are classified based on general criteria, which reflect ecological status and include chemical, physicochemical, biological, and hydromorphological elements, as well as criteria for specific hazardous and toxic substances, in accordance with the EU Water Framework Directive (ODV EU, 2000).

Biological elements refer to the composition and diversity of aquatic flora, benthic macroinvertebrates, and fish fauna.

While physicochemical measurements indicate the current state of water quality, they do not provide information on longer-term conditions. Certain organisms serve as bioindicators, reflecting cumulative effects of environmental stress (Petrović *et al*., 1998). Biological assessment is particularly applicable to running waters, where pollution loads may be periodic (especially from diffuse sources) and, due to water flow, remain at a given site for a short time and thus may go undetected by chemical water monitoring (Wright, 1995).

Macrozoobenthos is a particularly suitable indicator in running waters, as it is widely distributed, sedentary, and sensitive to environmental changes. Life cycles of its constituents allow assessment of cumulative stress, and their taxonomy is well known, facilitating qualitative and quantitative analyses. Limitations include seasonal variations, influence of non-pollution factors, unresolved taxonomy in some groups, and insensitivity to specific stressors (Rosenberg and Resh, 1993).

Fish communities also provide important information on water quality. They respond quickly to accidental pollution, and their community composition reflects saprobic status and ecological disturbances. Fish are present in almost all surface waters, relatively easy to identify, and their ecological requirements, life cycles, and tolerance to stressors are well known. Different species occupy various trophic levels and habitats, offering a wide range of responses to environmental pressures. Limitations include labor-intensive sampling, migrations, and methodological biases (Karr, 1981; Grabarkiewicz and Davis, 2008; Stojković-Piperac, 2015).

The aim of this study was to assess the water quality and ecological status of the Krupa River by analyzing physicochemical parameters, macrozoobenthos, and ichthyofauna.

MATERIALS AND METHODS

Study area

The Krupa River is an extension of the Ponor River, which originates near the village of Podrašnica (Municipality of Mrkonjić Grad). After flowing for several kilometers, the Ponor River sinks beneath the southwestern slopes of Mt. Manjača in the Podrašnica Field through a typical karst sinkhole, and subsequently re-emerges at the surface as the Krupa River (Figure 1). The total length of the Ponor River is 12.4 km (Crnogorac *et al*., 2013; Lolić *et al*., 2017).

The source of the Krupa River is located approximately 3 km west of the settlement of Krupa na Vrbasu, and it joins the Vrbas River at Krupa na Vrbasu as a left tributary (Water Management Basis, 1989, cited in Pavlović *et al*., 2012). The Krupa River is subject to direct anthropogenic pressures, including alterations of riverbed morphology, bank modifications, and wastewater discharge. Part of the riverbank has been developed as a recreational area, and effluents from a salmonid fish farm are discharged into the lower section of the river (Pavlović *et al*., 2012).

According to the Amendments and Supplements to the Spatial Plan of the Republic of Srpska up to 2025 (Official Gazette of the Republic of Srpska, 15/15), the Krupa River is planned for protection under the category of a nature park (IUCN category VIa), covering

approximately 1,800 ha. Its conservation importance is further highlighted by the designation of the Krupa River area (excluding built-up zones) as a fishing district (Authors' group, 2022).

Sampling and analysis of selected physicochemical parameters and macrozoobenthos were conducted at three sites along the Krupa River in autumn 2023. The first site (L1), located near the river source at 44.620701° N, 17.112216° E, included sampling of water for physicochemical parameters and macrozoobenthos. The second site (L2), upstream of the waterfalls at 44.616463° N, 17.138977° E, was sampled for physicochemical parameters, macrozoobenthos, and ichthyofauna. The third site (L3), near the confluence with the Vrbas River at 44.615922° N, 17.148532° E, included sampling of physicochemical parameters and macrozoobenthos (Figure 1).

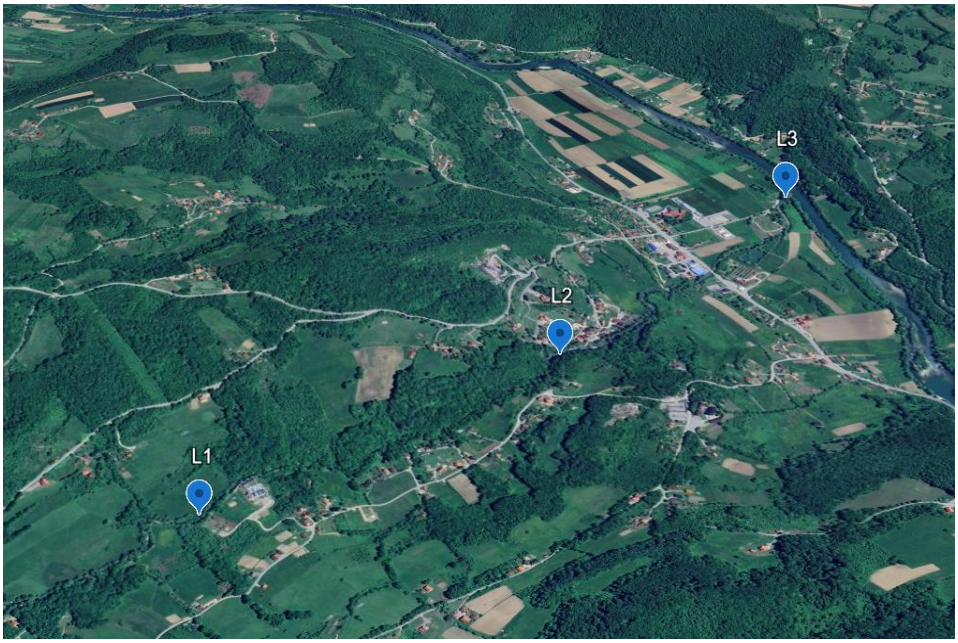

Figure 1. Location of the Krupa River with the sampling sites (L1 – near the source, L2 – upstream of the waterfalls, L3 – near the confluence with the Vrbas River)
(*Google Earth,* modified by Golub, 2025)

Physicochemical water quality parameters

The physicochemical water quality parameters measured in the field included water temperature, pH, oxygen concentration, and electrical conductivity. Water temperature, pH, oxygen concentration, and electrical conductivity were measured in the field using mercury thermometer, HI 98127 pH meter (±0.1), HI 9142 oximeter (±0.1 mg/L) and Nahita conductometer (±2 µS/cm), respectively. Laboratory analyses at the Faculty of Natural Sciences and Mathematics, Banja Luka, determined concentrations of orthophosphates, sulfates, nitrites, ammonia, and total suspended solids using HACH DR2800 spectrophotometer, following standard procedures, while oxygen saturation was calculated using water oxygen saturation calculator (WOW, 2024). BOD_5 was determined after five days of incubation at 20 °C with the HI 9142 oximeter. The obtained results were interpreted

according to the relevant water quality regulations of the Republic of Srpska (Official Gazette of the Republic of Srpska, 42/01).

Macrozoobenthos

Macrozoobenthos sampling was performed using a Surber net (NHBS GmbH) with a mesh size of 500 μm, frame dimensions of 25×25 cm, and net length of 50 cm. The net was placed facing the water current and stabilized by the sampler's weight to ensure stability during sampling. Sampling targeted representative microhabitats at each site. Samples were transported to the Faculty of Natural Sciences and Mathematics, University of Banja Luka, where they were analyzed under a LEICA EZ4D stereomicroscope. Organisms were identified using relevant identification keys (Macan, 1960; Kerovec, 1986; Gledhill *et al*., 1993; Waringer and Graf, 2013; Kriska, 2014; Živić and Marković, 2017). The Trent Biotic Index (TBI) was used as a parameter of the ecological conditions in the Krupa River, as the composition and sensitivity of benthic macroinvertebrate communities based on the number of defined taxa and the presence of six key indicator groups reliably reflect the impact of anthropogenic and natural factors on the quality of the aquatic ecosystem (Woodiwiss, 1964).

Ichthyofauna

Sampling of ichthyofauna was carried out using an electrofishing unit ELT62II GI HONDA GCV160, of the power of 3 kW, with the permission of the Ministry of Agriculture, Forestry and Water Management of the Republic of Srpska No. 12-03.2-330-359/22, dated 10.06.2022 and in accordance with the standard for sampling fish with electricity for the purpose of water quality analysis (CEN, 2023). Sampled fish were identified, weighed, counted in the field, and then released unharmed. Species identification of the sampled fish was accomplished using identification keys (Simonović, 2001; Kottelat and Freyhof, 2007; Sofradžija, 2009). The Pantle–Buck based saprobic index was calculated for the fish communities at sites using the formula $S = \Sigma(h \times s) / \Sigma h$, where h is the relative abundance and s the species saprobic value (Grginčević and Pujin, 1998).

RESULTS AND DISCUSSION

Physicochemical water quality parameters

The values of the analyzed general parameters, concentrations of nutrients and sulfates and oxygen indicate that water quality in the Krupa River is generally satisfactory, with most parameters corresponding to Class I or II standards. An exception is the oxygen saturation at the third site (near the confluence with the Vrbas River), which indicates Class IV water quality (130.81%). This is due to intense water turbulence downstream of the waterfalls, which temporarily increases oxygen saturation. This supersaturation is most likely temporary, as oxygen levels should stabilize further downstream. Oxygen supersaturation can affect aquatic organisms, potentially causing gas bubble disease in fish if combined with rapid temperature or pressure changes, while invertebrates are affected only at higher supersaturation levels than those lethal to fish (Meyer and Barclay, 1990) (Table 1).

Table 1. Physicochemical characteristics of the Krupa River

Parameter (unit)	**Measured values**			**Water quality class[1]**
GENERAL PARAMETERS				
	L1	**L2**	**L3**	
T_{water} (˚C)	12.0	13.1	15.7	-
Conductivity (µS/cm)	400	400	500	II/II/II
pH	8.37	8.24	8.64	I/I/II
Total suspended solids (g/m^3)	2	3	5	II/II/II
OXYGEN REGIME				
Dissolved oxygen (g/m^3)	11.8	9.1	13.0	I/I/I
Saturation (O_2) (%)	109.46	86.52	130.81	I/I/IV
BOD_5 (g O_2/m^3)	3.5	1.6	3.1	II/I/II
NUTRIENTS				
Orthophosphates (g/m^3PO_4)	0.16	0.35	0.25	-
Nitrite-nitrogen (g/m^{3}N)	0.008	0.007	0.009	I/I/I
Ammonia nitrogen (g/m^{3}N)	0.02	0.02	0.08	I/I/I
OTHER INORGANIC SUBSTANCES				
Sulphate (g/m^3)	20	40	40	I/I/I

According to the Regulation on the Classification and Categorization of Watercourses of the Republic of Srpska (Official Gazette of the Republic of Srpska, 42/01), Class I waters in their natural state can be used for drinking or in the food industry and are suitable for the growth and development of salmonid fish species. Class II waters, after appropriate treatment, can be used for drinking; in their natural state, they can be used for bathing and water sports, and are suitable for the growth and development of cyprinid fish species.

The Ponor River, which forms the upstream section of the Krupa River, exhibits generally good physicochemical water quality. Similar to the Krupa River, it shows slightly alkaline water and high oxygenation, with most parameters corresponding to Class I or II standards (Lolić *et al*., 2011; 2017). This comparison highlights that both rivers maintain satisfactory water quality along their courses, with the Krupa River reflecting conditions downstream of the Ponor Spring and incorporating additional local influences such as waterfalls

[1] Official Gazette of the Republic of Srpska, 42/01

Macrozoobenthos

Analysis of the macrozoobenthos revealed 15 taxa in total, with 14 taxa at site L1, 12 at site L2, and 11 at site L3. The community structure indicates spatial differences in composition and gradients in habitat quality along the course of the Krupa River (Table 2). The dominance of *Gammarus* sp. in terms of abundance at all sites indicates stable microhabitats and favorable ecological conditions, whereas the high abundance of Chironomidae at site L3 suggests local organic enrichment and potential alterations in water quality.

Table 2. Qualitative and quantitative composition of the macrozoobenthos community in the Krupa River

	L1		L2		L3	
Taxa	**No of individuals**	**%**	**No of individuals**	**%**	**No of individuals**	**%**
GASTROPODA						
Hydrobiidae	11	2.6	64	24.5		
Bithyniidae	4	0.9			43	13.7
ANNELIDA						
Oligochaeta	2	0.5	6	2.2	8	2.5
CRUSTACEA						
Isopoda						
Asellus aquaticus					14	4.5
Amphipoda						
Gammarus sp.	189	45	68	25.8	51	16.2
INSECTA						
Ephemeroptera						
Baetidae	6	1.4	7	2.6		
Heptageniidae						
Ecdyonurus sp.	8	1.9	29	11	6	1.9
Ephemerellidae	2	0.5	1	0.3		
Plecoptera						
Capniidae	3	0.7				
Perlidae	2	0.5	2	0.8	1	0.3
Coleoptera						
Elmidae	106	25.2	35	13.2	9	2.9
Trichoptera	7	1.7	4	1.5	14	4.5
Diptera						
Chironomidae	9	2.2	17	6.4	156	49.7
Simuliidae	63	15	28	10.6	5	1.6
Hydracarina	8	1.9	3	1.1	7	2.2

At site L1, the TBI was VIII, indicating very good ecological quality and the presence of sensitive indicator taxa. At sites L2 and L3, TBI was VII, reflecting a slight decline in ecological quality downstream, associated with habitat modifications and a higher proportion of tolerant taxa. Overall, the Krupa River exhibits good to very good ecological status along its course.

Compared to the upstream Ponor River (Authors' group, 2022), the macrozoobenthos community of the Krupa River is richer and more diverse, reflecting a wider range of habitats and environmental conditions. While *Gammarus* sp. dominates in both rivers, the Krupa River hosts a greater representation of Ephemeroptera, Plecoptera, Coleoptera, Trichoptera, and Diptera, whereas the Ponor River shows lower taxonomic richness and a simpler community structure, with limited occurrence of Elmidae, Trichoptera, and Diptera. The higher abundance of tolerant Chironomidae in the lower Krupa section indicates minor local organic enrichment, yet the continued presence of sensitive taxa such as Elmidae, Baetidae, and *Ecdyonurus* sp. supports good to very good ecological conditions. Overall, the higher diversity in the Krupa River, together with multiple sensitive insect taxa and a broader distribution of gastropods, annelids, and crustaceans, suggests a more heterogeneous habitat structure and a more complex ecological network, consistent with its high TBI values.

Our study was conducted at three sites during a single season, which limits the number of taxa recorded compared to the longitudinal and multi-season study of the Krupa River in 2010 (Pavlović *et al*., 2012), where samples were collected at four sites during winter, spring, and summer, and a total of 20 taxa were identified. Although our study recorded fewer taxa than previous multi-seasonal studies, the distribution of dominant taxa (e.g. *Gammarus* sp. and Ephemeroptera) was similar, reflecting the influence of longitudinal ecological gradients. In previous studies, Ephemeroptera were dominant in winter and spring, while *Valvata* snails were most abundant in spring and summer; our results are most compatible with the winter and spring patterns of the Krupa River, especially regarding longitudinal changes in abundance and taxa richness. Similarities in the occurrence of dominant taxa indicate that local ecological factors and longitudinal position strongly shape the community, even though seasonal effects in our study are less pronounced. Differences in the number of recorded taxa and density can be explained by the limited seasonal coverage and fewer sampling sites, highlighting the importance of multi-seasonal and multi-site studies for a more complete characterization of the zoobenthos.

The structure of the macrozoobenthos community recorded in the Krupa River is consistent with the findings of Balta and Bilbija (2015), who identified 18 taxa based on sampling at four sites during three sampling periods. Despite a broader spatial and temporal coverage in their study, both studies highlight the dominance of amphipods (*Gammarus* sp.) and the frequent occurrence of Ephemeroptera, Plecoptera, and Trichoptera as indicators of good ecological conditions and high oxygen availability. Balta and Bilbija (2015) also reported an increased proportion of Chironomidae in downstream sections as a response to localized organic enrichment, which corresponds to our results at site L3, where Chironomidae were abundant but sensitive taxa such as Elmidae, Baetidae, and *Ecdyonurus* sp. were still present. Such a community structure indicates moderate local pressure rather than a substantial deterioration of ecological status. Furthermore, the Trent Biotic Index values reported by Balta and Bilbija (2015) for comparable river sections generally corresponded to good or very good ecological status, which is in agreement with the TBI values obtained in the present study (VIII at the spring and VII downstream).

Ichthyofauna

The Krupa River ichthyofauna comprises six species across four families: Salmonidae, Cyprinidae, Leuciscidae, and Cottidae. Brown trout (*Salmo trutta*) was the most abundant species (54.46%) and dominated in biomass (74.80%), followed by bullhead (*Cottus gobio*, 21.78%) by abundance and grayling (*Thymallus thymallus*, 10.89%) by biomass (Table 3).

Table 3. Qualitative and quantitative composition of the ichthyofauna community in the Krupa River

Taxa	No of individuals	%	Mass (g)	%
Salmo trutta (Salmonidae)	55	54.46	5192	74.80
Thymallus thymallus (Salmonidae)	10	9.90	756	10.89
Barbus balcanicus (Cyprinidae)	5	4.95	123	2.98
Barbus barbus (Cyprinidae)	4	3.96	311	1.77
Squalius cephalus (Leuciscidae)	5	4.95	352	4.48
Cottus gobio (Cottidae)	22	21.78	207	5.07

Compared to the upstream Ponor River, which hosts only species typical at the uppermost stream's fish communities (*Salmo trutta, Thymallus thymallus*, and *Cottus gobio*) (Authors' group, 2022), the Krupa River exhibits higher species richness due to its downstream location, hydromorphological heterogeneity, and influence of the confluence zone, supporting both salmonid and more tolerant cyprinid species.

Comparison with previous surveys (1975–1996) showed that 9–13 species were reported historically, whereas the 2023 study recorded six species. This discrepancy is likely due to differences in sampling area and methodology, as earlier studies covered the entire river course while our study focused on a smaller upstream section above the waterfalls. Despite differences in total species number, the persistent presence of key indicators for the most upstream sections (*Salmo trutta, Thymallus thymallus, Cottus gobio*) suggests long-term stability of the Krupa River's salmonid character. The absence of certain species in the 2023 survey, particularly cyprinids that are either migratory, or more generalistic in habitat preference (e.g. *Alburnoides bipunctatus*, *Chondrostoma nasus, Rutilus virgo*), is most likely more related to differences in extent of sampling, habitat availability in a sampled stream sections and methodological approaches, than to a real decline of ichthyofaunal diversity. Differences in a composition of ichthyofauna between earlier studies and the 2023 survey may also partly reflect increasing anthropogenic pressures along the Krupa River, particularly in downstream sections, such as habitat modification and altered hydromorphological conditions. However, the continued presence and dominance of sensitive, oxyphilic fish taxa indicate that these pressures have not led to a significant deterioration of ecological conditions.

Table 4. Comparative overview of data on the ichthyofauna of the Krupa River from 1975 to 2023

Taxa	**1975 (Authors' group, 1975)**	**1984 (Authors' group, 1985)**	**1994-1996 (Radević, 2000)**	**2023 (our research)**
	upper, middle and lower course (6.5 ha)	upper, middle and lower course (6.5 ha)	upper, middle and lower course	upstream of the waterfalls (1140 m^2)
Eudontomyzon vladykovi (Petromyzontidae)	+			
Hucho hucho (Salmonidae)	+		+	
Oncorhynchus mykiss (Salmonidae)	+	+	+	
Salmo trutta (Salmonidae)	+	+	+	+
Thymallus thymallus (Salmonidae)	+	+	+	+
Alburnoides bipunctatus (Cyprinidae)		+	+	
Barbus balcanicus (Cyprinidae)		+	+	+
Barbus barbus (Cyprinidae)		+	+	+
Chondrostoma nasus (Cyprinidae)			+	
Gobio obtusirostris (Cyprinidae)			+	
Phoxinus phoxinus (Cyprinidae)		+	+	
Rutilus virgo (Cyprinidae)			+	
Squalius cephalus (Cyprinidae)			+	+
Cottus gobio (Cottidae)	+	+	+	+

The Pantle–Buck saprobic index, based on the ichthyofauna, was 0.91, indicating oligosaprobic (Class I) conditions and reflecting the high-water quality of the Krupa River. Oligosaprobic waters are clean or slightly polluted, with high dissolved oxygen content, low bacterial counts, and stable organic matter, supporting diverse communities of oxyphilic organisms, e.g., brown trout, bullhead, and various macroinvertebrates (Tedeschi, 1997; Cvijan, 2000; Pešić, 2011). This assessment is further confirmed by high TBI values, both at the source (TBI = VIII) and downstream (TBI = VII), indicating minimal anthropogenic impact.

CONCLUSIONS

The analyzed physicochemical parameters indicate that the water of the Krupa River corresponds to Class I–II, i.e., the high-to-good water quality along most of its course. The observed oxygen supersaturation at the most downstream sampling site most likely represented a local and temporary phenomenon associated with the hydromorphological characteristics of the river.

The structure of the macrozoobenthos community, with relatively high taxonomic diversity and the dominance of sensitive taxa, confirms a good-to-very good water quality of the river. A slightly increased proportion of tolerant taxa downstream indicates moderate habitat changes, but with still retained biological balance. Trent Biotic Index (TBI) values (TBI = VIII near the source and TBI = VII downstream) supports a good-to-very good water quality status of the watercourse.

The ichthyofauna of the Krupa River, dominated by brown trout (*Salmo trutta*) and with other oxyphilic species, represents a reliable indicator of preserved ecological conditions. The Pantle–Buck index (S = 0.91) confirms oligosaprobic status and Class I water quality.

An integrated assessment of physicochemical and biological water quality elements showed that the Krupa River, despite existing anthropogenic pressures, maintains a high-water quality status and represents an important and well-preserved aquatic ecosystem in the Vrbas River basin.

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INTEGRISANA PRELIMINARNA PROCJENA KVALITETA VODE I EKOLOŠKIH KARAKTERISTIKA RIJEKE KRUPE (REPUBLIKA SRPSKA, BiH)

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Sažetak

Rijeka Krupa, lijeva pritoka Vrbasa, izložena je različitim antropogenim pritiscima, a zbog svojih hidromorfoloških specifičnosti predstavlja pogodan model za procjenu ekoloških karakteristika. Cilj ove studije bio je utvrditi kvalitet vode kao i ekološke karakteristike analizom fizičko-hemijskih parametara, makrozoobentosa i ihtiofaune. Uzorkovanje je provedeno u jesen 2023. godine na tri lokaliteta koristeći standardne terenske i laboratorijske metode. Saprobni status je procijenjen korištenjem Pantle–Buck indeksa (ihtiofauna), dok je kvalitet vode na osnovu makrozoobentosa procijenjen korištenjem Trent Biotic indeksa. Većina fizičko-hemijskih parametara ukazivala je na vodu kvaliteta I i II klase kvaliteta. Analiza makrozoobentosa utvrdila je prisustvo 14 taksona na izvoru, 12 iznad vodopada i 11 u blizini ušća. Dominacija roda *Gammarus* potvrdila je stabilna mikrostaništa, dok je veća zastupljenost predstavnika porodice Chironomidae u blizini ušća ukazivala na povećano organsko opterećenje. Vrijednosti Trent biotičkog indeksa iznosile su VIII u blizini izvora i VII na nizvodija dva lokaliteta. Ihtiofaunu je činilo šest vrsta riba, pri čemu je dominirala potočna pastrmka (*Salmo trutta*), pouzdan indikator čistih i dobro oksigenisanih voda. Pantle–Buck saprobni indeks (0,91) potvrdio je oligosaprobne uslove, što odgovara kvalitetu vode I klase. Rezultati su pokazali da rijeka Krupa, uprkos antropogenim pritiscima, zadržava karakteristike veoma dobrog kvaliteta vode. Istovremeno, rezultati naglašavaju važnost

kontinuiranog monitoringa u cilju očuvanja kvaliteta vode i zaštite datog vodenog ekosistema.

Ključne riječi: bentoski beskičmenjaci, zajednica riba, tekućice, saprobni status, biotički indeksi, antropogeni pritisak

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