Annals of the Institute of Biology - University of Sarajevo
AIBUS: 47: 17-27
Original Article
UDC: 574.5:579.68(497.6)"2025.332.06"

ISSN: 0350-2163 (Print)
ISSN: 2831-0705 (Online)
http://aibus.pmf.unsa.ba/
DOI: 10.35666/2831-0705.2025.47.17

15 December 2025

Ecological Status Assessment of the Upper Una River: Physico-Chemical and Microbiological Perspectives

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Abstract

The aim of this study was to assess the ecological status of the upper course of the Una River at eight sampling locations, four of which are situated within the protected area of Una National Park and four downstream, beyond the tourist and urban zones of the City of Bihać. The sampling sites were selected according to the "post-pollution input" principle, aiming to identify which of the selected locations contribute most to the river's pollution. Physico-chemical parameters (pH, dissolved oxygen, permanganate index, BOD_5, TOC, ammonia, nitrates, total nitrogen, orthophosphates, and total phosphorus) were analyzed following Bosnia and Herzegovina's water legislation, alongside microbiological assessment.

The results indicated that most physico-chemical parameters correspond to a high ecological status, with slight local fluctuations in BOD_5 and TOC values classifying the river as having a good ecological status, and ammonia indicating a moderate status. Microbiological analysis revealed spatial heterogeneity in the concentrations of total coliforms, *Escherichia coli*, and *Enterococcus faecalis*, which were detected in urban

and tourist-influenced zones. Statistical analysis (ANOVA) showed no significant differences ($p > 0.05$) between locations inside and outside the protected area, whereas PCA analysis distinguished sites based on their chemical and microbiological composition and their contribution to pollution. The findings confirmed that local anthropogenic impacts mainly result from fecal contamination. Comparison with data from other rivers in Bosnia and Herzegovina and Europe suggests that the Una River still maintains favorable ecological conditions, although continuous monitoring remains crucial for preserving water quality.

Keywords: *Una River, ecological status, physico-chemical parameters, microbiology, anthropogenic impacts.*

1. Introduction

The Una River is one of the most valuable natural resources in the northwestern part of Bosnia and Herzegovina, both ecologically and socio-economically. Its exceptional hydrological, geomorphological, and biological diversity ranks it among the most important freshwater ecosystems in the region. The river's waterfalls and rapids in the upper course create a spectacular landscape, while tufa deposition and the formation of diverse relief features—including tufa barriers, islets, cones, caves, and tectonically influenced cascades at Martin Brod and Štrbački buk, make the Una a unique habitat characteristic of karst rivers. These geomorphological and ecological phenomena are recognized and defined as a target habitat type within Natura 2000 (Tufa cascades of karstic rivers of the Dinaric Alps, habitat type 32A0), giving the Una River significance at the European level [1]. In addition, the Una is notable for its high surface water quality and numerous other values, which led to the designation of its upper course as Una National Park (NP Una) in 2008, covering an area of 198 km² [2].

In the upper course of the Una, within the protected area, high water quality reflects minimal anthropogenic impact [3]. Downstream, in the settlements of Martin Brod, Kulen Vakuf, Orašac, and Bihać, human activities such as wastewater discharge, agriculture, inadequate waste disposal, and tourism can compromise water quality [4–6]. These impacts manifest at different spatial and temporal scales [7]. Tourism in ecologically sensitive areas, such as the Una, can increase fecal pollution, alter nutrient levels, and burden sediments [8], as confirmed by studies on the Cávado River in Portugal [9]. Intensive tourism often also leads to the removal of riparian vegetation and the construction of riverbank reinforcements, reducing natural soil protection and the ability of forests to retain sediment and nutrients [7,10].

Therefore, systematic monitoring, combining physico-chemical and biological indicators along with GIS analyses, is crucial for tracking and managing these impacts [11].

Considering that tourism has become an important economic factor in the Una River area in recent years, there has been an increase in the number of visitors, accommodation facilities, campsites, catering establishments, and water-based and water-related activities. In addition, the local population is present in the same area with all its associated activities. The aim of this study was to assess the quality of surface waters of the Una River at selected locations, both within the protected area of Una National Park and downstream, through the analysis of physico-chemical and microbiological parameters, with the purpose of determining how and to what extent human activities affect changes in water quality.

2. Material and Methods

2.1. Study Area

The study was conducted on the Una River, which originates near Donja Suvaja (Croatia) and flows northward through Bosnia and Herzegovina. The upper course, spanning 53 km, encompasses the protected area of Una National Park, while the downstream section of 25 km passes through the urban area of the City of Bihać.

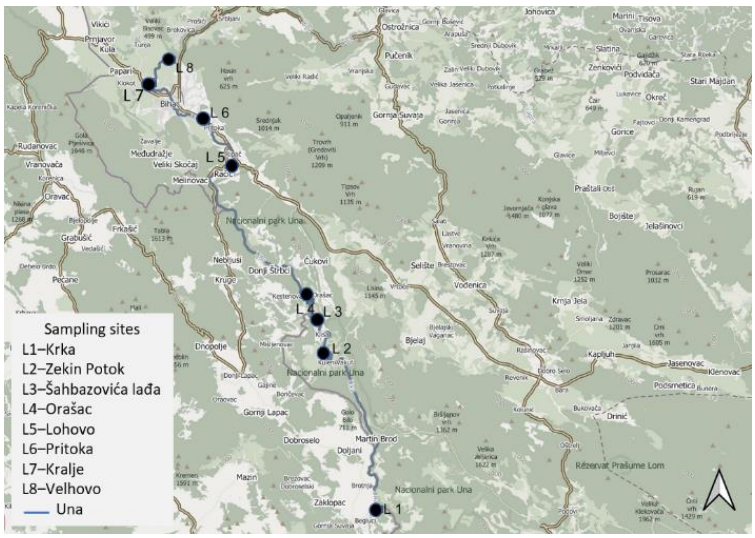

Figure 1. Sampling locations along the longitudinal profile of the Una River.

Along the banks of the protected area, there are four settlements with about 7,900 inhabitants, while Bihać has a total population of around 56,000 [12]. For the purposes of the study, eight sampling sites were selected (Figure 1), distributed

along the river's longitudinal profile. Four sites are located within the protected area, and four downstream, where human impact is more pronounced. The sites were chosen according to the "post-pollution input" principle, except for L1 (Krka – Una tributary), which represents a reference, pristine zone. L2 (Zekin Potok) reflects agricultural influence, L3 (Kulen Vakuf – Šahbazovića lađa) and L4 (Orašac) reflect the impact of the local population, L5 (Lohovo) and L6 (Pritoka – below Ripač) reflect tourism activities, L7 (Kralje) reflects the urban influence of Bihać, and L8 (Velhovo) is located downstream of a wastewater treatment plant that does not operate at full capacity.

2.2. Analytical Methods

Water samples for the analysis of physico-chemical and microbiological parameters were collected in June 2025 following standardized protocols [11], stored at 4°C, and processed in the laboratory within the prescribed timeframe. Bosnian-Herzegovinian standards (BAS EN ISO) [13] were applied as analytical methods, including the determination of pH (10523:2013), dissolved oxygen content (5814:2014), permanganate index (8467:2002), BOD_5 (5815-2:20024), total organic carbon (TOC) (Macherey-Nagel, 2020), ammonia (7150-1:2002), nitrates and total nitrogen (7890-3:2002), and orthophosphates and total phosphorus (6878:2006). Microbiological parameters included the enumeration of total coliform bacteria and *Escherichia coli* according to BAS EN ISO 9308-1:2015, and fecal streptococci (*Enterococcus faecalis*) according to BAS EN ISO 7899-2:2003. Based on the measured parameters, the ecological status of the sampled locations was assessed in accordance with the Decision on the Characterization of Surface and Ground Waters, reference conditions, and parameters for water status assessment and monitoring [14].

The results were analyzed using descriptive and multivariate statistics. PCA was applied to identify sites contributing to variability in physico-chemical and microbiological parameters, while ANOVA assessed significant differences ($p > 0.05$) between locations and between protected and non-protected areas. Analyses were conducted in Past 4.3 and Excel 2016. Interpretation focused on identifying critical sites, key contributing parameters, and spatial variability.

3. Results

The results of the physico-chemical and microbiological parameters of the Una River, with respect to the sampled locations, are presented in Table 1, while their descriptive statistics are shown in Table 2-3. The average pH value of the water along the Una River was 7.93, ranging from 7.8 (L3, L4) to 8.0 (L1, L6, L7, L8). Dissolved

oxygen concentrations ranged from 8.80 to 10.70 mg/L, with the highest value at L1 and the lowest at L3 and L7. The permanganate index was below 0.50 mg/L at all sites.

Table 1. Physico-chemical water parameters of the Una River by sampling locations

Parameters	L1	L2	L3	L4	L5	L6	L7	L8
pH	8.0	7.9	7.8	7.8	7.9	8.0	8.0	8.0
Dissolved oxygen content (mg/L)	10.7	9.8	9.7	8.8	10.1	9.8	9.7	9.5
Permanganate index (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
BOD_5 (mgO_2/L)	2.29	1.74	1.59	0.58	0.99	0.70	1.70	< 0.5
Total organic carbon (mg/L)	3.2	< 2	< 2	3.1	< 2	2.2	3.5	2.6
Ammonia (mg/L)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.056
Nitrates (mg/L)	0.078	0.30	0.14	0.46	0.16	0.18	0.27	0.23
Total nitrogen (mg/L)	0.6	1.00	0.,9	1.2	0.7	0.7	1.0	0.9
Orthophosphates (mg/L)	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025
Total phosphorus (mg/L)	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025

Table 2. Spatial variation (mean ± SD, CV) of physico-chemical parameters of the Una River

Parameters	Mean ± SD	Range	CV*
pH	7.93 ± 0.09	7.80–8.00	1.12
Dissolved oxygen content (mg/L)	9.76 ± 0.53	8.80–10.70	5.47
Permanganate index (mg/L)	0.50 ± 0.00	0.50–0.50	0.00
BOD_5 (mgO_2/L)	1.26 ± 0.66	0.50–2.29	52.08
Total organic carbon (mg/L)	2.58 ± 0.62	2.00–3.50	23.92
Ammonia (mg/L)	0.05 ± 0.00	0.50–0.60	4.18
Nitrates (mg/L)	0.23 ± 0.12	0.08–0.46	52.05
Total nitrogen (mg/L)	0.88 ± 0.20	0.60–1.20	22.65
Orthophosphates (mg/L)	0.03 ± 0.00	0.03–0.03	0.00
Total phosphorus (mg/L)	0.03 ± 0.00	0.03–0.03	0.00

**CV–Coefficient of variation*

The highest BOD_5 value (2.29 mgO_2/L) was recorded at L1, while the lowest (< 0.5 mgO_2/L) was observed at L8. Ammonia concentrations were < 0.05 mg/L at all locations. Total nitrogen varied from 0.60 mg/L (L1) to 1.20 mg/L (L4), while orthophosphates and total phosphorus were < 0.025 mg/L at all sites. The coefficient of variation (CV) for physico-chemical parameters ranged from 0.00 % to 52.08 %, indicating differences between locations. Most parameters showed low to moderate variability (CV < 30 %), suggesting stable conditions along the Una River. Higher CV values for BOD_5 and nitrates (> 50 %) indicated spatial heterogeneity, likely caused by local anthropogenic impacts and differences in land use.

PCA analysis (Figure 2a) revealed spatial differences in water quality along the Una River. Site L1 was distinguished by elevated BOD_5, dissolved oxygen, and pH values,

indicating active biological decomposition and good water oxygenation. Sites L5, L6, and L8 had lower pH and oxygen but higher nutrient levels (total nitrogen, nitrates), suggesting a mild anthropogenic influence. L7 stood out for higher TOC, while L4 showed lower BOD_5 and pH, indicating slower organic matter decomposition.

Table 3. Significant differences in water parameters across protected and unprotected areas of Una flow (ANOVA)

Parameters	Mean (Protected area)	Mean (Unprotected area)	t - test	*p* – value
Number of locations (n)	4	4		
pH	7.88	7.98	0.1294	$p > 0.05$
Dissolved oxygen content (mg/L)	9.75	9.78	0.9544	$p > 0.05$
Permanganate index (mg/L)	< 0.5	< 0.5	-	-
BOD_5 (mgO_2/L)	1.55	0.97	0.244	$p > 0.05$
Total organic carbon (mg/L)	2.58	2.58	1	$p > 0.05$
Ammonia (mg/L)	< 0.05	< 0.05	-	-
Nitrates (mg/L)	0.24	0.21	0,7214	$p > 0.05$
Total nitrogen (mg/L)	0.93	0.83	0.5237	$p > 0.05$
Orthophosphates (mg/L)	< 0.025	< 0.025	-	-
Total phosphorus (mg/L)	< 0.025	< 0.025	-	-

Table 4. Microbiological water parameters of the Una River by sampling locations

Parameters	L1	L2	L3	L4	L5	L6	L7	L8
Total coliforms (cfu/100 mL)	770	3500	3400	260000	880	450	850	1100
Esherichia coli (cfu/100 mL)	4	23	8	3100	12	1	62	41
Enteroccocus faecalis (cfu/100 mL)	NI	9	6	170	NI	3	38	36

**NI–Not isolated*

Table 5. Descriptive statistic and significant differences in water microbiological parameters across protected and unprotected areas of Una flow (ANOVA)

Parameters	Mean ± SD	Range	CV*	Mean**	Mean***	t - test	p – value
Number of locations (n)				4	4		
Total coliforms (cfu/100 mL)	33900 ± 91400	260–450	270	66900	20	0.3800	$p > 0.05$
Esherichia coli (cfu/100 mL)	406 ± 1090	1–3100	268	78400	29	0.4004	$p > 0.05$
Enteroccocus faecalis (cfu/100 mL)	32.8 ± 57.6	0–170	176	46.3	19.3	0.5662	$p > 0.05$

**CV–Coefficient of variation;* **Protected area; ***Unprotected area

One-way ANOVA did not reveal statistically significant differences in physico-chemical parameters between protected and non-protected areas ($p > 0.05$). Minor variations, such as a higher average BOD_5 in the protected zone (1.55 mgO_2/L), reflect natural variability rather than protection effects, indicating uniform water quality conditions across both zones.

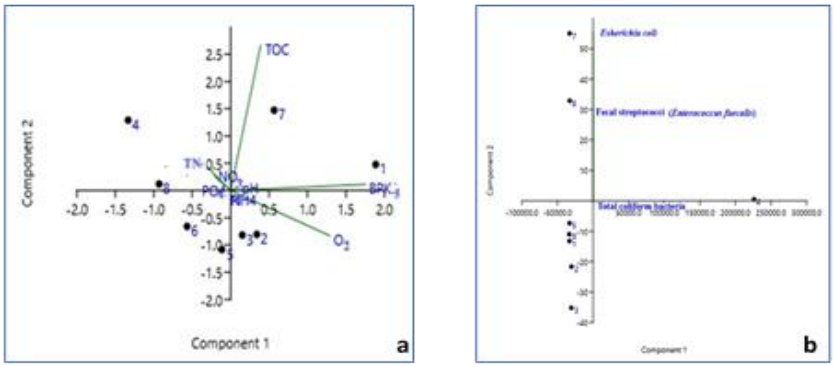

Figure 2. PCA biplot for physicochemical water parameters (a) and microbiological parameters (b).

Microbiological analysis showed variable levels of total coliforms, E. coli, and E. faecalis across the eight sites. Total coliforms ranged from 770 cfu/100 mL at L1 to 260,000 cfu/100 mL at L4. E. coli was low in less impacted areas (4 cfu/100 mL at L1) and higher in impacted sites (3,100 cfu/100 mL at L4), indicating fecal contamination. E. faecalis was absent at L1 and L5, with the highest values at L4 (170 cfu/100 mL). High spatial variability (total coliforms: 33,900 ± 91,400 cfu/100 mL; CV = 270 %) aligned with PCA results. Despite numerical differences between protected and non-protected areas, ANOVA showed no statistically significant differences ($p > 0.05$), likely due to high variability and small sample size.

4. Discussion

The results of the physico-chemical parameters showed relatively similar values between the protected and non-protected areas, which was confirmed by ANOVA analysis, as no statistically significant differences ($p > 0.05$) were observed either between locations or between the protected and non-protected areas. Although minor numerical differences were recorded, they were not large enough to be considered significant, suggesting that the physico-chemical composition of the water remains stable even in areas with more pronounced human impact. Spatial variability and PCA analysis clearly distinguished sites L4, L5, L6, L7, and L8 as potentially contributing to greater pressure on the Una River, while L1 remained a "pristine zone."

Microbiological parameters exhibited greater spatial variability and varied among locations, with the lowest values recorded at L1, in the undisturbed natural area, and the highest concentrations observed at L4 (influence of the Orašac settlement), L7 (urban area of the City of Bihać), and L8 (downstream of the wastewater treatment plant). The presence of total coliforms at L1 (770 cfu/100 mL) and other relatively undisturbed sites can be explained by natural sources, such as wildlife feces, soil

runoff, or organic matter decomposition. Despite higher numerical differences, no statistically significant differences ($p > 0.05$) were found either between locations or between protected and non-protected areas. This indicates that, although anthropogenic impacts locally and downstream increase the concentrations of fecal indicator bacteria, high spatial variability and the small sample size prevent these differences from being statistically significant. The combination of physico-chemical and microbiological data suggests that sites L4, L7, and L8 are most associated with anthropogenic impacts on the water quality of the Una River, and that local factors and natural variability contribute to spatial heterogeneity.

According to the Decision on the Characterization of Surface and Ground Waters [14], the results for pH, dissolved oxygen, BOD_5, nitrates, total nitrogen, orthophosphates, and total phosphorus indicate that all sites along the Una River have a high ecological status, with good oxygenation, low nutrient levels, and minimal anthropogenic influence. The permanganate index and TOC parameters indicate minimal organic pollution and naturally balanced biological decomposition, reflecting good ecological status, while ammonia shows a moderate ecological status, suggesting a mild but non-problematic input of fecal and agricultural waters.

In Bosnia and Herzegovina, it has been found that the Bosna River exhibits significantly higher pollution concentrations following wastewater input [15], suggesting a more favorable water quality in the Una River. Similar patterns of spatial variability have been observed in the Sava River basin [16], where higher nutrient concentrations are associated with lower oxygen levels, with temperature and circulation being key factors for oxygenation. At sites L2 and L3, moderate BOD_5, pH (7.93), and oxygen values were recorded, indicating stable conditions and a slightly alkaline reaction typical for carbonate areas. In contrast, L5, L6, and L8 showed lower pH and oxygen levels and elevated nutrients, suggesting organic pollution, consistent with findings from the Tisa River basin [17]. Similar microbiological values have been reported in the Buna and Neretva rivers [18]. At the European level, although EU policies have improved water quality, most rivers still do not achieve “good ecological status” [19]. Additionally, a report by researchers from 23 European countries indicates that most rivers have not yet reached the required ecological quality, which is indicative of the challenges in managing continental waters [20]. In this context, the relatively stable results for the Una River indicate comparatively favorable water quality, with risks present that are similar to those observed in other rivers in the region.

5. Conclusions

The results indicate that the Una River along the analyzed course largely maintains a high ecological status based on physico-chemical parameters. Values of pH, dissolved oxygen, BOD_5, TOC, and nutrients mostly meet the criteria for a "high status," with stable conditions and limited spatial heterogeneity in areas with higher anthropogenic influence. Coefficients of variation point to relatively stable conditions along most of the river, with spatial heterogeneity localized in areas of greater human impact. Microbiological parameters show greater variability, with the highest values observed at sites L4, L7, and L8, near urban and tourist areas. No statistically significant differences were found between protected and non-protected zones, highlighting the importance of local influences. Overall, the Una River is preserved in good ecological condition, with minimal risk of eutrophication; however, elevated values at certain sites indicate the need for continuous monitoring and responsible water resource management.

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7. Acknowledgement

We would like to thank Una National Park and the Environmental Protection Fund of the Federation of Bosnia and Herzegovina for their support and co-financing of the project “Identification of Pollutants in the Una National Park Area and Assessment of Their Impact Trends on Surface Water Quality,” which made this research and the preparation of this paper possible.

8. Author contribution

Author 1: Collected water samples, performed physico-chemical and microbiological analyses, interpreted the obtained results, and conducted statistical analyses with their interpretation.

Author 2: Conceived and designed the study, supervised the project, coordinated research activities, collected water samples, and interpreted results.

Author 3: Collected water samples, performed physico-chemical analyses, and interpreted the obtained results.

Author 4: Collected water samples, performed microbiological analyses, and interpreted the obtained results.

Author 5: Collected water samples, performed physico-chemical analyses, and defined the analytical methods.

Author 6: Participated in the research project, coordinated research activities, collected water samples, and entered results into the GIS database.

Avdić and all co-authors: Drafted the manuscript, reviewed and edited it, and approved the final version.

9. Conflict of Interest Statement

The authors declare no conflict of interest.