Research Article

Biochemical Biomarkers of Two Fish Species, *Barbus balcanicus* and *Chondrostoma nasus*, in Different Rivers of Kosovo

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Abstract | In this study, biochemical biomarkers in *Barbus balcanicus* and *Chondrostoma nasus* were assessed to provide baseline data for local water quality monitoring and pollution assessment in Kosovo. This study aimed to evaluate target physiological and biochemical biomarkers in two species of fish that usually inhabit freshwaters, *Barbus balcanicus* and *Chondrostoma nasus*, obtained from three rivers in Kosovo with different levels of pollution. Fish samples were selected from the Sitnica, Lepenci, and Lumbardhi i Prizrenit Rivers using standard electrofishing techniques. Plasma glucose, total protein, AST, and ALT were analyzed in 60 fish, comprising 30 specimens of *Barbus balcanicus* and 30 specimens of *Chondrostoma nasus*. Ten individuals of each taxon were collected from each river. . The results showed significant changes in GLU, TP, AST, and ALT levels in fish samples from the Sitnica River compared to samples from the Lepenci and Lumbardhi i Prizrenit Rivers ($p < 0.05$), indicating increased metabolic and hepatic stress. These findings in biochemical parameters show a significant correlation between river pollution, particularly heavy metal pollution, and the physiological response of fish to stress. These results confirm that the analyzed biomarkers show a real picture of environmental stress and the low level of water quality in these rivers. The study also confirms that the fish species *Barbus balcanicus* and *Chondrostoma nasus* can serve as adequate bioindicators for monitoring water pollution as effective biochemical biomarkers for the detection and timely sensitization to environmental pollution in freshwater ecosystems, as well as potential risks to human health through the trophic chain.

Keywords | Fish biomarkers, Water pollution, Heavy metals, Liver enzymes, Freshwater ecosystems, Kosovo

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INTRODUCTION

Freshwater ecosystems are among the most vulnerable environments to anthropogenic pollution, particularly in regions experiencing rapid urbanization and industrial development. Rivers receiving untreated municipal, agricultural, and industrial effluents are frequently exposed to complex mixtures of pollutants, including heavy metals, which pose significant risks to aquatic organisms and human health through the food chain (Panda *et al*., 2023; Sharma *et al*., 2025; Shetty *et al*., 2025). Monitoring water quality using biological indicators is therefore essential for assessing ecosystem health and identifying early signs of environmental degradation (Van der Oost *et al*., 2003; Adams *et al*., 2025).

Fish are widely recognized as reliable bioindicator organisms due to their sensitivity to changes in water quality, their position in aquatic food webs, and their ability to bioaccumulate contaminants (Van der Oost *et al*.,

2003; Zahran *et al*., 2025). Physiological and biochemical biomarkers measured in fish blood and tissues provide valuable information on sublethal stress responses induced by environmental pollution. Among these biomarkers, plasma glucose (GLU) and total protein (TP) are commonly used indicators of metabolic stress, while hepatic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) reflect liver function and cellular integrity. Alterations in these parameters have been consistently associated with exposure to heavy metals and other aquatic pollutants (Oudah, 2024; Zeng *et al*., 2025).

Despite Kosovos limited water resources, fish farming is still possible in some rivers, such as the Drini i Bardhe, Lumbardhi i Pejës, Lumbardhi i Prizrenit, Lepenci, Brodi, Restelica etc. Although fishing is not a significant economic activity, fish farming can make a valuable contribution to local aquaculture (Annual Report on the State of the Environment, 2021). Fishing is one of the most promising economic development areas due to the abundance of rivers and favorable environmental conditions. However, not only the mentioned but also other rivers with lesser water capacity are viable rivers for fish farming in the national level (Annual Report on the State of the Environment, 2021).

In recent decades, many fish species have suffered from changes in river water quality, while carp species have benefited from these changes (Subotić *et al*., 2019; Oroian *et al*., 2025). To maintain fish productivity, it is necessary to be careful about diseases, stress, and environmental pollution. Fish health can be affected by pathogen activity, stress, and food. Fish can experience stress due to various abiotic environmental factors, such as changes in water temperature, pH, oxygen content, and the presence of pesticides, herbicides, petroleum products, and heavy metals (Subotić *et al*., 2019; Oroian *et al*., 2025).

Barbus balcanicus Kotlík, Tsigenopoulos, Ráb and Berrebi, 2002, commonly known as the large spot barbel or Danube barbel, is a small rheophilic, lithophilic, and benthopelagic freshwater cyprinid distributed in parts of the Danube, Adriatic, and Aegean drainage systems. The species mainly inhabits mountainous and submountainous streams and rivers characterized by clear, well-oxygenated, fast- to moderately flowing water and gravel or stony substrates, where it is commonly associated with riffles and other high-velocity habitats. Spawning generally occurs from May to July, when adults migrate upstream in search of suitable spawning grounds with gravel and stone substrates. Because of its dependence on good water quality and structurally intact lotic habitats, *B. balcanicus* may be sensitive to pollution, flow regulation, and habitat degradation, making it a potentially useful species for assessing environmental changes in freshwater ecosystems (Bertoli *et al*., 2019).

The common nase (*Chondrostoma nasus*) is a medium-sized rheophilic freshwater cyprinid native to Central and Eastern Europe, typically inhabiting well-oxygenated, fast-flowing rivers and streams. This species has an elongated, laterally compressed body, a relatively small head, and a straight, inferior mouth with a thick cornified lower lip adapted for feeding on benthic substrates.. The skin color of its body is usually silvery, with a subtle olive-green or brown tint. The Common nase grows to a length of 60 cm and weighs up to 3 kg. These Common nase are benthic fish that feed mainly on invertebrates such as insects, crustaceans, and worms, as well as plant matter, especially algae and detritus. Their flexible body and strong, muscular tail make them well-suited to living in fast-flowing waters. This species usually lives in clean, oxygen-rich rivers with rocky or gravel bottoms, often preferring areas with moderate to strong currents and currents. Billfish are known for their unique reproductive behavior - spawning occurs in the spring (usually between March and July), when water temperatures rise (Jamil Emon *et al*., 2023; Zahran *et al*., 2025).

During spawning, females deposit adhesive eggs on shallow gravel substrates in flowing water. Embryonic development and hatching occur externally in the aquatic environment, while newly hatched larvae may remain temporarily within the interstitial spaces of the gravel before emerging into suitable nursery habitats.. These fish prefer warm water temperatures between 12 °C and 20 °C and are sensitive to pollution. Therefore, they are excellent indicator species for water quality in their habitats. In Europe, the common nase is mainly found in waters such as the Danube, Rhine, and Elbe, although populations elsewhere are threatened by habitat destruction, water pollution, and dam construction (Pal and Maiti, 2019; Pargi *et al*., 2024; El-Agri *et al*., 2022). Despite these challenges, the common nase remains an important species in European freshwater habitats, particularly in the food chain, where it supports higher trophic levels. With regard to species conservation, some populations of *Chondrostoma nasus* are considered threatened due to habitat fragmentation and severe pollution, which has led to efforts to protect and monitor their habitats (Pal and Maiti, 2019; Pargi *et al*., 2024; El-Agri *et al*., 2022). A lot of scientific programs envisage monitoring the exposure (with a focus on all aquatic organisms) to various pollutants (such as metals, PCBs, and pesticides) to track their concentrations in large rivers and lakes. Previous individual projects and programs for monitoring aquatic organisms have focused solely on the characteristics of specific parameters. However, other potential factors at the molecular level, which can have multifactorial impacts on the environment and humans,

also play an incredible role (Pal and Maiti, 2019; Pargi *et al*., 2024; El-Agri *et al*., 2022).

A modern, multidimensional monitoring program for aquatic organisms must incorporate a complex spectrum of biomarkers within the biocenosis of a large ecosystem. Fish are particularly well-suited as bioindicators to reflect changes and potential disruptions in vital functions, such as the effects of contamination. Various organisms in rivers and lakes can be used as bioindicators. These include fish, crustaceans, and mollusks, as well as aquatic animals and plants, algae, macrophytes, and macro invertebrates. In this context, the present study aims to evaluate the impact of river pollution on fish health by analyzing selected biochemical biomarkers in freshwater species from Kosovo. Specifically, the study investigates plasma glucose (GLU), total protein (TP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels in *Barbus balcanicus* and *Chondrostoma nasus* collected from rivers with different pollution levels (Sitnica, Lepenci, and Lumbardhi i Prizrenit). The objective is to assess the relationship between environmental contamination, particularly heavy metal pollution, and physiological stress responses in fish, and to determine the suitability of these species as bioindicators of freshwater ecosystem health.

MATERIALS AND METHODS

Study area and sampling sites

The investigation was carried out at several sampling sites along three rivers in Kosovo. Along the Sitnica River, site S1 was positioned near the river source in the village of Lumi i Madh (Vushtrri), while site S2 was located in Mitrovica, an area affected by intensive river flow and historical discharge of waste from the Battery Factory. These sections of the Sitnica River, including the stretch passing through Lumi i Madh, are considered among the most polluted areas in the country. Additional sampling sites were established on the Lepenci River in Brezovica (L1) and Brod (L2), as well as on the Lumbardhi i Prizrenit River at Mushnikova (LP1) and Reçan (LP2) (Figure 1). In addition to fish sampling, water samples were collected from all sampling sites along the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers for physicochemical and heavy metal analysis. Fish sampling was conducted at two locations in each river: S1 and S2 in the Sitnica River, L1 and L2 in the Lepenci River, and LP1 and LP2 in the Lumbardhi i Prizrenit River. A total of 60 fish were collected, comprising 30 specimens of *Barbus balcanicus* and 30 specimens of *Chondrostoma nasus*. Twenty fish were collected from each river, including 10 specimens of each taxon. Within each river, specimens of each taxon were distributed approximately equally between the two sampling sites. For the biochemical analyses, data from the two sampling sites within each river were pooled and analyzed at the river level; therefore, the sample size for each taxon in each river was n = 10.

Fish sampling and experimental design

Biochemical and heavy metal analyses were performed at the Institute of Clinical Biochemistry, University Clinical Center of Kosovo (Prishtina), and at the National Institute of Public Health of Kosovo. Following capture, the fish were transported to the laboratory in containers filled with water from the respective sampling river and were maintained under short-term holding conditions for no longer than 24 hours before blood collection.

Physicochemical and heavy metal analysis of water

Physicochemical parameters and heavy metal concentrations were determined in water samples collected from the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers. Water sampling and analysis were conducted following standardized protocols (APHA 3111B, 2018; ISO 5667-5:2000). Parameters including pH, temperature, conductivity, turbidity, nitrites (NO_2^-), and ammonium (NH_3) were measured. Heavy metal concentrations (Pb, Zn, Cu, Ni, Cd, Co) were analyzed prior to treatment using atomic absorption spectrophotometry. These additional environmental parameters were incorporated to better interpret the relationship between water quality and biomarker responses in fish.

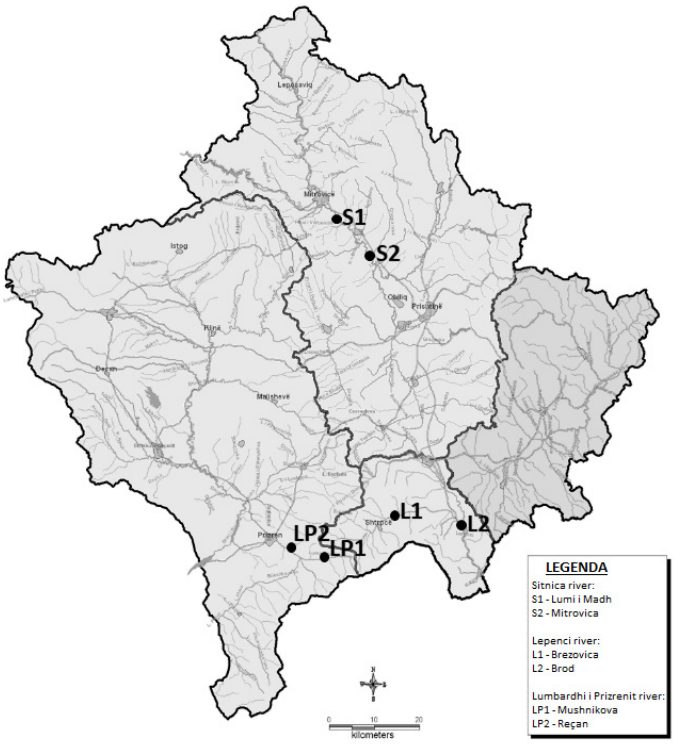

Figure 1: Map of sample sites.

Blood collection and sample preparation

Prior to blood collection, fish were anesthetized using ether, which is commonly applied as an anesthetic in an-

imal studies. Approximately 1 mL of blood was collected from the caudal vein of each anesthetized fish using a sterile syringe containing EDTA and fitted with a 0.8 × 38 mm hypodermic needle. The blood samples were centrifuged at 5,000 rpm, after which the plasma was separated and transferred into labeled microtubes for biochemical analysis.The caudal vein was chosen for blood collection because it offers advantages over other methods, such as the Cuvierian vein. This method is considered safe, easy to use, and allows for the rapid collection of a sufficient blood volume.

Biochemical analysis

The following methods were used to analyze the fish blood samples: Plasma total protein (TP) concentration was determined using the Biuret colorimetric method. This method utilizes a colorimetric reaction to measure the amount of protein in a solution. Glucose content (GLU) was determined using a Cobas 400 biochemical analyzer (Roche) based on the enzymatic measurement of glucose oxidase. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in plasma using enzymatic colorimetric methods on a Cobas Integra 400 biochemical analyzer (Roche).. Heavy metals were analyzed according to the method based on the American Public Health Association (APHA). The standard values presented in Tables 1 and 2 were obtained from established laboratory reference data for the respective fish taxa.

Statistical analysis

SPSS statistical software (version 22.0; SPSS Inc., USA) was used to analyze the results. Differences in biochemical parameters among fish collected from the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers were assessed using one-way analysis of variance (ANOVA), followed by an appropriate post hoc test for pairwise comparisons among rivers. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS

Overall, fish collected from the Sitnica River showed higher plasma glucose concentrations and AST and ALT activities, but lower plasma total protein concentrations, compared with fish collected from the Lumbardhi i Prizrenit and Lepenci Rivers. The results for each biochemical parameter are presented separately below.

Plasma glucose

Results of plasma glucose concentrations in *Barbus balcanicus* and Chondrostoma nasus from the Sitnica, Lumbardhi i Prizrenit, and Lepenci rivers are presented in Tables 1 and 2, respectively. The Sitnica River is one of the most polluted areas; therefore, the observed increase in plasma glucose values (197 mg/dl in *Barbus balcanicus* and 114 mg/dl in *Ch. nasus*) is likely a consequence of pollution-induced stress in fish from this river. At the Lumbardhi i Prizrenit and Lepenci River sampling sites, glucose levels were 152 mg/dl and 166 mg/dl in *Barbus balcanicus* and 61 mg/dl and 72 mg/dl in *Ch. nasus*, respectively, which were significantly lower than those observed in fish from the Sitnica River.

Total protein

Plasma total protein concentrations differed among the three rivers in both fish taxa, as presented in Tables 1 and 2. In both taxa, the lowest concentrations were recorded in fish collected from the Sitnica River. In *Barbus balcanicus*, plasma total protein concentrations were 3.87 g/dL in the Sitnica River, 4.95 g/dL in the Lumbardhi i Prizrenit River, and 4.38 g/dL in the Lepenci River. In *Chondrostoma nasus*, the corresponding concentrations were 4.87, 5.35, and 5.18 g/dL, respectively. (Figures 4, 5).

Liver enzyme activity (AST and ALT)

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are presented in Tables 1 and 2, and Figures 6-9. Fish from the Sitnica River showed significantly increased AST and ALT activity compared to fish from the Lepenci and Lumbardhi i Prizrenit Rivers ($p < 0.05$) (52.3 IU/L in *Barbus balcanicus* and 84 IU/L in *Ch. nasus* for AST). AST activity was consistently higher than ALT activity in fish from all sampling sites. No statistically significant differences in ALT and AST activity were observed between fish from the Lepenci and Lumbardhi i Prizrenit Rivers (38.6 IU/L and 42.0 IU/L in *Barbus balcanicus*, and 54.2 IU/L and 61.0 IU/L in *Ch. nasus*, respectively).

Table 1: Biochemical parameters analyzed in *Barbus balcanicus* from Sitnica, Lumbardhi i Prizrenit, and Lepenci rivers.

Parameter	Standard value	Sitnica (n=10)	Lumbardhi i Prizrenit (n=10)	Lepenci (n=10)
Plasma glucose (mg/dl)	185.27 ± 15.79	197 ± 13.15*	152 ± 10.12	166 ± 9.05
Total proteins (g/dl)	5.15 ± 0.91	3.87 ± 0.13*	4.95 ± 0.34	4.38 ± 0.25
AST (IU/L)	36.5 ± 5.2	52.3 ± 7.1*	38.6 ± 3.8	42.0 ± 4.2
ALT (IU/L)	18.4 ± 3.2	32.0 ± 5.1*	18.8 ± 2.43	21.3 ± 3.43

* Significant difference compared to standard/reference values ($p < 0.05$).

Table 2: Biochemical parameters analyzed in *Chondrostoma nasus* from Sitnica, Lumbardhi i Prizrenit, and Lepenci rivers.

Parameter	Standard value	Sitnica (n=10)	Lumbardhi i Prizrenit (n=10)	Lepenci (n=10)
Plasma glucose (mg/dl)	95 ± 2.3	114 ± 0.15*	61 ± 0.13	72 ± 0.12
Total proteins (g/dl)	6.70 ± 0.95	4.87 ± 0.13*	5.35 ± 0.34	5.18 ± 0.25
AST (IU/L)	52 ± 9.8	84 ± 5.11*	54.2 ± 4.3	61.0 ± 4.2
ALT (IU/L)	14.8 ± 3.9	42.6 ± 10.4*	16.2 ± 2.9	20.07 ± 3.8

* Significant difference compared to standard/reference values ($p < 0.05$).

Table 3: Physicochemical parameters of water samples from the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers.

Parameter	Unit	Reference limit	Sitnica river	Lepenci River	Lumbardhi i prizrenit river
pH	-	6.5–8.5	6.5–8.4	6.5–8.4	6.5–8.4
Temperature	°C	-	ND	ND	ND
Conductivity	µS/cm	-	ND	ND	ND
Turbidity	NTU	<5	ND	ND	ND
Nitrites (NO_2^-)	mg/L	0.1	Above reference limit	Near reference limit	Within reference limit
Ammonium (NH_3)	mg/L	0.5	Above reference limit	Near reference limit	Within reference limit

Sampling method: ISO 5667-5:2000. Ambient air temperature during sampling: 8–12 °C.

Heavy metal concentrations

Heavy metal concentrations measured in water samples from the Sitnica River are presented in Figure 2. The analysis included lead (Pb), zinc (Zn), cobalt (Co), copper (Cu), nickel (Ni), sodium (Na), potassium (K), and cadmium (Cd).

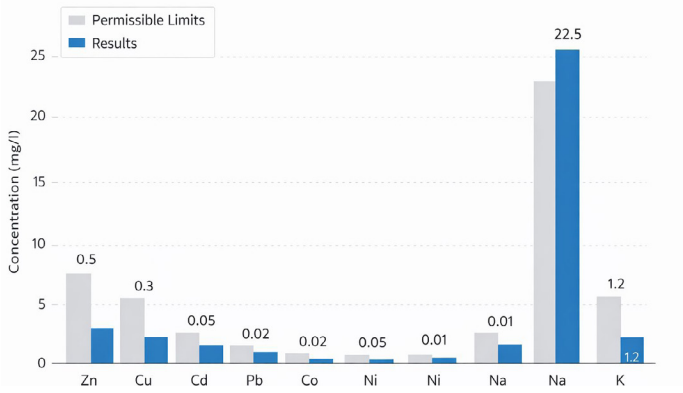

Figure 2: Heavy metal concentrations in water samples from the Sitnica River, determined using the APHA 3111B method.

The physicochemical parameters measured in water samples from the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers are presented in Table 3.

The available physicochemical data are presented in Table 3. The recorded pH values ranged from 6.5 to 8.4 in all three rivers. Nitrite and ammonia/ammonium levels were reported as being above the reference limits in the Sitnica River, near the reference limits in the Lepenci River, and within the reference limits in the Lumbardhi i Prizrenit River. Water temperature, conductivity, and turbidity values were not determined. Nitrite levels exceeding 0.1 mg/L are known to exert toxic effects on aquatic biocenosis, especially fish. These findings indicate early-stage pollution and possible microbiological degradation processes.

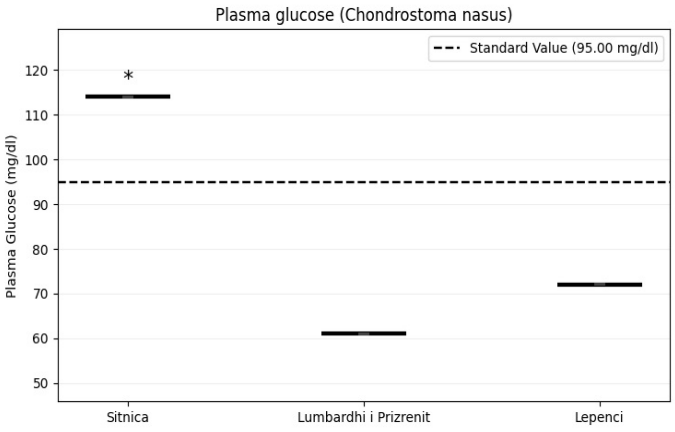

Figure 3: Plasma glucose analyzed in *Chondrostoma nasus*.

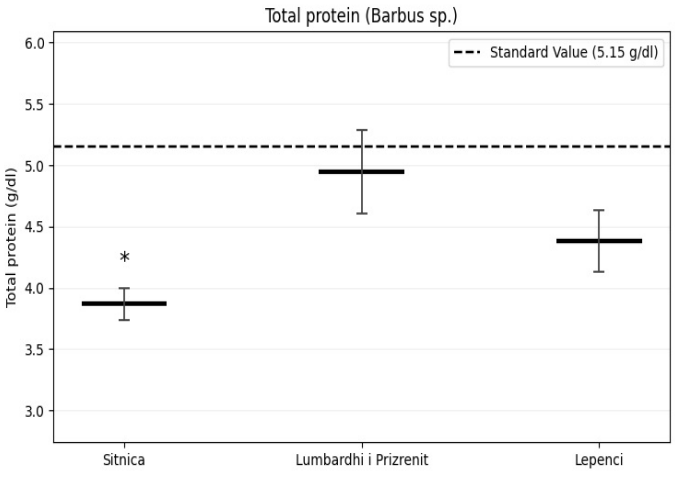

Figure 4. Plasma total protein concentration in *Barbus balcanicus*

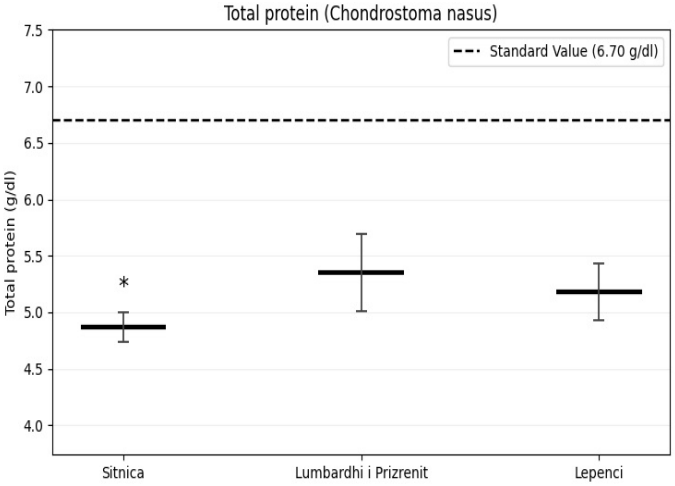

Figure 5. Plasma total protein analyzed in *Chondrostoma nasus.*

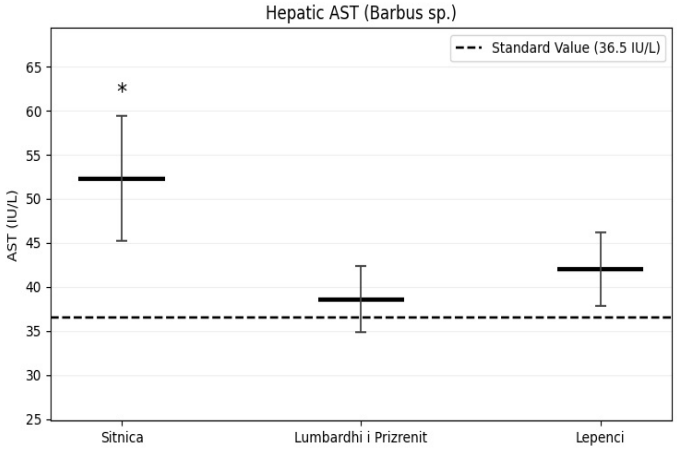

Figure 6. Plasma AST activity in *Barbus balcanicus*

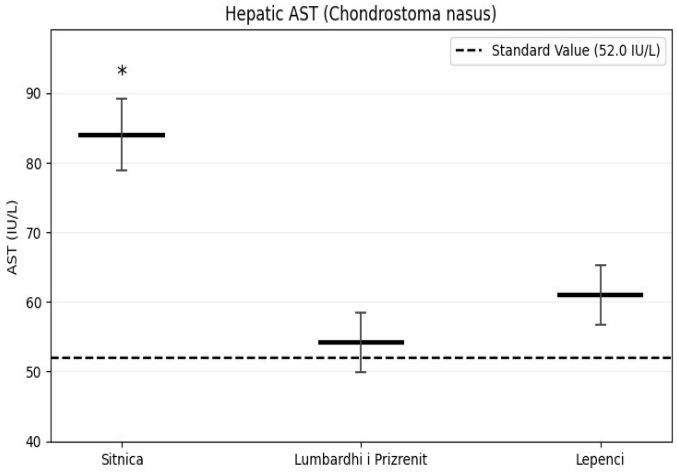

Figure 7. Plasma AST activity in *Chondrostoma nasus.*

The pH values observed (within 6.5–8.4) suggest a generally stable acid–base balance, although slight deviations may influence metal solubility and bioavailability, thereby indirectly affecting fish physiology.

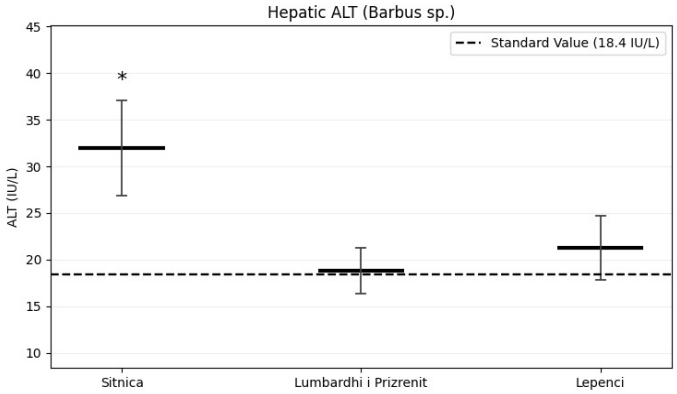

Figure 8. Plasma ALT activity in *Barbus balcanicus*

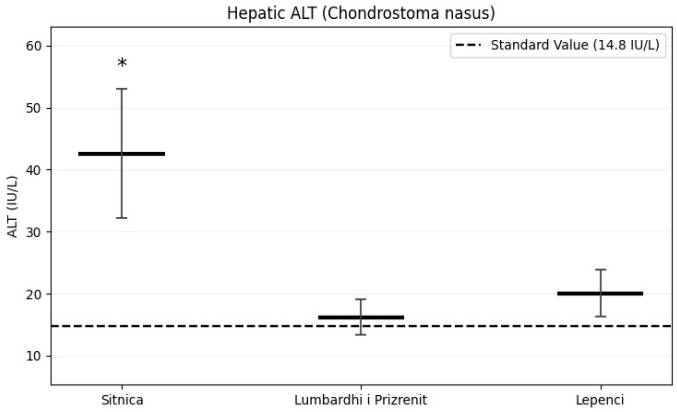

Figure 9. Plasma ALT activity in *Chondrostoma nasus.*

DISCUSSION

This study provides scientific explanations regarding the clear differences in physiological and biochemical biomarker responses of the fish species *Barbus balcanicus* and *Chondrostoma nasus* collected from rivers with different pollution profiles in Kosovo. Fish samples collected from the Sitnica River manifested significant changes in plasma glucose levels, total protein, and liver enzyme activities (ALT and AST) compared to the levels of these parameters from the Lepenci and Lumbardhi i Prizrenit Rivers, reflecting an increased level of metabolic and hepatic stress in correlation with the high level of environmental pollution.

Increased glucose values are correlated with the activation of the hypothalamic-pituitary-adrenal axis and the release of catecholamines, which activate the glycogenolysis process to compensate for the increased energy demands in stress conditions (Oudah, 2024; Zeng *et al*., 2025). Analogous results have also been shown in fish exposed to heavy metals and mixed pollutants, where it is accompanied by a process of hyperglycemia manifested as an adaptive reaction to maintain homeostasis in unfavorable environments (Van der Oost *et al*., 2003; Zahran *et al*., 2025).

The lower plasma total protein concentrations observed in

both fish taxa from the Sitnica River indicate an alteration in protein metabolism associated with environmental stress. Compared with fish from the Lepenci and Lumbardhi i Prizrenit rivers, fish from the Sitnica River showed consistently reduced total protein concentrations. This decrease may reflect disruption of protein synthesis and degradation pathways under pollutant-induced stress, including possible impairment of hepatic metabolic function. Previous studies have shown that exposure to heavy metals can alter protein metabolism by affecting hepatic protein synthesis and degradation processes (Jamil *et al*., 2023; Sharma *et al*., 2025).

Increased concentrations of alanine aminotransferase and aspartate aminotransferase activities in fish from the Sitnica River reflect liver and cell membrane degradation. The enzymes alanine aminotransferase and aspartate aminotransferase are well-known indicators of liver health and tissue denaturation in fish exposed to environmental pollutants. Hyperactivity of the enzymes alanine aminotransferase and aspartate aminotransferase correlates with increased permeability of hepatocyte membranes and the enormous release of these enzymes into the bloodstream after toxic exposure (Subotić *et al*., 2019; Oroian *et al*., 2025).

The hyperactivity of the enzymes alanine aminotransferase and aspartate aminotransferase (AST, ALT) reflected in this research is also in correlation with previous studies confirming that aspartate aminotransferase (AST) can serve as a biomarker with high sensitivity to systemic toxicity and multi-organ stress in fish (Van der Oost *et al*., 2003). High levels of heavy metal pollution have also affected fish from the Sitnica River, causing significant changes in biochemical and physiological biomarkers. Heavy metals such as lead, cadmium, nickel and copper bioaccumulate in fish tissues, manifesting oxidative stress reflecting reactive oxygen species reactions, thus degrading subcellular structures and metabolic functions (Panda *et al*., 2023; Pargi *et al*., 2024).

The present findings are consistent with previous studies conducted in Kosovo, particularly those reported by Morina *et al*. (2013a), who demonstrated that oxidative stress biomarkers in fish are significantly elevated in polluted sections of the Sitnica River. Similarly, Morina *et al*. (2013a) reported clear physiological alterations in fish exposed to contaminated waters, supporting the reliability of fish as bioindicators of environmental stress. Comparable results were also reported in regional freshwater systems (Morina *et al*., 2013b), where biochemical biomarkers showed sensitivity to varying pollution gradients, confirming that the observed values in this study reflect significant ecological stress rather than natural variability.

The elevated heavy metal concentrations detected in water samples from the Sitnica River may have contributed to the significant alterations observed in the biochemical and physiological biomarkers of fish from this river. Metals such as lead, cadmium, nickel, and copper are known to induce oxidative stress through the generation of reactive oxygen species, potentially disrupting cellular structures and metabolic functions in exposed fish (Panda *et al*., 2023; Pargi *et al*., 2024). Heavy metals can also cause biochemical dysfunctions by affecting the cumulative stress pathway in correlation with pollution in aquatic organisms.

The results of this study further indicate a clear relationship between physicochemical parameters, heavy metal concentrations, and fish physiological responses. The Sitnica River, which exhibited the highest levels of nitrites and ammonium together with elevated heavy metal concentrations, also showed the most pronounced alterations in biochemical biomarkers. Elevated plasma glucose levels in fish from the Sitnica River suggest a stress-induced metabolic response, while increased AST and ALT activities indicate hepatic stress and possible tissue damage, consistent with exposure to environmental pollutants. In contrast, fish from the Lepenci and Lumbardhi i Prizrenit rivers, where physicochemical parameters were closer to normal ranges and heavy metal concentrations were lower, exhibited significantly reduced biochemical alterations. This pattern supports a functional correlation in which increased NO_2^- and NH_3 are associated with physiological stress and impaired oxygen transport, elevated heavy metals with liver damage and enzyme activation, and combined exposure with an amplified biochemical response. Although causality cannot be definitively established, the consistent gradient observed across the three rivers strongly supports an ecological linkage between environmental quality and fish health status.

The elevated levels of nitrites and ammonium detected in this study further support the observed biochemical alterations. Nitrogen compounds such as NO_2^- and NH_3 are well-known stressors that disrupt oxygen transport and metabolic processes in fish. Their presence, particularly in the Sitnica River, likely contributes to the increased glucose and enzyme activity observed. Although most physicochemical parameters were within acceptable limits, their interaction with heavy metals and organic pollutants may produce synergistic toxic effects, amplifying physiological stress responses.

The largely stable relationships of the biochemical and physiological biomarker profiles analyzed in fish from the Lepenci and Lumbardhi i Prizrenit Rivers reflect reduced concentrations of environmental stress and at

the same time a healthier environment in the ecological aspect of these rivers. This reflection provides a more confirmatory overview of the application of biochemical and physiological biomarkers to evaluate the impacts of pollution, supporting the idea of bio-environmental assessment using the fish species *B. balcanicus* and *Ch. nasus* as sensitive bioindicator models in the freshwater biocenosis.

Overall, the results of this study are in line with other studies that confirm the importance of biochemical and physiological biomarkers in detecting sublethal effects of water pollution much earlier than escalating to the final stage of ecological biodegradation (El-Agri *et al*., 2022; Adams *et al*., 2025). The metabolic and hepatic biomarker assessment platform enables a multidimensional overview of the health assessment of the aquatic biocenosis and indicates a relevant ecological indicator of river waters at the national and wider levels.

CONCLUSION AND RECOMMENDATIONS

This study reflects scientific findings that freshwater fish that biocenose in rivers with different pollution profiles in Kosovo manifest significant physiological and biochemical reactions. Significant alternations in plasma glucose levels, total protein, alanine aminotransferase, and aspartate aminotransferase in *Barbus balcanicus* and *Chondrostoma nasus* from the Sitnica River manifest hyperactivity of metabolic and hepatic stress compared to fish from the Lepenci and Lumbardhi i Prizrenit Rivers. These alterations were associated with higher heavy metal concentrations and indicate physiological stress in fish exposed to poorer environmental conditions. Additionally, with elevated concentrations of nitrites and ammonium, particularly in the Sitnica River, indicating the combined effect of physicochemical and chemical stressors on the health of the aquatic biocenose. These results confirm that target biochemical and physiological biomarkers are adequate mechanisms for assessing reactions to sublethal stress in freshwater biocenoses. Also, from this research it can be concluded that *Barbus balcanicus* and *Ch. nasus* can be applied as effective bioindicator targets for monitoring environmental pollution as well as assessing the degradation of aquatic ecosystems, which can indirectly affect human health through the trophic chain.

It is recommended to increase and advance the methods and dynamics of the water quality monitoring process in the Sitnica, Lepenci, and Lumbardhi i Prizrenit Rivers, especially fish as an adequate bioindicator target. These studies should be expanded to other multitask research such as metabolomics, proteomics, and then genetic and histopathological analyses, which would provide a multidimensional understanding of stress mechanisms associated with environmental pollution. In addition, more integrated studies of environmental management and bioremediation are recommended to improve the condition and safety of freshwater ecosystems and their biota.

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AUTHOR'S CONTRIBUTION

The first author, RF has developed the study concept, field sample collection, laboratory analyses, and evaluation of the results. Co-author Mohamed Fawzy Ramadan has also contributed to the interpretation of data and critical evaluation of the paper. While co-author Qenan Maxhuni has given relevant contributions to the design of the study plan, statistical analysis of the results, manuscript review, and its final evaluation. All authors have approved the final version of this original scientific article.

Ethical approval

The experimental procedures carried out during this scientific research on fish were carried out in accordance with the appropriate international protocols for animal experimentation during scientific research. These study protocols were also reviewed and approved by the relevant institutional ethics committees. During the experiments, all conditions were provided to minimize animal stress in the experimental chain during capture, handling, and extraction of samples.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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