Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 9(1): 200-219 (2025) (http://www.wildlife-biodiversity.com/)

Research Article

Physiological and biochemical parameters in two fish species (*Scardinius erythrophthalmus* and *Cyprinus carpio*) in different rivers of Kosovo

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Received: 25 September 2024 / Revised: 18 November 2024 / Accepted: 31 December 2024/ Published online: 06 January 2025.

How to cite: Ferizi, R., Ramadan, M.F., Karataş, A., Maxhuni, Q. (2025). Physiological and biochemical parameters in two fish species (*Scardinius erythrophthalmus* and *Cyprinus carpio*) in different rivers of Kosovo, Journal of Wildlife and Biodiversity, 9(1), 200-219 **DOI**: https://doi.org/10.5281/zenodo.14634913

Abstract

The aim of this research was to analyze physiological and biochemical parameters in two fish species (Scardinius erythrophthalmus and Cyprinus carpio) taken in three different rivers of Kosovo. Fish samples were applying the electrofishing method (according to Hans Grassl GmbH) alongside the Sitnica, Lepenci, and Lumbardhi i Prizrenit rivers. The biochemical parameters—plasma glucose (GLU), total protein (TP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)—were analyzed in the blood samples of 135 fish of both species. The research results showed a significant increase in GLU concentration and a highly significant increase in the AST and ALT levels ($P < 0.05$), while a significant decrease in TP concentration ($P < 0.05$) was observed in two fish species taken in the Sitnica River, compared with the fish species taken in the Lepenci and Lumbardhi i Prizrenit rivers. Due to their rapid reactions and great sensitivity to changes in the aquatic environment, fish continue to be key bioindicators that play a crucial role in monitoring pollution in the aquatic ecosystem. These findings highlight the need to improve the dynamics and techniques of monitoring water pollution in the Sitnica, Lumbardhi i Prizrenit, and Lepenci rivers as well as their biota, particularly fish as bioindicator organisms.

Keywords: Fish, biochemical metabolite, GLU, TP, AST, ALT

Introduction

Along with many other resources, Kosovo holds promise for the fishing industry. Fishing is one of the most promising economic development areas due to the abundance of rivers and favorable environmental conditions. However, the Drini i Bardh, Lumbardhi i Pejes, Lumbardhi i Prizrenit, Lepenci, Brodi, Restelica and other rivers with a lesser water capacity are viable rivers for fish farming in the nation. Fishes are still a very dependable part of a system for monitoring aquatic life.

Rivers and river basins

The hydrography of Kosovo's watercourses is divided into four river basins: Drini i Bardh, Ibri, Morava e Binça, and Lepenci. Kosovo's rivers flow into three marine catchments: the Black Sea, the Adriatic Sea, and the Aegean Sea. The main rivers belonging to the Black Sea catchment, are: Ibri, Sitnica with branches; (Llapi, Drenica), and Morava e Binça. The Adriatic Sea includes: Drini i Bardh with its tributaries (Lumbardhi i Pejes, Lumbardhi i Deçanit, Lumbardhi i Prizrenit, Klina, Ereniku, Mirusha, Toplluha and Plava). While the Lepenci river with its main branch (Nerodime) belongs to the Aegean Sea. The watershed flows in different directions. The flow coefficient ranges from 3.93 l/sec/km^2 (Morava e Binça) to 42.46 l/sec/km^2 (Lumbardhi i Deçanit) (Table 1) **(**Hydrometeorological Institute of Kosovo, 2024**)**

Table 1. Data on the maximum, minimum and average values of annual water flow (Q = m^3/s) at several stations of the three rivers.

Name	Length (km) in Kosovo	Surface m^2	Flow direction
Sitnica	90 km	2.873 m^2	Black sea
Lepenci	53 km	679.0 m^2	Aegean Sea
Lumbardhi i Prizrenit	31 km	262.6 m^2	Adriatic Sea

Kosovo has significant sources of thermal water that are used for recreational and health purposes. The protection, conservation, and development of water resources are very important and one of the biggest environmental challenges in Kosovo.

Kosovo stands out for its rich biodiversity, geographical position, geological, pedological, hydrological factors, relief, and climate are some of the factors that have enabled Kosovo to have a rich biological diversity and landscape with a good variety of flora, vegetation, and fauna, where it is worth emphasizing the presence of species relicts, endemics, and species of special importance. In addition to many other resources, it is considered that Kosovo may have a prospective potential in terms of fish resources and suitable fishing conditions, as one of the promising sectors in the field of development.

The territory of Sharri is characterized by the flows of small streams and Mountain rivers, which meet the Lepenci river, flowing into the Aegean Sea, and the Lumbardhi (Bistrica) e Prizrenit, flowing into the Adriatic Sea. Based on current knowledge, the following types of fish also live in these rivers: river trout (*Salmo trutta* m. *fario* L.), common barbel (*Barbus barbus*), northern pike (*Esox lucius*), perch (*Perca fluviatilis*), etc. Fish continue to be bioindicators, which play an important role in monitoring pollution in the aquatic ecosystem, due to their effective response and high sensitivity to changes in the aquatic environment. The sudden death of fish as an aquatic species indicates the state of severe pollution in the respective waters; the effects of exposure to sub-lethal (non-lethal) levels of pollutants can be measured through biochemical and physiological parameters of fish behavior, and response. Fish are therefore very good biosensors for aquatic pollutants. The common rudd *Scardinius erythrophthalmus*, mainly living in brackish waters, belong to the family Cyprinidae and are permanent residents in most countries of Europe and Western Asia. *S. erythrophthalmus* is essentially a small fish, no more than about 30 cm in length. The body of this fish is blue-silver in color, with a white belly and a red tail. *Scardinius erythrophthalmus* is a species of freshwater fish in the family Cyprinidae (Yaqoob, 2021). Rudds typically have a slender and laterally compressed body. The size of rudd can vary, but in general they are smaller than carp. Adult rudds can reach an average length of 25 to 30 cm (10 to 12 inches). Rudd fish come in a variety of colors, although their body sides are usually golden to yellow-green. The species name "erythrophthalmus" comes from its dark red fins and often red or orange eyes. Ruddfish's dorsal fin is closer to the tail than to the head. The caudal fin is bifurcated, and the anal fin is relatively large. Rudd fish are covered with scales that are usually cycloid (smooth-edged), like most other fish. The rudd fish has a lip at the end of its beak, or terminal mouth. (Yaqoob, 2021). Rudd fish have teeth in their neck called pharyngeal teeth that they use to swallow food. Rudd fish have large eyes on both sides of the head. On both sides of the fish there is an operculum, a bony plate that covers the gills. Rudd fish, like other fish, have lateral lines, a group of sensory organs on the sides of their bodies that aid their ability to sense water vibrations. Although their normal environment is freshwater, they can survive in many bodies of water, including lakes, ponds, rivers, and reservoirs. Although they can thrive in some salinities, they are not limited to brackish water. (Yaqoob, 2021). Common rudd is widespread and can be found in many countries in Western Asia and Europe. They are very common in freshwater environments and are often associated with calm or slow flowing water. Common rudd can tolerate a wide range of environmental conditions, although they prefer freshwater environments.

Carp live in a variety of freshwater environments, including lakes, ponds, rivers, and reservoirs. They can thrive in different water conditions. Carp have a robust body with large scales. The color can vary, but is typically brownish or bronze. The eyes are relatively large, and the mouth is surrounded by four barbels (beard-like sensory organs). Carp can reach considerable size, with individuals over 22.7 kilograms (50 pounds) not uncommon. Carp are omnivores and feed on a variety of foods, including aquatic plants, insects, crustaceans, and small fish (Yaqoob, 2021). Carp reproduce by spawning, typically in spring or early summer. They lay sticky eggs on submerged vegetation, and these eggs hatch into young fish, called brood fish. In some regions, the introduction of carp has had negative ecological impacts. Carp are known to disrupt aquatic ecosystems by uprooting plants and stirring up sediment as they feed, which can reduce water clarity and impact other aquatic organisms. Carp are an important species in aquaculture. It is farmed for its meat in many countries and is also popular with anglers for sport fishing.

During the last decades, the changes in the water quality of the rivers have negatively affected many species of fish, while they have favored the survival of the carp species. Due to their adaptability and high ecological value, carp have been very successful in colonizing new environments. In order to maintain fish productivity, care must be taken against diseases, stress, and environmental pollutants. Stress, diet, and pathogenic activities can all have an impact on fish health. A number of abiotic environmental conditions, including variations in water temperature, pH, oxygen concentration, and water contaminants including herbicides and insecticides, petroleum products, and heavy metals, can put fish under stress (Chowdhury & Saikia, 2020). Metal toxicity severely causes the reduction and alteration of white blood cells, especially lymphocytes, causing degradation of the immune response in fish. The identification of molecular biomarkers, related to the prediction in early stages, diagnosis, and monitoring of major physiological changes, as well as the impacts caused by pollution, can contribute towards the conservation (in situ) of fish populations. As a priority, biomarkers, for a short time, show the result of biological effects, which with other methods can be observed only after a long time (Colin et al., 2016).

Numerous studies have focused on the results of hematological and biochemical parameters in Carp fish under normal conditions, compared to the results when exposed to stressors. Biomarkers provide biological responses to environmental chemicals (contaminants) from the molecular level, when changes from standard values are reflected. Biomarker responses result from the molecular level, the cellular level, and the whole organism. The importance of the application of biomarkers is important because they represent measurements of biological effects, which are related to the presence of environmental chemical micro-levels; they also provide insight into the interpretation and influence of environmental pollutants on biological effects (Vineis *et al*., 2020). Environmental biological responses are considered as pyramidal bioindicators from the organismal, population, community and ecosystem levels.

Fish infections, some aspects of the disease, also affect the economy, reducing the quality and productivity of fish and, on the other hand, increasing mortality (Assefa & Abunna, 2018). As a consequence of this, the efficiency of their metabolism toward food is reduced due to the great denaturation of amino acids, which are related to the synthesis of proteins, and therefore, the quality of their meat is degraded. The research results related to the biochemical and physiological parameters in the blood of fish are in line with our analysis, where the diagnostic value of the metabolism from the correlation is emphasized, including the integrity of the blood system related to liver function (Zeng *et al*., 2016).

Our research consists of analyzing the physiological and biochemical parameters in the blood of fish. The fish were taken during the months of March-July 2021, with electrofishing equipment (according to Hans Grassl GmbH) in the Sitnica, Lumbardhi i Prizrenit and Lepenci rivers. The results are of the biological parameters realized in two fish species: *Scardinius erythrophthalmus* and *Cyprinus carpio*, as a result of which the program is necessary to carry out the monitoring of contamination (with emphasis on all aquatic biota) from a number of pollutants (such as metals, PCBs, and pesticides) for monitoring their concentration in major rivers and lakes. Until now, individual projects and aquatic biota monitoring programs have focused only on the characteristics of specific parameters, becoming other potential elements at the molecular level that can have a multifactorial effect on the environment and man. The modern monitoring program of aquatic biota, to be multi-dimensional, should be included in a complex battery of biomarkers in the biocenosis of a large ecosystem. In this sense, fish, as bioindicators, best reflect the modifications and eventual changes of vital functions, such as the effects of contamination. Various organisms in rivers and lakes can be used as bioindicators. This includes fish, shellfish, and molluscs, as well as aquatic animals and plants, algae, macrophytes, and macroinvertebrates.

Fish as biomarker organisms

The advantages of applying bioindicators in relevant research are:

- Provides direct and rapid insight into eco-physiological and biochemical effects, including active and passive biomonitoring.
- Analysis of antagonistic and synergistic, multifactorial effects in the biocenosis of aquatic ecosystems
- High sensitivity, related to the escalation of negative effects on biota.
- Active biomonitoring includes methods that were applied by placing organisms under controlled stress conditions and then analyzing the effects that are reflected in living organisms.
- Easy application method, also economical and effective.

Biological biomarkers

So far, there are several definitions for the term 'biomarker'. These potential definitions focus on a measurement context (experimentation) that reflects the interaction between a biological system and a hazard, which may be chemical, physical, or biological. Biomarkers are also reflected as a biological reaction (from the molecular-cellular and physiological levels) related to exposure to or response to the effects of environmental pollutants. On the other hand, some scientists have redefined the terms biomarker-bioindicator and ecological factors, as their interactions at different levels of biological organization (Schmitz, *et al*., 2016).

Fish, as a bio-accumulative marker, can be applied to demonstrate the effects of environmental contaminants and the biota's response to them, as well as bio-concentrators to mark individual micro-substances that have an impact on aquatic organisms in small amounts of water. For this reason, it is practically impossible to determine the nature of xenobiotic substances by applying only simple models because the complexity of bioaccumulation is evaluated, including toxico-kinetics, metabolism, accumulating substances, biota, specific bioaccumulating, and residual organs (sediments). Since we cannot choose the right levels of bioaccumulation organization, even with their sophisticated model.

Most of the bioaccumulative markers in the fish organism are assimilated and denatured by persistent organic contaminants, such as polychlorinated biphenols (PCBs), polychlorinated dibenzofurans (PCDD), and dibenzop-dioxins (PCDF), and further still by easily biodegradable compounds as well as polycyclic aromatic hydrocarbons (PAH). Despite these degrading and contaminating compounds, chlorophenols do not have the affinity to accumulate in fish tissues at that exposure-influential level. Recently, devices have been successfully applied through which the processes of bioaccumulation of hydrophobic organic substances can be imitated in aquatic organisms, such as semi-permeable membranes (SPMDs) (Narváez *et al*., 2020). The term biomarker consists of a biological variation of the organism (at the molecular, biochemical, physiological, structural, or behavioral level) or reflection and response to different chemicals present in aquatic ecosystems.

According to research, the "battery" of biological biomarkers that is applied during biomonitoring includes biochemical and hematological biomarkers, as well as heavy metal effects.

Biochemical biomarkers

Biochemical biomarkers of pollution are indices of capability that were applied in fish toxicity tests as well as for field monitoring of water pollution. Biochemical biomarkers confirm the contact pattern of chemical compounds and clarify further metabolic dynamics (Fig. 1).

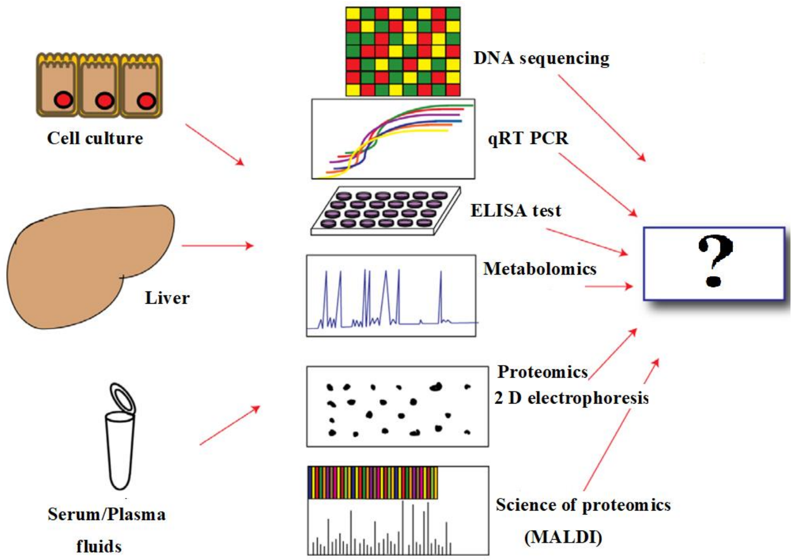

Figure 1. Biological biomarkers.

The liver (hepar) plays a key role in xenobiotic detoxification, as many xenobiotics penetrate the body, such as lipophilic ones, which have an affinity for lipids, etc. The polarity of xenobiotic growth is conveyed within two phases of metabolism: firstly, through oxido-reduction, and secondly, through the hydrolysis reaction and production of metabolites (Narváez *et al*., 2020).

The response of the biomarkers depends on many things, including those of the chemicals, the presence of other compounds, the duration of exposure, the temperature of the water, etc. A potential pollution of the aquatic environment is also the use of pesticides for the protection of agricultural crops, which is why, in the last decade, there has been no case reported of the poisoning of fish by means of pesticides. However, non-target organisms are exposed to pesticides, with many pesticide compounds being detected in surface waters (Nanusha *et al*., 2020). Other contemporary research has shown different possible accountings of protein composition and high levels of cortisol, glucose, and cholesterol in fish blood serum. There is also a lot of enzymatic activity depending on the type of contaminants, the fish, the quality of the water, and the duration of exposure.

Plasma glucose (Glu)

Glucose is a biochemical and metabolic component of activity, and as plasma glucose, it makes a constant and effective resource for keeping it continuously necessary throughout the work of the muscular system and the general organization. The amount of glucose in the blood varies very quickly, especially due to the influence of some sugar substances in the blood or outside. This aspect reflects the role of the components as a biochemical indicator, i.e., as a reference for the general physiological and metabolic state of the organization (Witeska *et al*., 2020).

Keeping the glycemic balance within limits is suppressed by a complex of mechanisms that maintain the homeostatic rule. In this program are, among others, the hepato-pancreas, some extra-hepatic tissues, and a series of endocrine glands. The determination and improvement of serum glycemia in high fish are referred to more quickly and efficiently, among other mechanisms of the state of stress.

All glycemic values in fish need to be considered during sampling and blood extraction because biochemical parameters, such as plasma glucose, can be sensitive in cases of functional physiological or pathogenic changes in their organization (Shahjahan *et al*., 2022). At the initial beginning of the action of the pathogenic agents, the fish did not show symptoms of the disease. The organism activated its defense mechanisms, reviewing its reserves to escape and fight the bacterial agents. Thus, glucose, being released from the tissues, began to function and pass into the peripheral blood, and then the amount of glucose was measured with quantitative methods of dosing. The rapid decrease in the amount of glycogen in the muscles and in the liver of fish serves as a bioindicator of the toxicity of the fish organization (Beghin *et al*., 2022). In a hypoxic state, the fish reaches the energy of glucose, which is present in the cellular storage, through the anaerobic metabolic line (pathway) of glycogenolysis. The increased demand for these molecules as a reserve and source of energy is sufficient in different cases due to different influences.

Total protein (PT)

The physiological and enzymatic suitability of fish has an organizational value in their diagnostics, which are manifested as a consequence of the contamination of the aquatic ecosystem. To monitor the reaction and response aspects of fish stress, blood serum parameters such as glucose, total proteins, or even ALT, AST, cortisol, etc. were applied as biomarkers (Moss *et al*., 2008). The weight that changes blood values in its protein values, the different species, and the variation of fish make it necessary to determine the total proteins in the serum and their components.

Blood proteins are associated with blood; their clinical importance has been considered to be important in fish as well as breast and human cells. It is considered that Lepkowski was the first to start the study of proteins in the blood serum of fish (Ravenscroft *et al*., 2008). After him, other researchers analyzed information about changes and blood serum proteins in different types of fish through different methods of fractionation of serum proteins by means of proteins.

This is one of the most commonly applied techniques related to the relevant substances (cellulose acetate, agarose gel, and polyacrylamide gel). The hyperactivity of protease enzymes increases proteolysis, resulting in an increase in amino acid synthesis. Free amino acids also act as precursors for energy and for the synthesis of necessary proteins, as a protective mechanism against pollution stress (Di Domenico *et al*., 2022).

Hepatic enzymes ALT and AST

Hepatic enzyme biomarkers (alanine transaminase-ALT, aspartate transaminase-AST, and alkaline phosphatase-ALP) are important to reflect the biochemical and physiological changes of the organization, especially the functions of the liver (Donkor *et al*., 2022). Everything, including the role of biochemical biomarkers and the special proteins of the blood serum such as albumin, ceruloplasmin, and transferrin, which are considered non-enzymatic antioxidants, in the interaction with metals and their metabolism (distribution) in the tissues, is being analyzed. Plasma ALT and AST concentrations in fish exposed to stress were compared to those in the control group in the natural environment. However, the activity of AST is clearly increased compared to ALT (Dong *et al*., 2022). Also, throughout the study, there was an increase in the activity of AST and ALT in the cytoplasm and an increase in the activity of ALT in the mitochondria after exposure to stress because the body's cells compensated as a result of the defense mechanism by increasing the activity of the metabolism. Therefore, when fish face a degradation of the liver, ALT and AST concentrations will increase due to their role in synthesizing and compensating amino acids, which are necessary for the body. As physiological changes can result in increased energy demand or malnutrition. Heavy metals, present in the flowing waters of rivers, damage the hepatocytes of the liver, which leads to the improvement of transnases in the serum (Tlenshieva *et al*., 2022). Research has shown that AST and ALT may remain good biomarkers of cellular damage, protein degradation, and liver damage.

Material and methods

The determination of total proteins (TP), plasma glucose (GLU), and transaminases (AST and ALT) was conducted on 135 fish samples belonging to the species Scardinius erythrophthalmus and Cyprinus carpio. These samples were collected using electrofishing methods from the Sitnica, Lumbardhi i Prizrenit, and Lepenci rivers. The analyses were performed at the Institute of Clinical Biochemistry at the University Center of Kosovo and the Faculty of Life Sciences, University of Vienna, Austria.

Sampling stations

Sampling site S1, which is the closest to the source of the Sitnica River, is located in the village of Lumi i Madh in the Vushtri City. Sampling site S2 is in Mitrovica, where the Sitnica River flows a lot and where the waste from the battery factory was discharged. Therefore, this part and the line where the Sitnica river passes through the villages of Lumi i Madh are characterized as contaminated areas. The sampling sites were in Brezovica (L1) and Brod (L2) on the Lepenc River, as well as Mushnikova (LP1) and Reçan (LP2) on the Lumbardhi i Prizrenit River (Fig. 2).

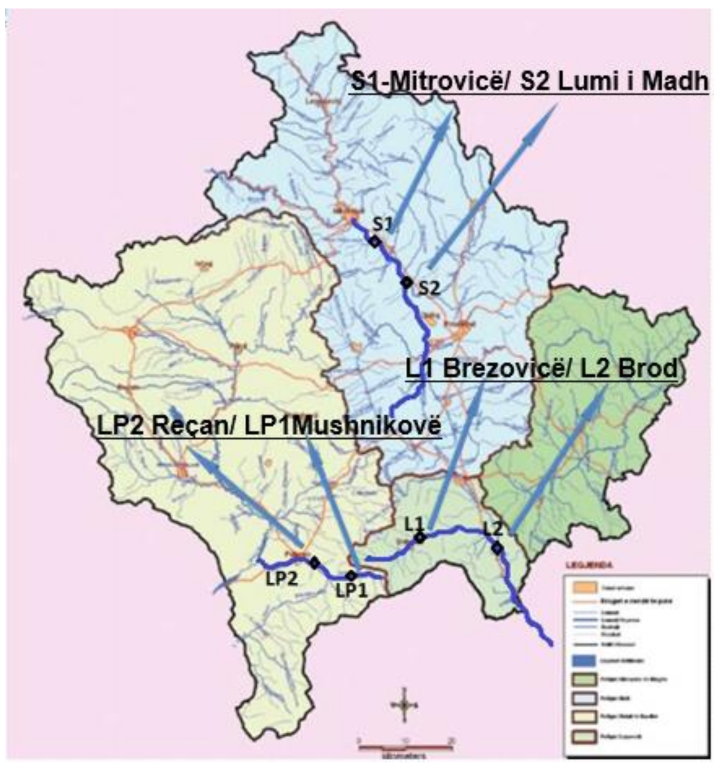

Figure 2. Map of sample sites.

Fish sampling

Fish samples were collected using electrofishing equipment (Hans Grassl GmbH) from three points in each river, with 45 samples taken per river, resulting in a total of 135 fish samples (n = 135)..

Biochemical and heavy metals analysis

For the realization of the study, the analysis of the samples was carried out at:

- Institute of Clinical Biochemistry, University Clinical Center of Kosovo, Prishtina,
- National Public Health Institution of Kosovo, Pristina,
- Department of Microbiology, Immunobiology & Genetics, University of Vienna, Austria.

Biochemical analysis procedure

The fish brought to the first laboratory are acclimatized for less than 24 hours to see the effect of stress after taking them out of the water and transporting them. Initially, the fish were treated with anesthesia, which is used as an anesthetic for animals (ether). Then 1 mL of blood was taken from the caudal vein of the fish with an adequate sterile ethylene di-amide tetraacetic acid (EDTA) syringe with a hypodermic needle 0.8 x 38 mm. Next, the blood serum is centrifuged at a speed of 5000 rpm per minute. Then the serum is taken and transferred, with the help of automatic tubes, to an Eppendorf tube to be applied for the improvement of biochemical parameters. Compared to other methods, the practice of taking blood through the caudal vein from the vena ductus Cuvieri provides better and more effective functions. This is safer and easier to apply, as well as, for a short time, to get the necessary volume of blood.

Applied methods

The analysis of fish blood samples was carried out according to the following methods:

The determination and concentration of PT were analyzed according to Integra Biuret measures.

- GLU was performed through the biochemical analyzer, Cobas 400 (Roche), according to the enzymatic measures of glucose oxidase.
- While AST and ALT were analyzed by enzymatic-colorimetric analysis according to the spectrophotometer model: Cobas Integra 400 (*Roche*).
- While the heavy metal was analyzed according to the APHA method.

Based on our results, there are some changes ($p<0.05$) in biochemical parameters in the river in the levels of total proteins (TP), glucose (Glu), alanine hepatic transaminases (ALT), and aspartate hepatic transaminases (AST) compared to the Lumbardhi i Prizrenit, and Lepenci rivers.

Results

Bioactivity of metabolites

Plasma glucose

The results of analyses (plasma glucose) in the blood serum of the fish *Scardinius erythrophthalmus* and *Cyprinus carpio* from the Sitnica, Lumbardhi i Prizren and Lepenci rivers (Table 2).

Table 2. Levels of Plasma glucose (Glu) analyzed in two species of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio* in the Sitnica, Lumbardhi, Prizren, and Lepenci rivers.

Sampling station	Number and type of fish (*Scardinius erythrophthalmus* and *Cyprinus carpio*)	Plasma glucose (mg/dl)
Standard values		75 ± 2.3
Sitnica	S1 (n=45)	92 ± 0.13*
Lumbardhi i Prizrenit	S2 (n=45)	86 ± 0.14
Lepenci	S2 (n=45)	80 ± 0.12

The Sitnica River is a most polluted area, so it can be indicated that the increase in plasma glucose value is a consequence of the effect of pollution stress on the fish of this river. Whereas, in sampling site S2, the Lumbardhi i Prizrenit and Lepenci rivers (S3), the amount of glucose level resulted in a value of 86 mg/dl, while in sampling site S3, the Lepenci River, the amount of glucose resulted in a value of 80 mg/dl, compared to the Sitnica River (Fig. 3).

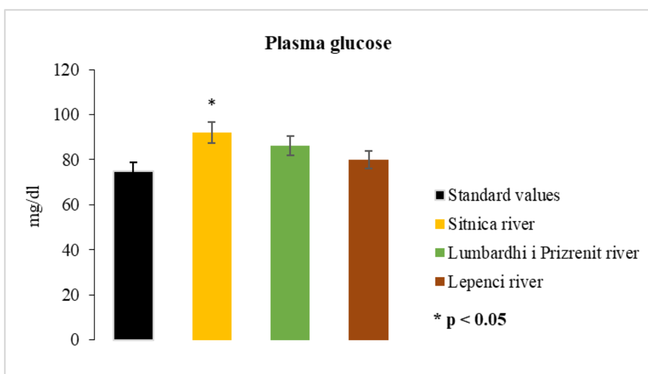

Figure 3. Plasma glucose analyzed in two species of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio.*

It is considered that this immediate increase in plasma glucose in Sitnica River fish is a non-specific response to pollution stress. Although the mechanism of this response in fish is not yet clear, it is thought to be related to the activation of the hypothalamus-sympathetic system-chromaffin cell act.

Also, our results are in correlation with the reflectance, or its amount, in the blood, which is a biosensor of environmental stress, changing glucose in carbohydrate metabolism under stress conditions. The amount of plasma glucose that comes into their blood in stressful situations is expected because its metabolism is related to the secretion of corticosteroid hormones and catecholamines from the adrenal gland. The mouths of rivers, water droplets, the amount of water, the flow of metabolic activity, and as a result that of glucose, also increase.

The different changes in plasma glucose in the Sitnica River, compared to the other two rivers, Lumbardhi i Prizrenit and Lepenci, interfered with the values of heavy metal ions: cadmium (Cd), which was present in the highest values, and lead (Pb), which showed increasing values. This condition is also argued with the experiments in fish treated for 7 days with the metal cadmium at a concentration of 0.5-2.00 ppm as well as with copper at a concentration of 0.2-0.5 ppm, resulting in an increase in the level of plasma glucose, compared to the control group.

Total protein (PT)

Concentrations of total proteins in the blood serum of fish *Scardinius erythrophthalmus* and *Cyprinus carpio* in the rivers Sitnica, Lumbardhi i Prizrenit and Lepenci showed a reduction effect compared to standard values (Table 3).

Table 3. Levels of Total proteins (PT), analyzed in two species of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio*, in the rivers Sitnica, Lumbardhi, Prizren, and Lepenci.

Sampling site	Number and type of fish (*Scardinius erythrophthalmus* and *Cyprinus carpio*)	Total protein (g/dl)
Standard value		3.93 ± 1.8
Sitnica	S1 (n=45)	2.33 ± 0.12*
Lumbardhi Prizrenit	S2 (n=45)	2.82 ± 0.36
Lepenci	S2 (n=45)	2.74 ± 0.26

The amount of total proteins in the blood plasma of Sitnica River fish shows a decrease ($p<0.05$) compared to the standard values. Sitnica is known as the most polluted area, and the results showed that the total protein level in this river was 2.33 g/dl. The decrease in the value of total proteins comes as an impact of the action of multifactorial stress on the fish of the Sitnica river, since pollution of different types is found in this river. On-site sampling in the Lumbardhi i Prizrenit river showed that the number of total proteins was closer to the normal values at 2.82 g/dl, while from the results of the on-site sampling in the Lepenc river, a total protein value of 2.74 g/dl (Fig. 4).

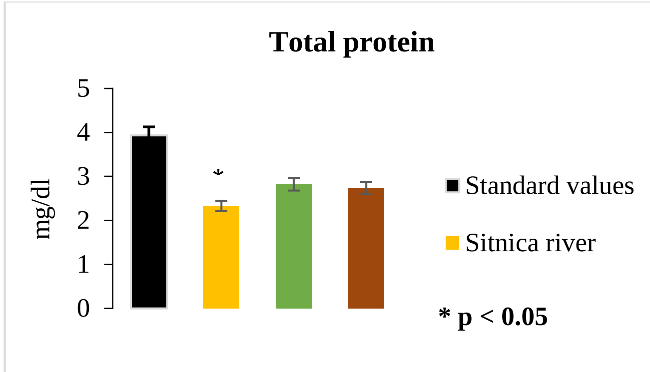

Figure 4. Total proteins analyzed in two types of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio.*

Since the Sitnica contamination includes perennial pollution, the fish found in it represent exposure to mixed heavy metal pollution. We consider that the bioaccumulation of heavy metals has induced oxidative stress in the cell through the production of micro-reactive radicals (ROS). The protective proteins counteract the toxic radicals, which leads to the elimination of the proteins from the liver cells. The decrease in the total protein level reflects protein synthesis and indicates the change in blood osmolarity. The values of total proteins in the plasma or blood serum of fish are considered metabolite biomarkers, as they reflect the metabolism of the fish, the state of the cardiovascular system, and the functions of the liver.

Also, similar studies showed the effects of herbicide pollution, which rapidly decreased the amount of total protein in fish. The decrease in the number of total proteins can also be caused by other physiological and health abnormalities that result from the influence of heavy metal pollution factors, such as blood plasma denaturation, renal and hepatic longevity, and hepatic blood flow diseases.

Synthesizing and producing output from the breakdown of carbohydrates is not enough for stress energy, so as a reserve, the organization begins to spend proteins.

Liver enzyme activity (ALT and AST)

The activity of hepatic aspartate transaminase (AST) analyzed in the blood of fishes of *Scardinius erythrophthalmus* and *Cyprinus carpio*, in the Sitnica, Lepenci and Lumbardhi i Prizrenit river (Table 4 and Fig. 5).

Table 4. Hepatic Aspartate transaminase (AST) levels analyzed in two species of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio* in Sitnica, Lumbardhi i Prizren and Lepenci rivers.

Sampling site	Number and type of fish (*Scardinius erythrophthalmus* and *Cyprinus carpio*)	Hepatic Aspartate transaminase-AST (IU/L)
Standard value		42 ± 1.0
Sitnica	S1 (n=45)	120 ± 3.11*
Lumbardhi i Prizrenit	S2 (n=45)	75.4 ± 2.30
Lepenci	S2 (n=45)	70.3 ± 2.20

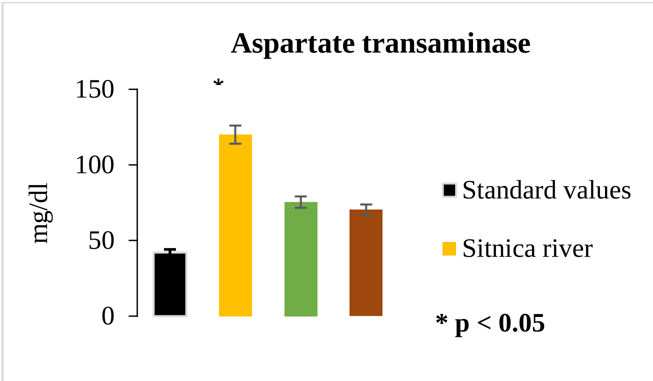

Figure 5. AST analyzed in two species of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio*

The activity of hepatic enzymes alanine transaminase (ALT) analyzed in the blood of fish, *Scardinius erythrophthalmus* and *Cyprinus carpio*, in the Sitnica, Lepenci and Lumbardhi i Prizrenit river (Table 5 and Fig. 6).

Table 5. Hepatic alanine transaminase (ALT) levels analyzed in two types of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio* in Sitnica, Lumbardhi i Prizren and Lepenci rivers.

Sampling site	Number and type of fish (*Scardinius erythrophthalmus* and *Cyprinus carpio*)	Hepatic alanine transaminase-ALT (IU/L)
Standard value		19 ± 0.23
Sitnica	S1 (n=45)	90.4 ± 1.08*
Lumbardhi i Prizrenit	S2 (n=45)	83.6 ± 2.43
Lepenci	S2 (n=45)	78.5 ± 3.43

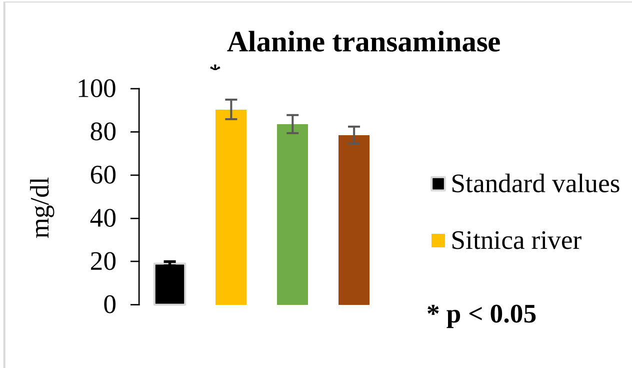

Figure 6. ALT analyzed in two types of fish: *Scardinius erythrophthalmus* and *Cyprinus carpio*.

Discussion

Based on our results, we found that, in the Sitnica River, biochemical parameters changed significantly ($p<0.05$), such as total protein level (TP), plasma glucose levels (Glu), hepatic alanine transaminase (ALT), and hepatic aspartate transaminase (AST). Transaminases play a major role in the metabolism of carbohydrates and amino acids in the tissues of fish and other organisms. The liver plays such a role in the processes of metabolism and detoxification of many xenobiotics and acute exposures to heavy metals, such as cadmium (Cd) and lead (Pb), all of which can bioaccumulate in the liver, causing abnormalities and damage of the tissues and blood of the heart, causing the degradation and breakdown of specific enzymes in the cells and tissues, which pass into their blood. The activity of transaminases (ALT and AST) in fish may also be due to the leakage (lysis or breakdown) of enzymes across the plasma membranes of the skin.

Elevated serum ALT and AST levels also reflect liver and heart damage. Pollution stress can be a dynamic process or a defense mechanism that requires a lot of energy. Animals, too, under conditions of stress, use this defense mechanism as a physiological reaction to its effects. The amount of plasma glucose is a very strong parameter for water stress in fish. Our results show AST and ALT activities are increased behaviorally ($p<0.05$) in the blood of fish in the Sitnica river compared to fish in the other two rivers, Lumbardhi i Prizrenit and Lepenci. Likewise, hepatic aspartate transferase (AST) dominated in higher amounts compared to hepatic alanine transferase (ALT).

The increase in the level of AST indicates the presence of toxicity and therefore increases the arterial pressure and blood flow rate. This enzyme also has functions in glycogen-converting proteins. The growth emphasized the activities of transaminases AST and ALT in the end areas of the world due to the penetration of enzymes from the plasma membrane of life. Our results showed the values of the parameters PT, Glu, ALT, and AST in the river Sitnica compared to the two rivers Lumbardhi i Prizrenit, and Lepenci, which shows that the values of pollution with heavy metals and contamination from industrial and urban discharges have significant differences in related biochemical and physiological parameters of fish. Hepatic transaminases play a role in the processes of carbohydrate and amino acid metabolism, as well as in the physiology of fish tissues and other organisms. This enzyme showed a significant increase in the Sitnica River ($p<0.05$), while in the Lumbardhi i Prizrenit and Lepenci rivers, it showed the same value ($p<0.001$).

Similarly, hepatic aspartate transferase (AST) showed high levels ($p<0.05$) in the Sitnica River, while in the Lepenc and Lumbardhi i Prizrenit River it showed lower values ($p<0.001$). In addition, the healthy level of these transaminases in the body's degradation of certain organs, such as the liver, can also interfere with the release of specific enzymes from the tissues in the blood. The different changes in the values of the biochemical and physiological parameters, the level of total proteins, plasma glucose, ALT, and AST, in the fish of the Sitnica River, compared to the fish of the Lepenci and Lumbardhi Prizrenit river, showed that the Sitnica River is at a critical level of contamination from heavy metals as well as from other urban and industrial contaminants. In the Sitnica river values of water pollution (sewage waste collector) from heavy metals (Pb, Zn, Co, Cu), especially Ni and Cd, have resulted in an increase in the level of transaminases as a response from the level of stres. (Table 6).

Table 6. The results of heavy metal concentrations in the water samples (realized with APHA 3111B methods) in Sitnica river. Ambient temperature: 09-24- 14 (°C).

Parameters	Standard method	Permissible Limits		Results
		Unit	Value	
Zinc (Zn)	APHA311B	mg/l	3.0	0.030
Copper (Cu)	APHA311B	mg/l	2.0	≤0.007
Cadmium (Cd)	APHA311B	mg/l	0.05	≤0.019
Lead (Pb)	APHA311B	mg/l	0.5	≤0.029
Cobalt (Co)	APHA311B	mg/l	/	≤0.0018
Nickel (Ni)	APHA311B	mg/l	0.001	≤0.0015
Sodium (Na)	APHA311B	mg/l	0.150	24.2
Potassium (K)	APHA311B	mg/l	12	1.20

The water quality in the Sitnica River fish is endangered due to heavy metals and phenols. Glucose levels in blood plasma are higher in stressed animals, which secrete catecholamine and corticosteroid hormones. Transaminases (AST and ALT) activity in polluted fish is significantly increased, with AST showing a higher increase than ALT. This indicates an adaptive response to water toxicity and transforming protein to glycogen, a major reserve fuel during stress-induced toxicity in the liver.

Conclusion

Based on this research, the levels of total protein (TP), glucose levels (GLU), hepatic alanine aminotransaminase (ALT), and aspartate transaminase (AST) significantly changed in the Sitnica River ($P<0.05$) compared with Lumbardhi i Prizrenit and Lepenci rivers as a control group. Also, the heavy metal concentrations, which showed an increased level of cadmium (Cd) and lead (Pb) in the water at the Sitnica River station that was higher than allowable standard values of 0.05 mg/L. It is known that the level of heavy metals is reflected in the trophic chain (level of stress in fish) and could have an biological correlation effect on human health. Furthermore, we can conclude that these parameters are a good approach to be used as biochemical metabolites of physiological responses to water pollution and, as a consequence, can be used as bioindicators of early detection of pollution effects on biological species inhabiting rivers, which could affect environmental and human populations.

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