Biomarkers as Bioindicators to Early Detection of Pollution Effects in Environmental and the Human Health

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Abstract

The aim of this research was to analyze biochemical parameters in two fish species (Chondrostoma nasus) and (Salmo trutta m. fario) taken in two different rivers of Kosovo. Fish samples were collected during March-July-October 2018 applying the electrofishing method (according to Hans Grassl GmbH) alongside Llapi (samples were taken near to Sitnica river) and Lepenci rivers. The biochemical parameters: plasma glucose (GLU), total protein (TP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed in the blood samples of 60 fishes of both species. GLU was analyzed by Glucose-Oxidase Enzymatic method, while determination and concentration of TP was analyzed with Biuret Method. Meanwhile enzymes analysis, AST and ALT respectively were conducted according the Enzymatic-Colorimetric method, with kit (Roche) and analyzer Cobas Integra 400-Roche. The research results showed a significant ($P<0.05$ and $P<0.01$) increase in GLU concentration in two fish species taken in the Llapi and Lepenci river compared with the normal values, while a significant decrease ($P<0.05$ and $P<0.01$) in TP concentration. There is also a highly significant increase in the AST and ALT levels in the blood of fish species ($P<0.05$ and $P<0.01$) compared to the normal values for. Statistical data were calculated One-way ANOVA using Minitab software.

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Introduction

Besides many other resources, Kosovo is promising a potential for the fisheries sector. Lots of rivers and suitable conditions make fishery the promising sectors in the field of economic development. However, potential rivers for fish farming in the country are the Drini I Bardhë, Lumbardhi I Pejës, Lumbardhi I Prizrenit, Lepenci, Lumi I Brodit, Lumi I Restelicës and other rivers with a lower water capacity.[1] Pisces are continued to be an extremely reliable component of an aquatic monitoring system because they integrate the effect of detrimental environmental changes as consumers which are relatively high in the aquatic food chain. The fishes as a bioindicator species play an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of a fish indicates heavy pollution; the effects of exposure to sub-lethal levels of pollutants can be measured in terms of biochemical, physiological or behavioral responses of the fish. Fish are very good biosensors of aquatic contaminants.[2] In order to maximize fish productivity, farmers need to be aware of the factors that influence fish performance such as nutrition, diseases, environmental stresses, and pollutants.

Exposure to environmental contaminants can affect the survival of aquatic organisms via numerous mechanisms, including direct toxicity

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(both short- and long-term). More often the effects are more notable, ultimately modulating organism fitness.[3] Standardized laboratory measures of growth, reproduction and survival can be an unreliable estimation of the many indirect stressor effects in the field. More convincing changes in normal physiological function, such as appropriate reproductive behavior, resilience to disease, and prey-capture abilities, may better indicate impacts on longer-term organism survival, reproductive output and ultimately, ecosystem health are also noted .[4] In order to better manage contaminants, it is desirable to be able to predict changes in ecosystem composition and function, as well as organism health, given a known set of environmental parameters and contaminant concentrations. It is also advantageous to have the ability to avoid losses in ecosystem structure and function before they occur, as opposed to conducting a retrospective analysis once an organism or system function has declined. However, this proposition is far more challenging than it seems, largely because of ecosystem complexity (for example, regular changes in the species composition and health of organisms in the field with tidal and seasonal cycles) and the presence of multiple stressors. In the direction of further complicated environmental risk assessments, many contaminant exposures are episodic in nature. Impacts such as oil spills, storm water runoff following floods or heavy rains, release of antifoulants following ship groundings, release of material due to resuspension following dredging etc., are all likely to have a large influence on the health of organisms and their ability to recover even if they contribute minimally to overall contaminant concentrations over a larger spatial and temporal scale.[5,6] Increasingly, environmental toxicologists are borrowing from the field of medicine to predict ecosystem health and function. Biomarkers are defined as detectable biochemical and tissue-level changes that indicate altered physiology.[7]

Water quality guidelines typically recommend that biomarker studies are best used to indicate exposure, as it is difficult to extrapolate between test results and ecosystem effects, even though they acknowledge that biomarker assays may be the best indicator of chronic stress.[8,9] However, biomarkers, in general, have not yet been fully exploited in the context of an integrated monitoring system, and there are additional under-utilised advantages to incorporating a biomarker-based approach. Carefully chosen biomarkers may be the best approach to identify an early response to contaminants and are much more sensitive for identifying organism stress than whole animal responses although it is not always clear the origin of the stress, and there are often multiple stressors present.[10]

Biomarker responses can be measured in organisms collected from, or deployed in field sites, to integrate the effects of chemical and non-chemical stressors, reducing the need for complex laboratory exposure scenarios. To achieve these goals, however, it is important that a biomarker-based approach links mechanistic knowledge with a consideration of whole-animal physiology and population-level effects.[11,12] (**Figure 1**).

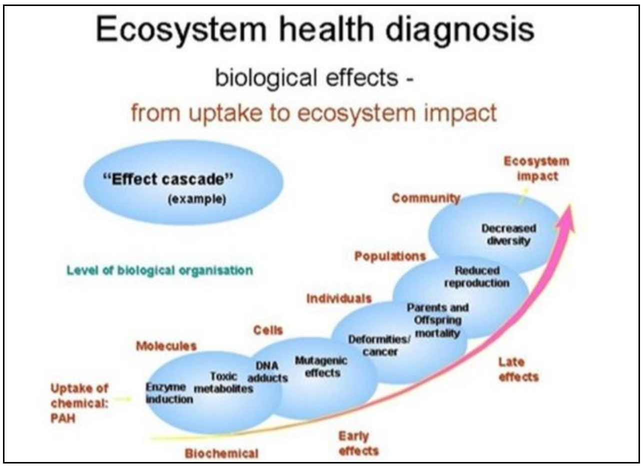

Figure 1. Level of biological evaluation-correlation effects from molecules to ecosystem.[1]

Biomarkers have been classified by the extent that they reflect exposure to environmental stressors, or adverse health effects from contaminant exposures. Some biomarkers can also indicate susceptibility to adverse outcomes from environmental contaminants, although these have not been developed or incorporated in ecological assessment frameworks due to the fact that they largely rely on well-defined genetic databases and the incorporation of epidemiological studies, which are areas of future development in the field of aquatic toxicology. However, biomarkers that reflect both exposure and biological effects have been widely incorporated in field and laboratory studies, although the examples are few.[13]

Exposure Biomarkers

Biomarkers of exposure to single or multiple contaminants with similar modes of action can show an early response to contaminants and are typically specific to a particular class of contaminants, e.g. biliary fluorescent aromatic compounds (FACs) for exposure to oil, or the induction of the egg-yolk precursor protein vitellogenin (vtg) for environmental estrogens. [14]

Some studies indicate that biomarker responses could be used to make field-based measurements of endpoints that are difficult to capture in laboratory-based studies. In laboratory-based studies, exposure to the pesticide esfenvalerate at ng/L concentrations altered swimming behaviour in the delta smelt (Hypomesus transpacificus).[15]

These changes could be correlated to changes concentrations of genes involved in neuromuscular processes, immune responses, apoptosis and redox and metal binding. Some genes, such as aspartoacylase and creatine kinase, could be mechanistically linked to the disrupted physiological processes.[15] Exposures to environmentally relevant concentrations of copper were also shown to decrease swimming speeds in the endangered delta smelt, these behavioral changes also appeared to be related to changes in gene expression.[15] The impact of low level exposures to certain contaminants, most notably pesticides and metals, has been linked to loss of olfactory ability in several fish species.[16,17,18,19] Recently, linked molecular biomarker encoding olfactory receptors and signal transduction components to histological injury and loss of behavior in *Coho salmon* exposed to cadmium.[20] This study is consistent with studies using the model organism zebrafish showing that olfactory antioxidant defense and signal transduction gene expression is quantitatively altered on exposure to the model trace metals copper and cadmium. [21,22] In the case of copper, the zebrafish study provided molecular insights into the ability of copper to impair sensory function in coho salmon, which has been linked with increased susceptibility to predation.[23] Fish welfare in general is a comprehensive issue integrating physiological, behavioral and cognitive responses, indicating adaptive responses to stressful stimuli. Thus have stated that poor welfare may provide a strong indication for the existence of infectious and non-infectious stressors, leading to impairment in maintaining homeostasis.[24] Assessing the impacts of contaminants on the health of aquatic organisms and ecosystems is challenging due to the presence of multiple stressors and the complexity of ecosystems.[25] (**Figure 1**) To improve the quality of the product with increasing its market value and to achieve a positive public perception, weak fish must be avoided, and healthy animals must be raised in accordance with welfare standards.[26]

Biomarker responses may be at the molecular, cellular or “whole organism” level. An important thing to emphasize about biomarkers is that they represent measurements of effects, which can be related to the presence of particular levels of an environmental chemical; they provide a means of interpreting environmental levels of pollutants in biological terms. Biological responses at higher organizational levels-population, community, and ecosystem are considered as bioindicators.

The measurement of total protein, albumin, and globulin in serum or plasma is of considerable diagnostic value in fish as it relates to general nutritional status as well as the integrity of the vascular system and liver functions.[26] Also, blood glucose level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions reported the significant increase in plasma glucose of immature gilthead sea bream, Sparus auratus after short-term and long-term stress conditions.

Alanine transaminase (AST) and aspartate transaminase (ALT) which participates in transamination reactions found predominantly in liver, cardiac cells, and striated muscle tissues. Cellular damage releases the ALT and AST into the blood stream and the levels of these enzymes have the potential to indicate hepatic-toxicity.[26]

The purpose of this study consists to analyzing some biochemical parameters as an indicator of fish health’s and indicated from water pollution, especially the total protein (TP) glucose (GLU) alanine aminotransferase (ALT) and aspartate transaminase (AST) also known as serum glutamic pyruvate transaminase-SGPT in Llapi and Lepenci rivers, which may have been indicated by heavy metals in the mentioned rivers.

Materials and methods

Subjects

The subjects of this research were two fish species: *Chondrostoma nasus* and *Salmo trutta m. fario*. For the research purpose were used 60 fish individuals of both species. Live fishes were taken with the containers of 20 liters' water and quickly transported to the laboratory with constant aeration, the random sampling which was repeated 3 times, in March-July-October 2017.

Experimental procedure

Blood was collected from fishes, using of ether for animal anesthesia. Blood (1 ml) without EDTA, was taken by caudal vein with cardiac puncture using 2 ml sterile plastic disposable syringes fitted with 0.8x38mm hypodermic needles and used to prepare blood films to determine the serum biochemical glucose levels, AST and ALT enzyme. Blood serum was separated by centrifugation (5000 rpm /10 min). In contrast to the blood sampling from the caudal vein or the cardiac puncture with drawl from the *Ductus Cuvieri* provide the most convenient procedure. It was safe for the fish and easy to handle.

Evaluation of TP was analyzed with Biuret method, while GLU by Glucose Oxidase Enzymatic method. AST and ALT were analyzed with an Enzymatic-colorimetric method with the kit (Roche) and analyzer used by model: Cobas Integra 400-Roche.

The total plasma proteins were measured by using the standard Biuret method as described by Lawrence (1986), which is based on the reaction between the peptide bonds of protein and Cu^{2+} (from copper sulfate solution) that produces a blue-violet colored complex in alkaline solution.

The measurements were done using the Biuret method (Chronolab) where 100 ml of blood plasma and standard protein solution were diluted into 500 ml of the Biuret reagent in a test tube. The Biuret reagent without a sample being added was used as a blank. After mixing of serum it stayed for 60 minutes in the test tubes, then the absorption values were read through the 540 nm wavelength spectrophotometer. The total plasma proteins obtained were expressed in g/dl. The intensity of response is not always caused by a specific stressor in any experiment; instead, it may be modulated or affected by alien factors that are not considered as direct stressors. The values of total plasma proteins obtained were expressed in g/dl.

Results

The research results showed significant changes in the analyzed parameters GLU, TP, AST and ALT in fish species (Chondrostoma nasus) and (Salmo trutta m. fario) taken in Llapi and Lepenci rivers. Results related to GLU concentration show the highly significant increase ($P<0.05$ - $P<0.01$) in fish species compared with normal values (Table 1, Figure 2).There is a significant decrease in TP concentration in fishes of Lepenci river ($P<0.01$) and the high significant decrease in fishes of Llapi river ($P<0.05$) in comparison with normal values (Table 2, Figure 3). Enzymes activity of ALT resulted from a significant increase ($P<0.05$ - $P<0.01$) in their activity on fish species taken form Llapi and Lepenci rivers, compared to their normal values (Table 3 and Figure 4). Enzymes activity of AST respectively resulted in a significant increase ($P<0.05$ - $P<0.01$) in fish species taken form Llapi and Lepenci rivers compared to their normal values (Table 4 and Figure 5).

Sampling station	Type of fishes (*Rutilus rutilus and Carassius gibelio*)	Plasma glucose (mg/dl)
Normal value	**Number**	75 ± 2.3
Ll-river	S1 (n=45)	90±0.15*
Le- river	S2 (n=45)	78±0.12

Table 1. Data of plasma glucose (GLU) analyzed on two fish species, Chondrostoma *nasus and Salmo trutta m. fario* in the Llapi and Lepenci rivers.

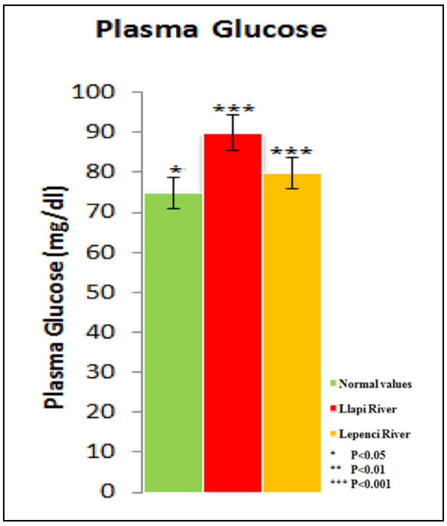

Figure 2. Results related to GLU concentration show a significant increase in fishes of Llapi river (P<0.05) and in Lepenci river (P<0.01) compared with normal values.

Sampling station	Type of fishes (*Rutilus rutilus and Carassius gibelio*)	Total Protein (g/dl)
Normal value	Number	3.93±1.8
Ll-river	S1 (n=45)	2.48±0.12*
Le- river	S2 (n=45)	2.62±0.33

Table 2. Data of total protein (TP) analyzed on two fish species, *Chondrostoma nasus and Salmo trutta m. fario* in the LLapi and Lepenci rivers.

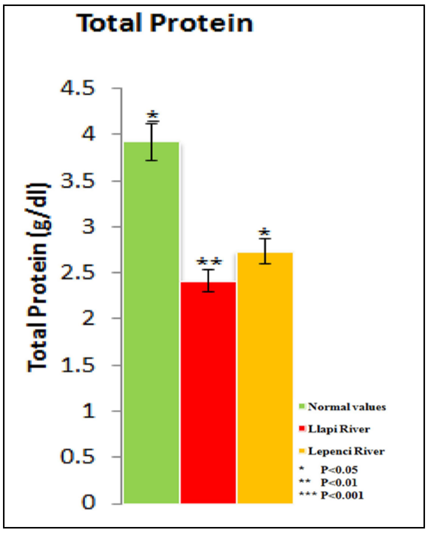

Figure 3. The high significant decrease in TP concentration in fishes of Llapi river (P<0.05) and the significant decrease in fishes of Lepenci river (P<0.01) in comparison with normal values.

Sampling station	Type of fishes (*Rutilus rutilus and Carassius gibelio*)	Aspartate Transaminase-AST (IU/L)
Normal value	Number	42 ±1.0
Ll-river	S1 (n=45)	118±3.13*
Le- river	S2 (n=45)	64.5±2.19

Table 3. Data of AST levels analyzed on two fish species, Chondrostoma nasus and Salmo trutta m. fario in the Llapi and Lepenci rivers.

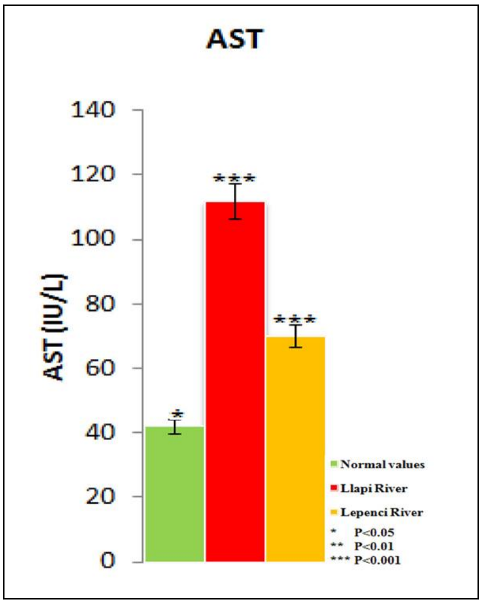

Figure 4. Enzymes activity of ALT resulted in a significant increase in Llapi river (P<0.05) and Lepenci river (P<0.01) in comparison with normal values.

Sampling station	Type of fishes (*Rutilus rutilus and Carassius gibelio*)	Alanine aminotransferase-ALT (IU/L)
Normal value	Number	19±0.23
Ll-river	S1 (n=45)	80.4±1.07*
Le- river	S2 (n=45)	76.3±1.93

Table 4. Data of ALT levels analyzed on two fish species, *Chondrostoma nasus and Salmo trutta m. fario* in the Llapi and Lepenci rivers.

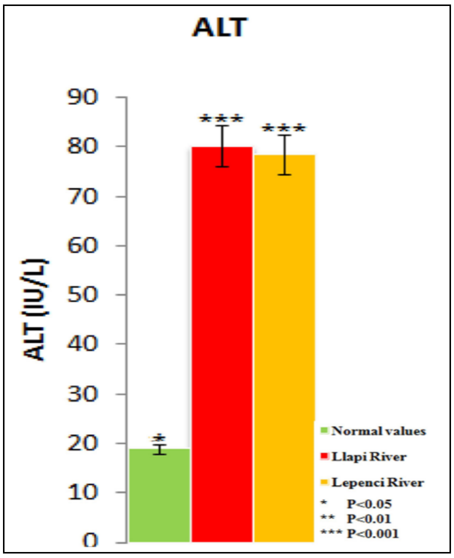

Figure 5. Enzymes activity of AST resulted in a significant increase in Llapi river (P<0.05) and Lepenci river (P<0.01) in comparison with normal values.

Parameters	Standard method	Permissible limits		Results
		Unit	Value	
Zinc (Zn)	APHA 3111B	mg/l	3.0	0.035
Copper (Cu)	APHA 3111B	mg/l	2.0	≤0.010
Cadmium(Cd)	APHA 3111B	mg/l	0.05	≤ 0.021
Lead (Pb)	APHA 3111B	mg/l	0.5	≤ 0.039
Cobalt (co)	APHA 3111B	mg/l	/	≤ 0.020
Nickel (Ni)	APHA 3111B	mg/l	0.001	≤ 0.017
Potassium(Na)	APHA 3111B	mg/l	150	34.4
Kalium (K)	APHA 3111B	mg/l	12	1.40

Table 5. The results of heavy metal concentrations in the water samples (realized with APHA 3111B methods) in Llapi river. Ambient temperature: 8-23- 13 (°C) March-July-October 2018.

Discussion

Increased levels of glucose are normal in the animals under stressing; it is related to secretion of catecholamine and corticosteroid hormones from surreal glands. Stress is an energy demanding process since the animal under stress conditions use energy to fight the stress effects. Our present data support very well these facts, also according to another study, the glucose levels can be a very sensitive parameter of water stress in carp fish.[28] There is a significant decrease in TP concentration to the fishes of Lepenc River and highly significant on the fishes of Llapi River. AST and ALT values also show a highly significant increase in both fish species of two rivers, Llapi and Lepenci compared to the normal values.Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other organisms. Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures of chromium may lead to accumulation in the liver and cause pathological alterations. Transaminase enzymes, including alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP) are important for amino acid and protein metabolism in fish tissues, and are key metabolic enzymes released into the blood from damaged hepatocytes and also have increased synthesis due to induction.[3] Thus, alterations in plasma levels of transaminase enzymes could be a sign of hepatocytes damages. Thus, alterations in plasma levels of transaminase enzymes could be a sign of hepatocytes damages. With heavy metal pollution, decreases or increases of some other easily measured liver detoxification enzymes, such as glucuronosyltransfrase (UDP-GT) [Castrén M] and δ-aminolevulinic acid dehydratase (ALAD) can also be used as suitable biomarkers, although they may not have been heavy metal specific.[4] ALT is a key metabolic enzyme released on the damage of hepatocytes. Increased level of ALT indicates an adaptive response to its leakage into the bloodstream due to the presence of water toxicity. It also has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress-induced toxicity in the liver.

Lack of published species-specific normal reference ranges remains the primary reason that blood testing is not routinely performed in fish health evaluations. Fish hematology is gaining great attention in fish culture because of its importance in monitoring the health status of fish. Our results showed significantly higher values of TP, GLU, and ALT, AST in the Llapi river compared to the Lepenci rivers, which indicates that values of contamination pollution increasing the affected consequently these relevant physiological parameters of fish. Similar results have been reported by response of *Nile tilapia, Oreochromis niloticus* exposed to individual and combined mixtures of pollution was expressed by a significant increase in glucose, total protein, albumin, AST, and ALT as well as a significant decrease in cholesterol levels. [24]

This strongly supports the present findings where the significant increase in values of total protein, albumin, and globulin was associated with increasing the stocking density of silver carp. This coincides with the recorded increase in values of glucose, total protein, AST and ALT with increasing the stocking density of silver carp in the present study. [25]

Acclimation of fish to different environmental factors results in increased metabolic activity correlated with changes in the quality and quantity of certain enzymes involved in energy metabolism and with compensating modifications in the rate of protein synthesis. AST and ALT are the most frequently tested enzymes in fish for indication of cyanobacterial toxicity. The possibility that infection in one part of the body can impact the health of other organs or tissues has been gaining increasing attention.[26] The acute toxicity of microcystins (known cyanobacterial toxin) is unlikely to occur in silver carp and chronic exposure to cyanobacteria will not be detected by changes in AST and ALT in the blood plasma.[27]

Moreover, measurement of plasma glucose, total protein, ALT, and AST proved to be more sensitive and usable in the detection of stress response of fish. Alanine transaminase ALT is a key metabolic enzyme released from the damaged hepatocytes. The enzyme shows a consistent increasing trend significantly in Llapi river ($p<0.05$)) and in Lepenci river ($p <0.01$). The overall increase of AST and ALT which is quite high suggesting acute heart and liver damage.[28] Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures of chromium may lead to accumulation in the liver and cause pathological alterations. Moreover, cell injury of certain organs like liver and heart to the release of tissue-specific enzymes into the bloodstream. Significant increases in transaminases (ALT & AST) activity in the fish could be possible due to leakage of enzymes across the damaged plasma membranes. Changed of the biochemical parameters on the fish in the Llapi river, higher than the level of total protein and plasmatic glucose, ALT and AST, compared with the control group of Lepenci river, indicates that in the Llapi river value of water pollution (especially Cd and Pb) have been resulted to increase the level of transaminases (ALT and AST) as a response mechanism from the level of stressful. Our results are in accordance with the results of previous researchers on freshwater fish.[29] As we know, the variability of biochemical parameters on the blood serum of the fish reflected for research and water pollution on the different rivers.

Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other organisms.[30] Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures of chromium may lead to accumulation in the liver and causing pathological alterations. Moreover, cell injury of certain organs like liver and heart to the release of tissue specific enzymes into the blood stream.[31] Significant increases in transaminases (ALT and AST) activity in the fish could be possible due to leakage of enzymes across the damaged plasma membranes. As we mentioned, increased serum ALT and AST activities reflect a situation of liver and heart damage.[32] Changed of the biochemical parameters on the fish in the Llapi river, higher than the level of plasma glucose (GLU), total protein (TP), ALT and AST, compared with the Lepenci rever, indicated that in the Llapi river (especially near the line of the Sitnica river) value of water pollution (sewage waste collector) from heard metal (Pb, Zn, Co, Cu) especially Ni and Cd have been resulted to increase the level of transaminases as a response mechanism from the level of stressful. (**Table 6**).

Parameters	Standard method	Permissible limits		Results
		Unit	Value	Results
Zinc (Zn)	APHA 3111B	mg/l	3.0	0.030
Copper (Cu)	APHA 3111B	mg/l	2.0	≤0.007
Cadmium (Cd)	APHA 3111B	mg/l	0.05	≤ 0.019
Lead (Pb)	APHA 3111B	mg/l	0.5	≤ 0.029
Cobalt (co)	APHA 3111B	mg/l	/	≤ 0.018
Nickel (Ni)	APHA 3111B	mg/l	0.001	≤ 0.015
Potassium(Na)	APHA 3111B	mg/l	150	24.2
Kalium (K)	APHA 3111B	mg/l	12	1.20

Table 6. The results of heavy metal concentrations in the water samples (realized with APHA 3111B methods) in Lepenci river. Ambient temperature: 09-24- 14 (°C) March-July-October 2018.

Experimental results show that quality of this water is endangered from heavy metals (Pb, Cd, Cu, and Zn) and phenols.[33] The levels of glucose in blood plasma of fish from Sitnica river were higher ($p<0.05$) compared with the control group. Elevated levels of glucose are normal in the animals under stressing; it is related with secretion of catecholamine and corticosteroid hormones from surreal glands. Stress is an energy demanding process since the animal under stress conditions use energy to oppose the stress effects. Glucose levels can be a very sensitive parameter of water stress in carp fish.[34] Our results show that the activity of AST and ALT were increased significantly ($p<0.05$) while AST exhibited a higher increase than ALT. ALT is a key metabolic enzyme released on the damage of hepatocytes. Increased level of ALT indicates an adaptive response to its leakage into the blood stream due to the presence of water toxicity. It also has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress- induced toxicity in liver. This result is in accordance with the results of previous researchers on fresh water fish.[35] Significant increase in transaminases (AST and ALT) activity in fish of polluted areas, could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver.[36]

Conclusions

Based on this research analyzed in Llapi river were significantly changed the level of total protein (TP), glucose levels (GLU), hepatic alanine aminotransaminase (ALT) and aspartate transaminase (AST), ($P<0.05$) compared with Lepenci river as a control group. From evaluated of the heavy metals concentrations show increased level of Cadmium (Cd) ≤ 0.019mg/ L and Lead (Pb) ≤ 0.039 mg/ L in water at stations Llapi river and average concentrations of Cd ≤ 0.021 mg/ L and Pb ≤ 0.029 mg/ L in the Lepenci river, were higher than allowable standard values 0.05 mg/l according to FAOAs it is known that the level of heavy metals reflected in the trophic chain (level of stressful of fishes) and have a biological correlation effect on human health. Furthermore, we can conclude that these parameters are a good approach to be used as a biochemical metabolite of physiological responses to water pollution, as a consequence can be used as bioindicators of early detection of pollution effects on biological species inhabiting rivers, which could affect in the environmental and human population.

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Declaration of Interest

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