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Biochemical Evaluation of *Rutilus rutilus* and *Carassius gibelio* in different Rivers (Kosova)

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Abstract
The objective of this study was to determine some biochemical parameters, analyzed in two different Kosovo rivers. Fish samples were collected during March - July - October 2014 applying the electrofishing method alongside Sitnica and Lumbardhi i Prizrenit rivers. We have analyzed those biochemical parameters on 30 fish individuals on two fish species: *Rutilus rutilus* and *Carassius gibelio*. Analyses of these parameters were conducted according these methods: determination and concentration of TP was analyzed with Biuret method, GLU was analyzed by Glucose Oxidase Enzymatic Method, while AST and ALT was analyzed with Enzymatic-Colorimetric Method, with kit (Roche) and analyzer used by model: Cobas Integra 400-Roche. Based on our results it was observed in Sitnica river were significantly changed (p<0.05) in level of total protein (TP), glucose levels (GLU), hepatic alanine transaminase (ALT) and aspartate transaminase (AST) compared with control group.

Keywords: Fish, total protein, glucose, AST, ALT

1. Introduction
Besides many other resources, Kosovo has a potential for the fisheries sector. Lots of rivers and suitable conditions make fishery one of 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 lower water capacity [1].

Fish 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 fish as a bioindicator species plays 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 sublethal 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].

The purpose of this study was analyzing some biochemical parameters as an indicator of fish healthy and indicated from water pollution, especially the Total Protein (TP), Glucose (GLU), Transaminase, Aspartate amino-transferase (AST) and Alanine amine - transferase (ALT) also known as Serum Glutamic Piruvat Transaminase (SGPT) in Sitnica and Lumbardhi i Prizrenit revers.

Identification of molecular biomarkers associated with the early prediction, diagnosis and monitoring of major physiological alterations and diseases of fish caused by pollution, may contribute towards *in situ* conservation of fish populations. As a consequence, biomarkers can be taken as short-term indications of biological effects that will be seen in the long term [2].

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 environmental chemical, they provide a means of interpreting environmental levels of pollutants in biological terms.

The United States Centers for Disease Control and Prevention reported that fish and shellfish account for 5% of the individual cases and 10% of all foodborne illness outbreaks [3].

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 blood stream and the levels of these enzymes have the potential to indicate hepatic-toxicity [4].

Serum ALT and AST activities used in diagnosis of damage fish tissues (i.e. gill, muscle, liver) [5]. Determinations of transaminases (AST and ALT) have been useful in the diagnosis of liver and kidney diseases in fish [6]. While the ALT and AST activities increased in fish exposed to only lead concentrations at both exposure periods, there are numerous study in this serum activity of fish [7, 8]. The researchers concluded that necrosis or disease of liver caused to leakage of this enzyme into blood stream, might be responsible for increase of this enzyme in blood.

Materials and Methods

The determination of total protein TP, glucose GLU and transaminases AST and ALT on the average of 30 live fishes of *R. rutilus* and *C. gibelio* were taken with the containers of 20 liters water and quickly transported to the laboratory with constant aeration, random sampling which was repeated 3 times, in March-July-October 2014.

These samples, 15 fish from each rivers, were obtained from two rivers - Sitnica and Lumbardhi i Prizrenit.

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 0.8x38-mm 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. An adequate volume of blood could be obtained in a short time; no injuries in contrast to the other methods could be detected [9].

Determination and concentration of TP was analyses with Biuret method, GLU was analyzed by Glucose oxidase enzymatic method, while AST and ALT where analyzed with Enzymatic-colorimetric method, with kit (Roche) and analyzer used by model: Cobas Integra 400-Roche.

Result and Discussion

The objective relevant of this study was to determine some biochemical parameters such are level of total protein (TP), glucose (GLU), transaminase AST and ALT, analyzed from 30 fish samples from two species *R. rutilus* and *C. gibelio* in two Kosovo rivers. Fish samples were collected during March–July-October 2014 applying the electrofishing method alongside Sitnica and Lumbardhi i Prizrenit rivers.

Table 1: Data of plasma glucose (GLU), total protein (TP), AST and ALT levels, analyzed from two fish species: Rutilus rutilus and Carassius gibelio in the Sitnica and Lumbardhi i Prizrenit rivers.

Fish group	GLU (mg/dl)	TP (g/dl)	AST (IU/L)	ALT (IU/L)
Normal value	75 ± 2.3	3.93±1.8	42 ±1.0	19±0.23
Sitnica river (n=15)	92±0.13	2.33±0.12	120±3.11	90.4±1.08
Lumbardhi i Prizrenit river (n=15)	86±0.14	2.82±0.36	75.4±2.30	83.6±2.43

Based on our results it was observed in Sitnica river were significantly changed ($p<0.05$) in level of total protein (TP), glucose levels (GLU), hepatic alanine transaminase (ALT) and aspartate transaminase (AST) compared with control group in Lumbardhi i Prizrenit river.

Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other organisms [10, 11, 12].

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 [13].

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 [14].

Changed of the biochemical parameters on the fish in the Sitnica river, higher than the level of plasma glucose (GLU), total protein (TP), ALT and AST, compared with the Lumbardhi i Prizrenit revers, indicated that in 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.

Experimental results show that quality of this water is endangered from heavy metals (Pb, Cd, Cu, and Zn) and phenols [15].

The levels of glucose in blood plasma of fish from Sitnica River were significantly 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 [16]. Our results show that the activity of AST and ALT were increased significantly ($p<0.05$) (Table1). 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 [16].

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 [17].

From evaluated of the heavy metals such are: Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb), Potassium (K), Nickel (Ni) and Sodium (Na), we can conclude that heavy metal were on the allowable standard value according to the Food and Agriculture Organization (United Nations) FAO.

But average concentrations of Cadmium (Cd) $\leq$ 0.019mg/ L and Nickel (Ni) $\leq$ 0.018 mg/ L in water at stations Sitnica river and average concentrations of Cd $\leq$ 0.013 mg/ L and Ni $\leq$ 0.012 mg/ L in the Lumbardhi i Prizrenit river were higher than allowable standard values 0.05 mg/l according by FAO.

As it is know, variability of biochemical parameters on the blood serum of the fish reflected for research and water pollution on the different rivers.

Table 2: The results of heavy metal concentrations in the Sitnica and Lumbardhi i Prizrenit rivers, water samples analyzed with APHA 3111B methods. Ambient temperature: 8-23-13 (°C).

Partamers tested	Standard method	Permissible limits		Sitnica River	Lumbardhi i Prizrenit River
		Unit	Value	Results	Results
Zinc (Zn)	APHA 3111B	mg/l	3.0	0.036	0.024
Cooper (Cu)	APHA 3111B	mg/l	2.0	≤0.019	≤0.017
Cadmium (Cd)	APHA 3111B	mg/l	0.05	≤ 0.019	≤ 0.013
Lead (Pb)	APHA 3111B	mg/l	0.5	≤ 0.048	≤ 0.028
Cobalt (Co)	APHA 3111B	mg/l	/	≤ 0.029	≤ 0.014
Nickel (Ni)	APHA 3111B	mg/l	0.001	≤ 0.018	≤ 0.012
Sodium (Na)	APHA 3111B	mg/l	150	37.4	23.7
Potassium(K)	APHA 3111B	mg/l	12	1.568	1.386

Conclusion

Based on our results observed in Sitnica river were significantly changed ($p<0.05$) in level of total protein (TP), glucose levels (GLU), hepatic alanine transaminase (ALT) and aspartate transaminase (AST) compared with control group in Lumbardhi i Prizrenit river. Heavy metals such are: Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb), Potassium (K), Nickel (Ni) and Sodium (Na) were on the allowable standard value in Sitnica and Lumbardhi i Prizrenit rivers, except average value of Cadmium (Cd) and Nickel (Ni) concentrations were higher than the standard values 0.05 mg/l. Furthermore, we can conclude that, total protein TP, glucose GLU, ALT and AST are good approach to be used as biomarkers 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.

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