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- RESEARCH ARTICLE -

Effect of water quality in hematological and biochemical parameters in blood of common carp (*Cyprinus carpio*) in two lakes of Kosovo

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Abstract

The aim of the current investigation is to evaluate the impact of water quality in hematological and biochemical blood profile in two habitats of common carp *(Cyprinus carpio*) captured in Batllava and in Radoniqi Lake. It has been found that Radoniqi Lake is characterized with increased levels of physicochemical parameters (T, DO, PO_4^{-3}, NO_3-N, NO_2-N). In Radoniqi Lake were recorded also heavy metal content such as Cd, Pb, Ni and Cu while in Batllava Lake only presence of Cu was recorded. Given the developed agriculture in the region where Radoniqi Lake is located, increased level of PO_4^{-3}, Cd and Cu can be attributed to agricultural pollution. Blood is taken for hematological (RBC, Hgb, MCV, MCH, MCHC, WBC), and biochemical (ALP, AST, ALT, Glucose, Cholesterol, Triglyceride, Total lipid, Total protein) analysis from twenty fish of each lake. The results of hematological parameters show that there is a difference between investigated fishes of respective lakes. There are slight differences in the values of Hgb, MCHC and WBC, while the changes are significant in RBC, MCV and MCH. By contrast, the biochemical parameters have shown significant differences, with the exception of the amount of cholesterol, where the differences are insignificant. Given the water quality of the two lakes, the results of this research show that water quality affects the health condition of fish. The high values of hematological and biochemical parameters in the fish of Radoniqi Lake are indicative that these fish suffer from stress caused by water pollution with heavy metals.

Keywords: Fish, Water Quality, Hematological Parameter, Biochemical Parameters, Lake.

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Introduction

Natural water systems can be largely contaminated with a wide range of pollutants, among which also by bacterial contamination, oil pollution, organic and inorganic contamination. Within organic pollutants pesticides are considered to be the most dangerous for aquatic organisms, while within inorganic contaminants heavy metals are considered the most common. Water pollution has negative effects on all kinds of organisms, whether plant or animal. Also physicochemical features of water such as temperature, pH, dissolved oxygen, nitrites, nitrates, carbon dioxide, pesticides, phosphates, etc. have an impact on fish health (Svobodova et al. 1993). Among the aquatic organisms, fish represent the largest and most diverse group that are closely related to the aquatic environment, hence the quality of water certainly affects their health. They respond with great sensitivity to direct as well as indirect changes in the aquatic environment (Borkovic et al., 2008). A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems (Law, 2003; De La Torre et al., 2010). Physical and chemical changes in the aquatic environment often induce changes in blood causing physiological disturbances in fish, therefore when research is conducted on the impact of habitat on fish health, it is important to consider the habitat as fully. The use of biochemical and enzymatic blood serum biomarkers in fish are a very good tool to assess the condition of aquatic habitat. It has been proven that the health condition of animals can be evaluated through the biochemical and enzymatic parameters of blood (Ismail et al., 2017; Zhou et al., 2009; Faggio et al., 2012). Changes in the activity of liver enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serve as an indicator for a normal liver function, and they also can be used as biomarkers for tissue damage (Almeida et al., 2002). Thus, it can be concluded that these enzymes are sensitive biomarkers for the stress determining in fish subjected to various pollutants present in waters (Adhikari et al., 2004; Yancheva et al., 2015). Hematological studies are also important for environmental monitoring and help to understand the relationship between the characteristics of blood to the habitat and adaptability of the species to environment (Fazio et al., 2013; Francesco et al., 2012). The purpose of this research is to investigate the impact of pollution and influence of the water quality in fish health, through some hematological and biochemical parameters in blood of common carp (*Cyprinus carpio*) in two lakes of Kosova, Batllava Lake and Radoniqi Lake.

Study areas

Batllava Lake is an artificial lake built in the 1970s. This lake is located in the region of Podujeva, situated in the north-eastern part of Kosovo. The lake is deep at maximum 48 meters. This lake is also used for water supply of Kosovo Capital (Prishtina) including some neighbor cities (Podujevo, Obiliq etc.) and for other activities such as rest, fishing and relaxation.

Radoniqi Lake is also an artificial lake created in the 1980s, located in western part of Kosovo in the municipality of Gjakova. The lake was named after the village, which was overthrown for its creation. The lake extends to a length of 6 km and a width of 2 km. The greatest depth is 55 m. Radoniqi Lake takes second place in Kosovo from the surface. Radoniqi supplies about 214,000 inhabitants of the towns of Gjakova and Rahovec and the surrounding villages. It is also used for irrigation.

Figure 1. Hydrographic map of Republic of Kosovo (adapted from https://www.goruma.de/, 2018).

These two lakes in which research was conducted, are the main water suppliers in Kosovo therefore the quality of water is continuously monitored.

Materials and Methods

Water samples for analysis of physicochemical parameters are collected in plastic bottles, while the tests are repeated three times. The soluble fraction of heavy metals in water is determined by atomic absorber spectrophotometry, according to standard method ISO 8288: 1986. The water sample was prepared by transferring of water from plastic bottles into a 100 ml glass conical bottle, adding 5 ml of concentrated HNO_3 in a liter of water sample and kipping the sample at 4 °C until analysis were performed. Reading is done by PERKIN ELMER atomic absorber spectrophotometer. The temperature, pH and dissolved oxygen (DO) are measured with the HANNA HI-9828 Multi-Parameter Water Quality Portable Meter. The determination of phosphorus is done by the spectrometric method with ammonium molybdate (NS 4725). Determination of nitrites was done according to the photometric method with sulfanilamide and N- (1-naphthyl) ethylene diamine, while nitrates are determined by the 2, 6-dimethylphenol spectrometric method. Heavy metals content (Cd, Pb, Ni and Cu) was determined by atomic absorption spectrophotometry (AAS). Fish were collected with the help of fishing nets, 20 fish in Batllava Lake and 20 fish in Radoniqi Lake. The age and gender of fish were not considered in the current research. Blood was taken from caudal vein with heparinized syringes. The collected blood is used for hematological and biochemical parameters. Hematology blood counter "SFRI" was used to determine the complete blood count: red blood cell count (RBC), hemoglobin

concertation (Hgb), mean corpuscular volume MCV, mean corpuscular hemoglobin MCH, mean corpuscular hemoglobin concertation MCHC and white blood cell count (WBC). Plasma concentrations of glucose (mmol/l), triglyceride (mmol/l) and total cholesterol (mmol/l) were measured using a commercial kit (Linear chemicals, Barcelona, Spain). Total lipid (g/l) and total protein (g/l) content was determined calorimetrically according to Joseph et al. (1972) respectively Gournall et al. (1949). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman and Frankel (1957). Alkaline phosphatase activity (ALP) was determined by the enzymatic method using the commercial kit (Linear chemicals, Barcelona, Spain). Statistical analysis of results were carried out by using of one-way analysis of variance (ANOVA).

Results

Table 1 show values of physicochemical parameters and heavy metal content of samples from both lakes. Results are presented as average values with standard deviation and p value. From the data presented in Table 1 it is obvious that in Radoniqi Lake higher values of physicochemical parameters (temperature, pH DO, NO_3-N, NO_2-N and PO_4^{-3}) at significant levels are recorded compared to values valid for Batllava Lake. Even though the differences are significant, all values of these parameters are within the allowed limits. Among the investigated heavy metals (Cd, Pb, Ni and Cu), in Batllava Lake only presence of Cu is recorded while in Radoniqi Lake the presence of all these heavy metals is noted. Taking into consideration data reported in the relevant literature, obviously the value of Cd found in Radoniqi Lake is higher than allowed limit.

Table 1. Mean values of physicochemical parameters of water in two investigated lakes.

Parameters	Batllava Lake	Radoniqi Lake	P value
T (°C)	12. 5 – 17.8	14. 1 – 20.4	-
pH	7.39 ± 0.12	7.81 ± 0.07	$p<0.05$
DO (mg/L)	5.38 ± 0.11	9.60 ± 0.10	$p<0.01$
PO_4^{-3} (mg/l)	0.08 ± 0.01	0.91 ± 0.02	$p<0.01$
NO_3-N (mg/l)	1.66 ± 0.06	1.42 ± 0.02	$p<0.05$
NO_2-N (mg/l)	0.0060 ± 0.0001	0.0071 ± 0.0001	$p<0.01$
Cd (mg/l)	ND	0.003	-
Pb (mg/l)	ND	0.05	-
Ni (mg/l)	ND	0.001	-
Cu (mg/l)	0.0083 ± 0.0006	0.0200 ± 0.0018	$p<0.01$

ND- no detected

Value of hematological (RBC, Hgb, MCV, MCH, MCHC and WBC) and biochemical (ALP, AST, ALT, Glucose, Cholesterol, Triglycerides, Total lipid and Total protein) parameters are presented in table 2 respectively 3 as mean value with ± standard deviation. Data presented in table 2 indicates that the differences for Hgb, MCHC and WBC are insignificant. Difference are significant for RBC ($p <0.05$), where the highest values have been recorded in fish from Batllava Lake, while for MCV ($p <0.01$) and MCHC ($p <0.01$) higher values are recorded in fish of Radoniqi Lake. Results related to biochemical parameters (table 3) show significant differences between almost all of these parameters in fishes of two habitats. The highest values of these

parameters have been recorded in fish of the Radoniqi Lake ($p< 0.01$; $p< 0.05$), with the exception of cholesterol levels where the difference is insignificant.

Table 2. Hematological parameters in the blood of *C. carpio* in two investigated lakes.

Hematological Parameters	Batllava Lake	Radoniqi Lake	P value
RBC ($x10^6/\mu L$)	1.51 ± 0.05	1.19 ± 0.11	P< 0.05
Hgb (g/l)	105.50 ± 3.59	108.80 ± 5.52	NS
MCV (fl)	197.67 ± 2.33	242.67 ± 3.24	P<0.01
MCH (pg)	69.95 ± 0.75	95.85 ± 3.10	P<0.01
MCHC (g/l)	35.82 ± 0.46	37.19 ± 1.67	NS
WBC ($x10^3/\mu L$)	68.82 ± 2.27	66.21 ± 2.95	NS
Neutrophils %	91.21 ± 0.82	91.17 ± 0.41	NS
Lymphocyte %	91.21 ± 0.82	91.17 ± 0.41	NS
Monocyte %	5.15 ± 0.23	5.43 ± 0.25	NS

NS- non significant

Table 3. Biochemical parameters in the blood of *C. carpio* in two investigated lakes.

Biochemical Parameters	Batllava Lake	Radoniqi Lake	P value
ALP (U/I)	7 1.24 ± 3.98	146.40 ± 3.67	p <0.01
AST (U/I)	477.47 ± 6.76	996.11 ± 3.37	p <0.01
ALT (U/I)	38.68 ± 1.97	62.57 ± 2.08	p<0.01
Glucose (mmol/l)	8.58 ± 0.54	21.55 ± 0.95	p <0.01
Cholesterol (mmol/l)	4.82 ± 0.21	5.44 ± 0.24	NS
Triglyceride (mmol/l)	1.83 ± 0.16	3.63 ± 0.27	p <0.01
Total lipid (g/l)	8.72 ± 0.28	9.86 ± 0.39	p <0.05
Total protein (g/l)	37.34 ± 1.12	28.20 ± 0.66	p <0.01

NS- non significant

Discussion

Lot of research done by various authors have confirmed that water quality affects the health of fish (Faggio et al., 2012; Francesco et al., 2012; Demke & Tassew, 2016; Arthanari & Dhanapalan 2016). Aquatic ecosystems are characterized by a complex combination of physical, chemical and biological properties that depend on the surrounding environment. So, it is normal that the quality of water in two different habitats belonging to the same category have different physicochemical and biological properties (Demke & Tassew, 2016). In this respect, the results of current research show that the water quality in the two explored lakes differs by the

physicochemical properties and different level of presence of metals. Water in the Radoniqi Lake is characterized by higher levels of parameters T, DO, PO_4^{-3}, NO_3-N, NO_2-N, even though within the allowed limits. Regarding the presence of heavy metals (Cd, Pb, Ni and Cu), only the presence of Cu was recorded in Batllava Lake, while in Radonqi Lake there were present Pb, Cd, Cu and Ni. Based on Svobodova et al., (1993), it can be concluded that the lead and nickel concentrations are within the allowed limits, but the concentration of cadmium and copper exceeds the maximum allowed limits. Admissible value of Cd for cyprinids is considered 0.001 mg/l and the maximum admissible copper concentration in water for the protection of fish is in the range of 0.001 to 0.01 mg/l, depending on the physicochemical properties of water (Svobodova et al., 1993). There may be a correlation between the high amount of PO_4^{-3} and the concentration of Cd and Cu in the Lake of Radoniqi. One of the sources of water pollution with these metals is the widespread use of phosphate fertilizers that contain cadmium (Levit, 2010, Kumar & Singh, 2010) and copper based pesticides which are widely used in viticulture. It means this lake is filled from the rivers that pass through the agricultural fields of this region, which is known for its developed agriculture especially for the vineyard. Hence, the high amounts of PO_4^{-3}, Cd and Cu may be attributed to agricultural contamination. While phosphorus is necessary for the growth of living beings and its presence in the form of phosphates in water is not toxic for animal and plants, heavy metals (Cd, Pb, Hg Ni, Cu, Fe, Cr, Co, Mn, As and Zn) are reported to be toxic. At the same time some heavy metals such are Cu, Ni, Fe, Co, Mn, Cr, and Zn are essential elements necessary for cellular metabolism.

Blood is a very sensitive tissue that is affected by environmental changes so, by the current comparative research related to the hematological and biochemical profile of the blood serum of fish, the evaluation of environmental pollution in these two different lakes has been carried out. In this relation, the comparative studies of different authors have shown that these parameters are good indicators for assessing the quality of water, respectively water pollution with heavy metals (Kopp et al., 2013; Parrino et al., 2018; Osman et al., 2018; Khan et al., 2015). Water contamination with heavy metals can cause changes in hematological parameters and this is noted in the current research. The data from this research show that RBCs count is significantly lower in fish of Radoniqi Lake compared to fish in Batllava Lake. This difference in the RBCs may be due to chronic exposure to heavy metals. The effect of Cd in reduction of RBCs has been noted also in the work of Al-Asgah et al., 2015 and Gill & Epple, 1993 who found significant reduction of RBCs in fish exposed to Cd. Other blood parameters such as MCV, MCH and MCHC are important in diagnosing animal anemia (Coles, 1986). The increasing values of these indicators appear in the case of various anemia, though one of the first indications of metal poisoning might be anemia. The findings of this research indicate the significant increase in the values of such indicators (MCV and MCH) in fish of Radoniqi Lake. Increase of these values can be attributed to the reduction of RBCs caused by heavy metals toxicity. These results are in agreement with the results of Al-Asgah et al. 2015, who reported that exposure of carp with Pb and Cd causes significant increase in MCV, MCH and MCHC.

Fish health condition can be indicated also by monitoring changes in the levels of AST, ALT and ALP enzymes in the fish blood. The use of these enzymes for monitoring stress or even to assess the impact of environmental pollution on fish has been noted by many researchers (Mohiseni et al., 2016; William, 1997; Adham et al. 1999). It was noted that exposure to different concentrations of copper sulfate (0.25 – 4 mg/l) after 14-day resulted in increased plasma / serum

ALT, AST and ALP activity of *Cyprinus carpio* (Karan et al., 1998). Also Firat et al. (2010) found alterations in serum enzyme activities of ALT, AST, ALP and LDH in fish *Oreochromis niloticus* exposed to cypermethrin (CYP), 0.05 mg / l of Cu and 0.05 mg / 1 of Pb for 4 and 21 days. In the present research, a significant increase of activity of ALP, AST and ALT in the blood of fish from Radoniqi Lake ($p < 0.01$) is noted compared to those of Batllava Lake. This may be due to chronic exposure by heavy metals especially Cd and other heavy metals (Cu, Ni and Pb) found in Radoniqi Lake. Heavy metals can be accumulated in various organs and may cause histopathological damage resulting in changes in the activity of the enzymes (Thirumavalavan, 2010). Therefore, increase in ALP, ALT and AST levels in blood may be explained due to hepatocellular damage (Zimmerman, 1978). The elevated activity of these enzymes in the plasma of the various types of fish after heavy metals exposure have also been reported by other authors (Khan et al., 2015; Saravanan et al., 2011; Abedi et al., 2013).

Stress caused by toxins affects carbohydrate metabolism by lowering glycogen levels in muscle and liver and causing hyperglycemia. These has been evidenced by many researches that have confirmed that exposure to water pollutants, including pesticides and heavy metals, has affected the metabolism of carbohydrates resulting in hyperglycemia (Mohiseni et al., 2016; Adham et al., 2002; AL-Atar 2005). This is also evidenced in the current research, where the level of glucose in the blood of fish in Radoniq Lake is significantly higher ($p < 0.01$) than that recorded in Lake Batllava. Given the presence of heavy metals recorded in Radoniq Lake, hyperglycemia may be attributed to glycogenolysis induced by stress caused by contamination of water with heavy metals. These results are also consistent with the results of Khalesi et al., 2016 and Al-Asgah et al., 2016 that found an increase in glucose levels of fish treated with Cd.

Studies in exposure of fish to heavy metals has showed increased levels of triglycerides in the blood (AL-Atar 2005; Mohiseni et al., 2016). Increase in TG levels may be related to hypothyroidism or dysfunction of liver caused by heavy metal contamination (Hontela et al. 1996; Levesque et al., 2002). Differences between fish of Radoniqi Lake and those of Batllava Lake are also noted in the lipid profile of serum. The highest level of total lipids and triglycerides was recorded in the fish of Radoniqi Lake ($p < 0.05$; $p < 0.01$). Similarly, Shalaby, 2007 and Al-Asgah et al., 2016 found increase of TL and triglycerides in fish exposed to Cd depending on dose and duration of exposure. The level of total protein is often used as a parameter to evaluate not only the toxicity of heavy metals but also the stress caused by environmental pollution. The results of many researchers (Al-Asgah et al., 2015; Coles, 1986; Mohiseni et al., 2016) show a decrease of total protein level in cases of heavy metal intoxication and this may be due to protein breakdown induced by heavy metal stress. In this sense the results of current research are consistent with the results of other authors since in the fish of Radoniqi Lake the decrease of the total protein level at significant levels ($p < 0.01$) was found in comparison to fish of Batllava Lake.

The results of the physicochemical parameters of water have shown that this lake is suffering from pollution that might be attributed to agriculture. This conclusion is based on high amount of PO4-3 and heavy metals such as Cd and Cu recorded in this lake, since all three elements (P, CD and Cu) are constituents of fertilizers and pesticides. The region in which Radoniqi Lake is located is known for its developed agriculture, and especially for its vineyard. We can also conclude that fish health is affected by the quality of water. This confirms the results of hematological and biochemical parameters that have been used as tools for assessing the health

of fish. The high values of these parameters of the Radoniqi Lake fish compared to those in Batllava Lake are indicators of water pollution.

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