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Original Research

Evaluating Environmental Pollution by Applying Oxidative Stress Biomarkers as Bioindicators of Water Pollution in Fish

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Abstract

Antioxidant enzyme activities of fish (*Cyprinus carpio* L.) were determined in order to detect the effects of pollution of the Sitnica River in Kosovo on fish physiology and biochemistry. Activities of three antioxidant defense enzymes as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) in the blood of fish were chosen as bioindicators. Fish (n=21) were sampled during March-May and August-September 2010-12 from Ferizaj, Vragoli, and Plemetin (Sitnica River). Plasmatic CAT, SOD, and GST were significantly higher ($P<0.001$) in fish from polluted Vragoli and Plementin sites than Ferizaj. Ferizaj is a source of the river site, so is less contaminated than the two other sites. These three enzymes constitute a sensitive biochemical bioindicator and can be used for detection of chemical pollution in fish. Based on results of our research we can conclude that the Sitnica River is polluted mainly by industrial and urban discharge of liquid waste products. As a consequence, legal actions need to be taken in order to prevent environmental pollution on the site.

Keywords: fish, *Cyprinus carpio* L., catalase, superoxide dismutase, gluthatione-S transferase, Sitnica River pollution

Introduction

Aquatic pollution is a major contributor to oxidative stress in fish, resulting from the redox cycling of pollution.

Exposure to contaminants in aquatic ecosystems can enhance the intracellular formation of reactive species of oxygen, which induce oxidative damage to biological systems. Oxidative stress happens when an imbalance occurs between production and elimination of ROS. The ROS can be detoxified by an enzyme defense system, comprising superoxide dismutase (SOD) and catalase (CAT), while organic peroxides can be detoxified by the activity of glutathione-S-transferase (GST). Several studies demonstrated that changes in the levels of antioxidant enzyme activities can be used as possible biomarkers in different aquatic organisms [1, 2].

Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissues directly from water through respiration and also through their diet. Fish are frequently

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subjected to prooxidant effects of different pollutants often present in the aquatic environment [3-5].

Recently the Sitnica river has been converted into a natural recipient of waste waters like the ones coming from Kosovo power plants in Obiliq, and waters from bigger and smaller central urban canalization systems through which the river flows down, and which have unresolved problems of treatment and purification of discharged waters. Experimental results have shown that Sitnica River water quality is endangered by heavy metals (Fe, Mg, Cr, Zn, Ni, Pb, Cd, and Cu) and phenols [6, 7]. Anomalous values of Fe, Mg, Cr, Ni, and Zn far exceeded the allowed values of the fourth category of the quality of surface waters, causing significant toxic effects in sediments at Mitrovica (about 50 km upstream from the border of Serbia) [8].

The objective of the present study was to evaluate the biochemical changes of the enzymatic defense systems in the Sitnica carp, which are of commercial importance and widely found in the river. The activity of antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) in fish blood were estimated.

Materials and Methods

Study Areas and Collection of Specimens

Three sites (Ferizaj, Vragoli, and Plementin) were chosen for active biomonitoring and investigation of water pollution in the Sitnica.

Animals

Carp fish species *Cyprinus carpio* L. (in total 21 fish individuals) were sampled with electro fishing from three Sitnica sites – Ferizaj (n=8), Vragoli (n=5), and Plemetin (n=8) – during the period March-May and August-September 2010-12. The animals were transported to the laboratory in containers with constant aeration.

Sample Preparation

Blood was collected from caudal vein and cardiac puncture using 2 ml sterile plastic disposable syringes fitted with 0.8×38-mm hypodermic needles. Blood samples were collected and preserved in tubes with EDTA as anticoagulant.

Antioxidant Enzyme Assays

Catalase Activity (CAT)

Catalase activity was determined with colorimetric assay (Biovision) were catalase first reacts with H_2O_2 to produce water and oxygen. Then the unconverted H_2O_2 reacts with a OxiRed™ probe to produce a product that can be measured at 570 nm. 0.2 ml erythrocytes were homogenized on ice in 0.2 ml cold Assay Buffer and were centrifuged at 10,000 × g for 15 min at 4ºC; The supernatant have been collected for assay and kept on ice. Liquid samples have been tested directly and CAT activity was calculated in terms of nmol/min/ml.

Superoxide Dismutase (SOD)

Superoxide dismutase activity was determined using colorimetric test (Biovision). Blood samples were collected using EDTA centrifuged at 1,000 × g for 10 min at 4ºC. The plasma layer was transferred to a new tube without disturbing the buffy layer and stored at -80ºC until ready for analysis. The buf layer was removed from the red cell pellet. The erythrocytes were resuspended in 5X volume of ice-cold distilled water and centrifuged at 10,000 × g for 10 min to

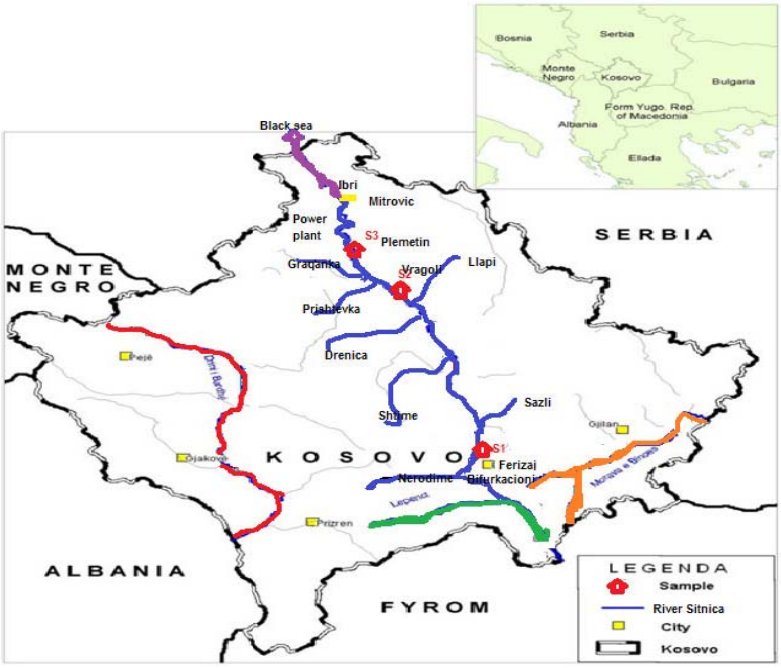

Fig. 1. Study area with sampling stations.

Table 1. Heavy metal concentrations in water of the Sitnica River.

Metals (mg/L)	Site 1 (Ferizaj)		Site 2 (Vragoli)		Site 3 (Plemetin)	
	April	August	April	August	April	August
Iron (Fe^{2+})	0.070*	0.058*	0.126*	0.985*	0.166*	0.134*
Magnesium (Mg^{2+})	0.027*	0.124*	0.491*	0.234*	0.331*	1.436*
Cromium (Cr^{6+})	<0.001	<0.001	0.045*	0.054*	0.027*	0.031*
Zinc (Zn^{2+})	0.012	0.012	0.048	0.010	0.089*	0.019*
Nikel (Ni^{2+})	<0.001	<0.001	<0.001	0.016	<0.001	0.021*
Lead (Pb^{2+})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cadmium (Cd^{2+})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Coper (Cu^{2+})	<0.001	<0.001	0.083	0.022	<0.001	<0.001

P<0.01

pellet the erythrocyte membranes. The supernatant was stored at -80ºC until ready for analysis. SOD activity was calculated in terms of units (U/ml).

Glutathione-S-Transferase (GST)

GST activity was measured spectrophotometrically (Titertek Multiscan R MCC/340) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The enzyme activity was calculated as (µmol/min/ml). The top plasma layer was transferred (without disturbing the white buf layer) to a new tube and stored on ice for assay; the buf layer (leukocytes) was removed and discarded. The erythrocytes (red blood cells) were lysed in 4 times its volume of ice-cold GST assay buffer, and after that it was centrifuged at 10,000 × g for 15 min at 4ºC and the supernatant (erythrocyte lysate) was transferred to a new tube and used for the GST assay. GST activity was calculated in terms of units (µmol/min/ml).

Statistical Analysis

All results were expressed as arithmetic mean values (mean) and standard deviations (±S.D). All data were tested for normality using T test and the significance level of P<0.001 were accepted.

Results

Heavy Metal Levels

Table 1 shows the levels of heavy metals in the water of three sampling sites from the Sitnica. According to the present results the heavy metal ions like Fe, Mg, Cr, Zn, Ni, Pb, Cd, and Cu, were found in Ferizaj, Vragoli, and Plemetin sampling sites. The Fe and Mg ions were higher than the permissible limit in all sites, while Cr, Ni, and Zn ion concentrations were higher that permissible limits in all sites and their values far exceeded the allowed values of the fourth category of the quality of surface waters.

Table 2. Activity of different antioxidant enzymes in fish blood.

Antioxidant Enzyme	Measuring unit	Ferizaj/S1	Vragoli/S2	Plemetin/S3
SOD (n=21)	U/ml	3.2±0.01	4.1±0.2	5.02±0.3
CAT (n=21)	nmol/ml	83.57±3.2	105.9±2.3	329.02±4.1
GST (n=21)	µmol/ml	0.5±0.02	0.7±0.01	0.81±0.1

Values are expressed as mean ±SE, significant level: P<0.001

The mean activities of plasmatic antioxidant enzymes CAT, SOD, and GST are shown in Table 2. The CAT, GST, and SOD activities were found to be significantly higher (P<0.001) in the fish collected from polluted water (site 2 and 3) compared with data from the fish from river water sources (site 1).

The estimated antioxidant enzyme activity of superoxide dismutase (SOD) in the carp blood were in the range of 3.2±0.01 to 5.02±0.3. Vragoli and Plementin sampling sites are more polluted than the Ferizaj site. The activity of plasmatic catalase (CAT) in carp was found to be 83.57±3.2 in Ferizaj, 105.9±2.3 in Vragoli, and 329.02±4.1 in Plementin. The glutathione-S-transferase (GST) activity in fish blood was found to increase from 0.5±0.02 in Ferizaj to 0.7±0.01 in Vragoli and 0.81±0.1 in Plementin. These values were statistically significant for P<0.001.

Discussions

Fish blood is often recommended as an environmental indicator of water pollution. The toxicants cause a disturbance in the physiological state of the fish, which affects the enzyme activity. Also, it can cause distortions in the cell organelles, which may lead to the elevation in the activity of various enzymes. The heavy metal toxicity stimulates the oxidative stress and antioxidant enzymes are induced as a defense mechanism [5]. Oxidative lesions in various organs of the common carp (*Cyprinus carpio* L.) have recently

been related to liver tumor formation in fish from polluted environments [2, 9-12].

The Sitnica is known to be polluted due to industrial and urban activity. In the last few decades attempts have been made regarding the evaluation of aquatic pollution in certain parts of the Sitnica [7, 8], but the assessment of metal contamination of fish has been neglected and there are no data regarding the effects of heavy metal pollution in carp physiology and biochemistry. The heavy metals found in Sitnica River water were Fe, Mn, Cr, Zn, Ni, Pb, Cd, and Cu. Our data indicate that Vragoli and Plemetin sampling sites are more polluted than Ferizaj. This is because Ferizaj is near a river source were waters are clean; meanwhile, in Vragoli subjoined small rivers like Graçanka, Drenica, Llapi, and Prishtevka, which also are recipients of the waste waters of different urban and industrial centers, is near a ferro-nikel complex and mine. Plemetin is near the Kosova Energetic Coorporate (KEK). Similar findings are reported also by other authors [7, 8].

It is also known that heavy metal toxicity stimulates oxidative stress and the antioxidant enzymes are induced as a defense mechanism. Activities of antioxidant enzymes and levels of free radical scavengers have been found to correlate with various physiological or pathological conditions, including stress. It is well known that stress leads to a series of biochemical, physiological and behavior changes, thus altering normal body homeostasis. The generation of ROS in blood impairs the antioxidant defense system to eliminate oxidative stress [13].

The defensive free radical scavenger, superoxide dismutase (SOD), triggers an induction response in heavy metal intoxicated groups [14]. This indicates that more protein is required to protect cells against superoxide radicals. As our results show, the superoxide dismutase level was found to increase in blood of fish collected from the Vragoli and Plementin sites, compared with Ferizaj.

The increased superoxide dismutase activity in the blood of the common carp (*Cyprinus carpio* L.) may be explained as a compensation mechanism against heavy metal intoxication, which was similar with increased superoxide dismutase activity after exposure to pollutants [13, 15-17]. SOD catalitically scavenges the superoxide radical, which appears to be an important agent of toxicity of oxygen [13, 18].

The H_2O_2 radical was trapped by catalase that primarily occurs in peroxisomes. The target function of catalase is

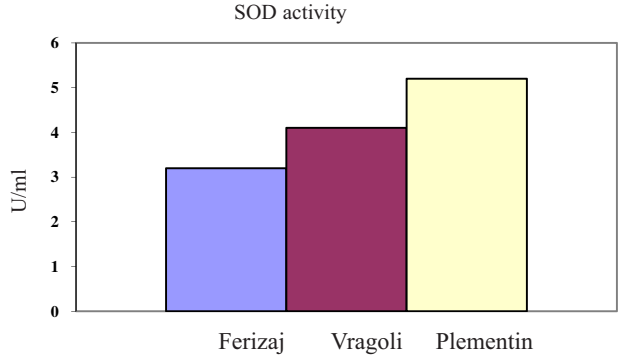

Fig. 2. Level of SOD in fish blood.

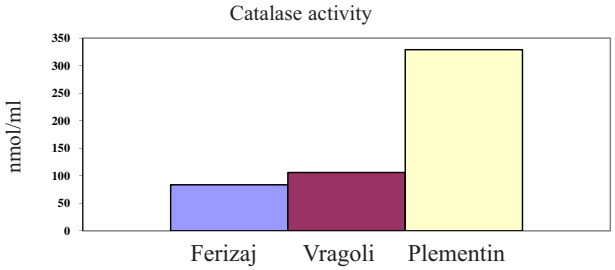

Fig. 3. Level of catalase in carp blood.

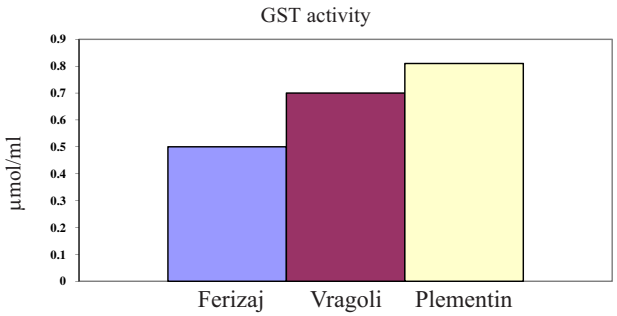

Fig. 4. GST activity.

to protect the cells from the accumulation of H_2O_2 by dismuting it to form H_2O and O_2, or by using it as an oxidant where it works as a peroxidase. We observed an increase of catalase activity by 4-fold in the blood of fishes collected at Plemetin (Fig. 3).

Our findings are supported by the fact that Plementin was reported to have the highest level of heavy metals [7, 19]. It was reported that the enhanced superoxide dismutase and catalase activities in the hepatocytes of the common carp (*Cyprinus carpio* L.) could be induced by microcystin [20-23]. The induction of catalase and superoxide dismutase in the blood is an adaptive response of the cells to mitigate toxicity for the prolonged exposure of fish to polluted waters. Evidence suggested that the high concentration of copper inhibited catalase activity in fish blood [24, 25].

Glutathione-S-transferase (GST) is an abundant cytosolic antioxidant involved in conjugation of toxic reactive metabolites. The higher tripeptide content is involved in the activation of the γ-glutamylcysteine syntethase, one of the enzymes involved in glutathione synthesis [13, 20]. The blood enzyme activity increased (Fig. 3).

The higher glutathione-S-transferase activity observed in the blood of fish specimens from the Vragoli and Plementin sites indicates an augmented detoxification activity. The glutathione-S-transferase detoxifies a number of environmental carcinogens, reactive nucleophile, and epoxides intermediates. The increased GST assay was suggested as a useful tool for biomonitoring oxidative stress [15, 18, 26]. The findings suggest that the heavy metal pollution creates harmful effects by generating reactive oxygen species that damage the cells by disturbing the fluidity balance. However, heavy metal toxicity was counter balanced by the production of antioxidants to suppress the free radicals and protect the blood cells against oxidative damage.

Our findings also show that the carp population of the Sitnica River is suffering toxic effects of heavy metal pollution and that the activity of the plasmatic antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) can be useful bioindicators of oxidative stress.

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