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ANTIOXIDATIVE ENZYMES IN FISH AS BIOCHEMICAL INDICATORS OF AQUATIC POLLUTION

L. VELKOVA-JORDANOSKA [1], G. KOSTOSKI [1] and B. JORDANOSKA
[1]*SI Hydrobiological Institute, Naum Ohridski, 50, 6 000 Ohrid, R. Macedonia*

Abstract

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Antioxidant enzyme activities of fish (*Barbus m. petenyi* Heck.) were determined to establish possible environmental impact of toxic effect on anthropogenic pollution on Lake Ohrid. Activities of superoxide dismutase (SOD) and catalase (CAT) in the blood of fish were chosen as bioindicators. Fish were sampled during May 2007 from locality Kalista (Lake Ohrid). After sacrificing the fishes freshly blood samples were collected and transported on ice. Superoxide dismutase (SOD) activity was measured by the ferricytochrome c method using xantine/xantine oxidase as a source of superoxide radicals. Catalase (CAT) activity was determinated by measuring the decrease of hydrogen peroxide concentration at 240 nm. Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to prooxidant effects of different pollutants often present in the aquatic environment.

Key words: B.m petenyi Heck., antioxidant enzymes, SOD, CAT, pollution

Introduction

Superoxide dismutatase (SOD) and catalase (CAT) have been detected in a wide variety of mammalian cells. These enzymes play important roles in protecting the cell against the potentially toxic effects of environmental pollutants (Kuthan et al., 1986). Superoxide dismutase catalyzes the dismutation of the superoxide ion (O_2^-) to hydrogen peroxide and oxygen molecule during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. The level of antioxidant enzymes have been extensively used as an early warning indicator of lake pollution (Lin et al., 2001). Based on such reasoning, the study of SODs and their application as biomarkers have become important areas in environmental imbe classified into 3 types, Cu/Zn, Mn, and Fe SODs, depending on the metal fount in the active site. Cu. Zn SOD is predominantly associated with the cytosolic fraction of eukaryotes and is very sensitive to cyanide and hydrogen peroxide. Mn SOD is associated with mitochondria and is insensitive to cyanide and hydrogen peroxide. Fe SOD is found in prokaryotes and is not sensitive to cyanide but is inhibited by hydrogen peroxide (Kuthan et al., 1986)

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E-mail: lidvejo@yahoo.com

The analysis of Lake Ohrid nutritient balance clearly point to an eutrophication process, whish has led to more than a threefold increase in average P concentration over past century. Although Lake Ohrid is a slow-reacting (P residence time is about 5 years) and oligotrophic system, the ongoing eutrophication was traced and quantified with monitoring, and combination of information on river inputs, lake concentration, sediment cores, and population development in the catchments (Matzinger et al., 2007).

Material and Methods

Fish were sampled during May 2007 from locality Kalista (Lake Ohrid). After sacrificing the fishes freshly blood samples were collected in monovetes with EDTA and transported on ice. Livers were dissected, stored in ice-cold 0,6% NaCl and transported on ice. Blood samples were centrifuged and plasma fractions were divided for each analysis separately. Then, blood cells were hemolized with dH$_2$O and DNA-se (1 mg/ml), and cells were frozen over night. Next day were centrifuged and hemolisate fractions were divided for each analyses. The specimens of hemolisate were frozen and stored at -20^{0}C until use.

Superoxide dismutase (SOD; EC 1.15.1.1) activities were measured by the ferricytochrome c method using xantine/xantine oxidase as a source of superoxide radicals. Enzyme activity will be reported in units of SOD per milligram of Hb or protein. One unit of activity was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome c reduction rate (McCord and Fridovich, 1969). Catalase (CAT; EC 1.11.1.6) activities were determinate by measuring the decrease of hydrogen peroxide concentration at 240 nm according to Aebi (1984). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH=7) and 10.6 mM H_2O_2 freshly added.

Results and Discussion

Increased levels of superoxide dismutase enzyme have been detected in several samples (No. 2, 7, 8, 9, 10) and catalase in samples 1, 3 and 11. The present data in samples 1, 3 and 11 show decrease of superoxide dismutase activity and increase of level of catalase enzyme. The response to environmental pollution and toxic impact of the pollutant in the aquatic environment represents one of the possible reasons. According some author (Jovanovich, 1993) antioxidant enzymes level depends of age, nutrition and spawning of the fish samples. According to Zikic (2001) cadmium induces the appearance of anemia and alters the metabolism of carbohydrates and proteins in goldfishes. Their results also show the decreased activity of SOD in erythrocytes of goldfishes during acute exposure to cadmium, which indicates the presence of ROS-induced peroxidation, which leads to the destruction of RBC membrane (Table 1).

The caught individual samples were of a healthy appearance and solid health condition. Our early investigation study of the condition of fish population of Lake Ohrid and histopathological analysis of the microscopic preparations, evidenced presence of granulomatous inflammation at the level of hepatocellular parenchyma in liver of *Barbus meridionlis petenyi*. The presence of eggs of the parasite *Capillaria sp*. was the cause of this type of lesion (Sima et al., 1996;

Table 1
Enzyme activity (SOD and CAT) in erythrocytes of *B. m. petenyi* from Locality Kalista (Lake Ohrid)

Samples	Species	Locality	SOD, U/mg Hb	CAT, U/mg Hb
1	*B.m.petenyi*	Kalista	0.0162	301.09
2	*B.m.petenyi*	Kalista	8.011	30.899
3	*B.m.petenyi*	Kalista	0.0076	250.283
4	*B.m.petenyi*	Kalista	0.001	27.514
5	*B.m.petenyi*	Kalista	0.002	49.444
6	*B.m.petenyi*	Kalista	0.003	69.354
7	*B.m.petenyi*	Kalista	9.434	23.924
8	*B.m.petenyi*	Kalista	3.753	16.949
9	*B.m.petenyi*	Kalista	7.890	27.098
10	*B.m.petenyi*	Kalista	4.919	26.539
11	*B.m.petenyi*	Kalista	0.0016	126.05

Velkova-Jordanoska, 2002; Roganovic-Zafirova et al., 2003). Inflammatory processes were evidenced in some of the investigated individuals, but with even distribution in all investigated localities. The link between the environmental pollution and the stress response in fish indicates that infectious diseases arise when the host is exposed to certain conditions of environmental pollution. Faunal investigation of the fish *Barbus meridionalis petenyi* Heck. revealed a number of various pathological changes in the billiary tree including bile duct proliferation and holangiofibrosis. The hepatic-toxic impact of the pollutants in the aquatic environment upon liver in barbel represents one of the possible reasons (Velkova-Jordanoska, 2003). Although Lake Ohrid generally resists the negative influences of the anthropogenic factor for the time being, certain localities of the littoral region display loading with contaminants from the ground, especially in the course of the summer period. This implies the need of a greater seriousness in terms of protection of the lake and more efforts towards eliminating the constant sources of pollution. Lake Ohrid with its spring waters, its old age, the endemic species and its beauty deserves the utmost attention.

Conclusions

Fish (*Barbus m. petenyi* Heck) were subject of activities of superoxide dismutase (SOD) and catalase (CAT) in the blood of fish, and marker to prooxidant effects of different pollutants present in the aquatic environment. The present data in our paper show decrease of superoxide dismutase activity and increase of level of catalase enzyme in several samples. Our results display that it is needed much more investigation on barbel populations in Lake Ohrid, to resolve the question, about pollution impact of this lake.

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