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Seasonal Changes of Superoxide Dismutase Activity in Erythrocytes of *Abramis brama* from Two Different Types of Poland Lakes

Tersa Gabryelak[1] and Nagy Sadek Tawfek[2]

[1]Chair of Biophysics, University of Lodz, Poland and [2]Department of Zoology, Faculty of Science, El-Minia University, Egypt

The toxic role of heavy metals in lakes, rivers and marine ecosystems has been very often investigated during recent years (Ashraf and Jaffar, 1988; Graham et al., 1986; Zamojski et al., 1986). The toxicity of the individual metals and their ability to promote various metabolic changes of fish organisms are detected (Verbost et al., 1989; Babich et al., 1986). It has been suggested that heavy metal ions stimulate lipid peroxidation and O_2^--dependent formation of $^{\cdot}OH$ free radicals (Suderman, 1986). Peroxidation of phospholipid unsaturated fatty acid residues led to functional changes of membranes (Diaz-Munoz, 1985). An important aspect of free radical - mediated toxicity is that it is moderated by several cellular defence mechanisms including enzymatic (superoxide dismutase - SOD; EC 1.15.1.1, catalase; EC 1.11.1.6, glutathione peroxidase; EC 1.11.1.9) and non-enzymatic (vitamin E, ß-carotene, ascorbate, glutathione, etc.) systems (Oberley, 1985). The aim of present work was to investigate the effect of seasonal variations of erythrocyte SOD, lipid peroxidation (LP) as well as water heavy metals and environmental conditions on bream fishes from two different lakes.

MATERIALS AND METHODS

Sexually mature bream fishes (Abramis brama L.) weighting 800-1200 g were netted in two different lakes (Spierewnik lake and Grochowskie lake), during the period of Spring, Summer and Autumn of 1989. Venous heparinized blood was draw from living fishes immediately after catching. The blood was centrifuged for 10 min at 3000 g (4^0C). Blood from one fish was used for one sample. After the removal of plasma, erythrocytes were washed three times with 0.6% NaCl and then lysed by the

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Send reprints request to Teresa Gabryelak at the above address.

addition of the equal volume of distilled water. Deoxyribonuclease 1 - DNase 1 (50 µg/mL) was added to the lysate, which was then incubated for 2 hr at room temperature and subsequently frozen overnight at -20^{0}C. After the centrifugation haemoglobin content was estimated by the method of Drabkin (1946). The SOD activity was measured in the hemolysate by the standard (xanthine oxidase/Nitro Blue Tetrazolium (NBT)) method of Beuchamp and Fridovich (1971) at 25^{0}C. Inhibition of the rate of NBT reduction (expressed in %) recorded at 540 nm was presented in the function of SOD concentration. One Unit of SOD activity was defined as the amount of the enzyme which is necessary to inhibit the rate of reaction by 50%. The method of Placer et al. (1966), based on a colorimetric thiobarbituric acid (TBA) test, was used to evaluate the level of the tissue lipid peroxidation and the amount of TBA-reactive substances was given as the indicator of lipid peroxidation proces. The results of enzyme activities are expressed as Units per mg of haemoglobin. The determination of metals (Cu, Zn, Cd, Pb) in water were carried out by atomic absorption spectroscopy. The absorption was recorded the wavelengths of 228.8, 213.9, 324.8 and 217.0 nm for Cu, Zn, Cd and Pb respectively, using the Carl Zeiss Jena spectrophotometer AAS-1 (Pinta, 1971). All the measurements were performed in triplicate. Individual means of given samples were averaged and the results were expressed as the mean ± S.D. The normal distribution of the data was checked out by means of Shapiro-Wilk test. The data were analyzed using the two-way analysis of variance model 1 in order to assign intervariable differences the multiple comparison analysis of variance according to Newman-Keuls was performed (Zar, 1984).

RESULTS AND DISCUSSION

Fishes were netted in two different lakes in Tuchola Forest district. Spierewnik lake is larger and deeper than Grochowskie lake and the characteristic of the lakes is given in Table 1. The lakes differ in the thermal stratification of the water column during Spring and Summer. The thermal structure is completely different in small lakes, in which water exchanges and mixes during Spring, Summer and Autumn. The mixing of water in a lake has a fundamental influence on the physical, chemical and biological processes that take place in the water column (Mazumder et al., 1990). Both lakes are eutrophic but Grochowskie lake has much higher level of degradation.

The aquatic environment has numerous parameters that may influence the physiological and chemical toxicity of fresh water organisms (Oikari, 1987). The toxic effect of pollutants may be decreased or increased by various water quality factors. For instance pH, temperature, hardness and saturation of water with oxygen (Cai and

Table 1. Values of the analysed parameters characterizing the environmental conditions in the investigated two lakes (Spring, Summer, Autumn). Parameters were determined under 1 m of water surface.

Parameter	Lake					
	Spierewnik			Grochowskie		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Area (ha)		138.9			71.7	
Mean depth (m)		5.2			2.2	
Temperature (^{0}C)	10.3	24.0	10.9	10.0	20.0	10.0
pH values	8.7	8.5	7.8	8.1	8.4	8.1
Total hardness (as $CaCO_3$)	215.0	210.0	210.0	300.0	310.0	290.0
Oxygen (mg/dm^3)	11.7	11.0	7.3	9.3	8.3	10.0
Cu ($\mu g/dm^3$)	4.1	3.9	5.1	3.7	3.4	5.7
Cd ($\mu g/dm^3$)	20.1	16.4	18.6	20.8	22.1	17.1
Zn ($\mu g/dm^3$)	9.5	14.5	52.0	49.0	8.1	27.6
Pb ($\mu g/dm^3$)	9.9	9.6	13.0	11.4	12.7	11.1

Table 2. Seasonal variations of the haemoglibin (Hb) contents, SOD activity and lipid peroxidation (LP) values in bream erythrocytes from the two investigated lakes (means ± SD).

Season	Lakes					
	Spierewnik			Grochowskie		
	Hb g/100 mL	SOD U/g Hb	LP nM MDA/g Hb	Hb g/100 mL	SOD U/g Hb	LP nM MDA/g Hb
Spring	7.31±1.19 n=27	36.69±6.01 n=47	1773.8±260.6 n=27	6.03±0.91 n=53	39.67±6.01 n=43	2547.9±281.9 n=53
Summer	5.45±0.58 n=40	48.91±5.43 n=40	4942.1±345.1 n=40	6.01±0.82 n=29	48.15±5.28 n=29	4547.8±541.2 n=29
Autumn	4.33±0.78 n=27	53.20±8.21 n=27	6570.1±816.9 n=27	4.71±0.87 n=32	60.08±5.84 n=32	4135.4±458.9 n=32

Adelman, 1990) are of the utmost importance. Temperature has a marked influence on metal ion toxicity to fishes and microinvertebrates (Carrier and Bettinger, 1988). Seasonal changes of water temperature (10-20^{0}C) of the two lakes are presented in Table 1. All metals investigated were generally equal to, or less than the most chronic values reported for fishes (Giattina and Garton, 1983). It should be remembered that the toxic effects of metals depend on fish species and water hardness. Heavy metal toxicity is known to be inversely related to water hardness (Carrier and Bettinger, 1988). Therefore, the toxic effects of heavy metals in Spierewnik lake (water hardness - 210.0) might be expected to exceed those occurring in Grochowskie lake (water hardness - 290.0-310.0).

The results of our analyses demonstrated significant seasonal and inter-lake differences (ANOVA, $P < 0.001$) in SOD activities and the extent of lipid peroxidation in the erythrocytes of breams (Table 2). The toxic effect of heavy metals was expressed, in our studies, by the increase in the level of malonyl dialdehyde (MDA), which is considered one of the main degradation products of polyunsaturated fatty acids. We also found the increase in the activity of superoxide dismutase. The enzyme plays an important protective role in the fish erythrocytes. The data obtained for different seasons of the year confirm the regular increase of SOD activity in fish erythrocytes from, Autumn through Summer and Spring in both lakes. The MDA level increased progressively from Spring to Autumn, but it occurred only in Spierewnik lake. In the second lake, MDA level decreased very clearly in Autumn. Possibly, the conditions in Autumn create in fish organism very strong protective enzymatic mechanisms against free radicals. Autumn is the season when fishes activity is still elevated. Breams feed with animal organisms living at the bottom of the lake. Noteworthy, the contamination of the sediments may be higher than that of water depth. There is a scarcity of literature data on the effect of heavy metals on the peroxide metabolism enzymes in erythrocytes of fish species living in different lake ecosystems. Therefore, the mechanism of this effect, under conditions studied, is hard to explain at present.

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