

The Effect of Aquatic Cadmium and Lead Pollution on Lipid Peroxidation and Superoxide Dismutase Activity in Freshwater Fish

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Received: 23 March 2010

Accepted: 9 June 2010

Abstract

The aim of this study was to estimate the effect of aquatic cadmium and lead pollution on superoxide dismutase (SOD) activity and lipid peroxidation in fish. The study used the fish species *Cyprinus carpio* L., *Oncorhynchus mykiss* Walbaum, and *Acipenser baeri* Brandt. SOD activity was measured by the adrenaline method. Lipid peroxidation was measured by determining malondialdehyde (MDA) concentrations. Cd and Pb were determined by graphite furnace spectrometry (GF-AAS). In the examined fish, measured concentrations were: Cd (0.002-0.022 mg kg⁻¹ w.w.), Pb (0.002-0.021 mg kg⁻¹ w.w.), SOD (1.20-5.84 U mg⁻¹ protein), and MDA (0.83-6.71 nmol mg⁻¹ protein). At low metal levels in the blood and muscle, higher SOD activity occurred, which reduced lipid peroxidation. Increased metal concentrations increased lipid peroxidation in the three fish species and simultaneously reduced SOD activity.

Keywords: SOD, MDA, cadmium, lead, freshwater fish

Introduction

Common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walbaum), and Siberian sturgeon (*Acipenser baeri* Brandt) are cosmopolitan species found as native or introduced species in rivers of Europe, North America, and Northern Asia. The aquatic environment is characterized by marked temporal and spatial heterogeneity in the oxygen content due to water features such as temperature, salinity, and flows [1-3]. Therefore, aquatic organisms are exposed to oxygen levels with daily and seasonal variation. Various metals can be introduced to the natural environment through human activity. These metals pollute aquatic and terrestrial ecosystems, adversely affecting the environment and inhabiting organisms. High concentrations of metals in fish tissues can lead to redox reactions,

generating free radicals, especially reactive oxygen species (ROS), e.g. singlet oxygen; superoxides; peroxides; hydroxyl radical; and hypochlorous acid [4]. These highly reactive compounds, molecules, or ions formed by the incomplete one-electron reduction of oxygen, may induce alterations and change some physiological responses of fish [5, 6].

Oxygen is essential for many metabolic processes that are vital to aerobic life. However, dependence on oxygen forces aerobic life to withstand its considerable toxicity, as increased ROS levels can result in significant damage to cell structures [7, 8]. ROS and other pro-oxidants are continually detoxified and removed in cells by an antioxidant defensive system comprising both antioxidant enzymes (SOD, GSSG, CAT, GPx) and small molecular weight free radical scavengers. Exposure of organisms to pro-oxidant attack can increase antioxidant defenses by synthesis of antioxidant enzymes. If antioxidant defenses are effective in detoxifying ROS, then no harmful consequences result to

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Table 1. Comparison of mass (g), total length (cm), and condition (Fulton's condition factor) of study fish.

Fish species	Number of fish (n)	Season			
		Spring	Summer	Autumn	Winter
		Mass (g)			
Common carp	36	197.4±21.3	293.8±29.8	465.6±28.4	392.6±35.4
Rainbow trout	38	192.8±15.6	276.9±31.2	312.5±19.2	489.5±27.6
Siberian sturgeon	36	211.9±24.6	435.8±36.5	434.6±33.5	452.1±45.2
		Total Length (cm)			
Common carp	36	22.7±3.8	28.8±6.5	32.6±8.5	34.6±9.8
Rainbow trout	38	23.7±4.6	24.9±4.8	32.4±6.8	37.3±8.3
Siberian sturgeon	36	24.2±4.9	29.8±6.2	33.5±6.1	35.8±9.5
		Fulton's Condition Factor			
Common carp	36	1.69	1.23	1.34	0.95
Rainbow trout	38	1.45	1.79	0.92	0.95
Siberian sturgeon	36	1.50	1.65	1.16	0.84

the tissues. However, if the ROS attack severely, then the antioxidant system may be overwhelmed [9]. Heavy metals (Cd, Pb) and oxygen are responsible for oxidative stress. The oxygen paradox relies on it: that oxygen bears life and death simultaneously. Deleterious effects of oxidative stress include oxidation of proteins and DNA, and peroxidation of unsaturated lipids in cell membranes. These produce unstable lipid hydroperoxides, the products of which, on decomposition, are highly reactive and threaten cell integrity. In addition, these products can break down into free radicals that can perpetuate the destructive cycle of lipid peroxidation chain reactions. The antioxidant response in fish can be modulated by natural environmental influences just like seasonal variations: thermoperiod, photoperiod, and oxygen saturation. Therefore, physiological changes in fish can serve as biomarkers of environmental pollution [10]. Since the discovery of the importance of free radical damage in the mechanisms of toxicity of many environmental pollutants (xenobiotics), there has been an increased application of biomarkers of oxidative stress in living organisms [11]. Molecular biomarkers of oxidative stress found widespread applications in mechanisms of environmental toxicity and ecotoxicity in aquatic organisms exposed to a variety of chemical pollutants [12]. The aquatic environment daily receives substantial amounts of environmental pollutants that have the potential to cause oxidative stress in aquatic organisms through a free-radical mechanism. The uptake of these pollutants by aquatic organisms can occur from sediments, suspended particulate matter with toxic properties, and food sources. Exposure to these contaminants will depend on the particular dietary and ecological lifestyles of the aquatic organisms. Current knowledge and recent advances of oxidative toxicity by xenobiotics in aquatic organisms provide a fertile field for aquatic toxicology studies. Aquatic organisms were chosen as test species because

of their filtration capacity, ease of caging, and sensitivity to oxidative damage from chronic exposure at sublethal concentrations. Aquatic organisms can provide a model system for investigation of how ROS damage cellular components, how cells respond, how repair mechanisms ameliorate this damage, and how oxidative stress can lead to diseases. Aquatic organisms are more sensitive to exposure and toxicity compared to terrestrial organisms, including mammals, and in this respect they provide experimental data for evaluation of subtle effects of oxidative stress, mutagenicity and other adverse effects of pollutants [13].

This study encompassed three fish species: common carp, rainbow trout, and Siberian sturgeon. The fish were cultured in northwestern Poland (western Pomerania) for 12 months from January to December. The aim of this study was to estimate the effect of aquatic cadmium, lead, and oxidant status pollution on lipid peroxidation (MDA), and antioxidant status on superoxide dismutase (SOD) activity in freshwater fish. I determined additionally how the season of the year and the age of the fish influenced these parameters.

Experimental Procedures

Fish

To conduct the studies I had the approval of the Polish Local Ethics Committee nr 9/05. The study used three fish species: common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walbaum), and Siberian sturgeon (*Acipenser baeri* Brandt); a total of 110 fish, 36-38 individuals of each species, aged from 8 to 20 months, weighing from 192.8 to 722.1 g, and measuring (total length) from 22.7 to 53.8 cm (Table 1). The fish were sampled four times

Table 2. Hydrochemical parameters of water.

Water parameters	Rearing site 20 km from Szczecin				Statistically significant seasonal differences, $P \leq 0.05$
	Spring	Summer	Autumn	Winter	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Temperature (°C)	5.54±3.30	15.66±5.30	12.29±4.60	3.06±2.34	+
pH	4.85±1.36	5.85±2.26	8.85±1.56	7.85±1.16	+
Dissolved oxygen (mg l ⁻¹)	6.49±0.65	5.89±0.35	6.49±0.76	7.29±0.78	+
Oxygen saturation (%)	72.0±3.66	73.3±4.34	72.6±3.86	73.0±4.66	-
Alkalinity (mmol l ⁻¹)	1.68±0.57	1.62±0.45	1.58±0.46	1.64±0.23	-
Hardness of water (mg l ⁻¹)	8.45±1.16	8.32±1.05	8.65±1.48	8.48±1.39	-
Chemical oxygen demand (mg l ⁻¹)	1.79±0.48	1.91±0.77	1.89±0.58	1.97±0.47	-
NH ₄ -N (mg l ⁻¹)	1.30±0.41	1.31±0.51	1.29±0.76	1.30±0.81	-
NO ₃ -N (mg l ⁻¹)	7.02±0.65	7.12±0.95	7.26±0.55	6.98±0.65	-
NO ₂ -N (mg l ⁻¹)	0.64±0.16	0.71±0.19	0.65±0.43	0.68±0.34	-
PO ₄ P (mg l ⁻¹)	0.12±0.04	0.10±0.08	0.11±0.34	0.10±0.05	+
Ca (mg l ⁻¹)	5801±445	5856±476	5781±394	5887±380	-
Cd (mg l ⁻¹)	0.04±0.01	0.04±0.02	0.03±0.01	0.03±0.01	-
Pb (mg l ⁻¹)	0.04±0.02	0.05±0.01	0.05±0.02	0.04±0.01	-

Results are presented as mean±SD; “+” – significant difference, “-” – difference not found.

(spring, summer, autumn, winter). Fish behaviour was observed throughout the study. Fulton's condition factor (CF) was calculated using the means of total length (TL) [cm] and weight (W) [g], and the following formula: $CF = 100 \cdot W / TL^3$ [14].

Water and Feed

The fish were cultured in commercial fish breeding ponds 20 km from Szczecin in northwestern Poland (Fig. 1). Throughout the experiment, water temperature, dissolved oxygen content, pH, hardness, and other water para-

eters were monitored (Table 2). The fish were fed twice daily with Aller Aqua extruded feed (Table 3), and the daily food ration amounted to 3.4 ± 0.8 g per fish. The feed was designed to meet all the basic nutritional needs of the fish to ensure their health and growth. Various kinds of feed were adapted to different sizes of fish and feeding strategies. Aller 576 feed, containing a high energy level, was used for trout breeding; low energetic Aller 45/15 feed was used for sturgeon, while Aller Master feed, having high protein and fat content, was used for common carp. Table 4 presents the comparison of Aller Aqua feed rationing with regard to fish weight and water temperature.

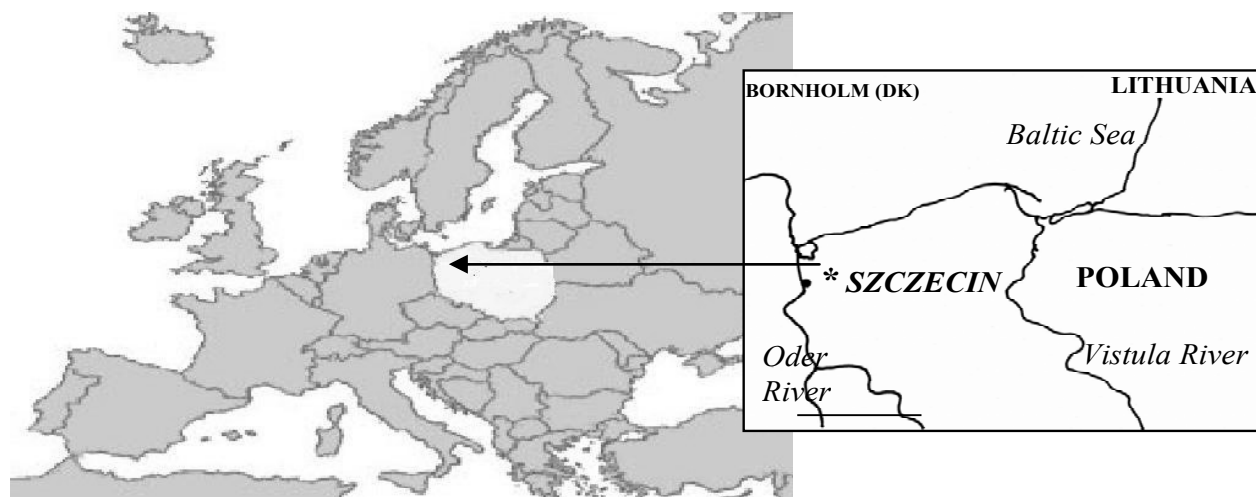


Fig. 1. The location of the breeding ponds about 20 km from Szczecin, Poland.

Table 3. Composition of Aller Aqua feed for three species of freshwater fish.

Nutritional parameters of feed	Fish species		
	Common carp	Rainbow trout	Siberian sturgeon
Feed Name	Aller Master	Aller 576	Aller 45/15
Composition			
Size of feed grain [mm]	5	5 and 6	5 and 6
Protein [%]	35.0	42.0	45.0
Fat [%]	9.0	30.0	15.0
Carbohydrates [%]	43.0	14.0	21.0
Ash [%]	7.0	7.5	8.0
Fiber [%]	5.0	1.0	2.5
All-out energy [Kcal/MJ]	4,325/18.1	5,823/2.3	4,924/20.5
Energy digestible [Kcal/MJ]	3,353/14.0	4,833/20.2	38,887/16.2
Nitrogen (N) [d.m*. %]	5.2	7.1	7.9
Phosphorus (P) [d.m. %]	1.3	7.0	1.2
Energy in dry mass [Kcal/MJ]	4,701/19.6	6,162/25.7	5,381/22.5

* d.m. – dry mass.

Table 4. Recommended daily rationing of Aller Aqua feed expressed as kg of feed/100 kg of fish/day for three freshwater fish species.

Fish species	Weight [g]	Feed size	Temperature of water [C°]						
			2	4	6	8	10	12	14
Common carp	300-2,000	5 mm	-	-	-	-	-	1.4	1.5
Rainbow trout	300-1,200	5 mm	-	-	-	-	-	0.5	0.5
	1000-2,000	6 mm	-	-	-	-	-	0.5	0.5
Siberian sturgeon	300-1,200	5 mm	0.3-0.5	0.4-0.6	0.5-0.6	0.5-0.8	0.6-0.9	0.7-1.0	0.8-1.2
	1,000-2,000	6 mm	0.3-0.4	0.4-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.6-0.8	0.8-0.9
			Temperature of water [C°]						
			15	16	18	20	22	24	>25
Common carp	300-2,000	5 mm	1.5	1.8	2.0	2.5	3.0	3.5	3.0
Rainbow trout	300-1,200	5 mm	0.5	0.8	1.0	1.2	1.4	1.3	-
	1000-2,000	6 mm	0.5	0.7	0.8	1.1	1.3	1.2	-
Siberian sturgeon	300-1,200	5 mm	0.8-1.2	0.9-1.2	0.9-1.2	-	-	-	-
	1,000-2,000	6 mm	0.8-0.9	0.8-0.9	0.8-0.9	-	-	-	-

Tissue Sampling

From each individual, samples of the blood, liver, kidney, and dorsal muscle were collected for biochemical and chemical assays. Before sampling, the fish were reared in 5 m x 20 m fish breeding ponds with water temperature of 12-16°C. Prior to dissection, the fish were gradually cooled

to induce their hibernation. In order to hibernate the fish, they were transferred to a separate tank with water temperature of 10°C. After 20 minutes the fish were transferred to a new tank with water temperature of 4-5°C. Blood was sampled from the caudal vessel (*a. et. v. caudalis*) with a heparinized syringe. Heparin in the amount of 50 IU sodium heparin per 1 ml blood was used for stabilization.

Table 5. Metal concentrations in the certified reference material (Fish-Paste 2) and experimental recoveries (n=6).

Element		Pb	Cd
Experimental value [mg kg ⁻¹]	Mean±SD	0.21±0.03	19.63±1.36
Certified value [mg kg ⁻¹]		0.20±0.02	20.81±0.51
Recovery [%]		95.23	94.33
Difference between the reference and experimental values [%]	Mean±SD	+4.76	+5.67

Directly after the blood collection, the fish were decapitated and dissected. No anaesthetics were used, as they produce inhibitory effects on biochemical and haematological parameters [15]. When dissecting the fish, anatomical observations of the organs and tissues were made.

Chemical Assays

Chemical assays were performed on the samples of liver, kidney, blood and dorsal muscle collected from each fish. The tissues were frozen and stored at -20°C until analyzed. Prior to the actual assay, 1-g tissue samples (weighed to the nearest 0.001 g) were mineralized wet in 3 ml concentrated HNO₃ in a CEM MDS 2000 microwave oven. The solution obtained was quantitatively transferred to polyethylene bottles. Total sample weight was brought up to 20 g with the addition of deionized water. Cd and Pb concentrations were determined with flameless graphite furnace atomic absorption spectrometry (GF-AAS) in a ZL 4110 Perkin Elmer spectrometer. The content of heavy metals in tissues was calculated from relevant calibration curves after correcting the data with blank results. Elemental tissue concentrations are reported in mg kg⁻¹ wet weight (mg kg⁻¹ w.w.). To test the accuracy of the methods applied, the Fish Paste-2 certified reference material (CRM) was analyzed. Cd and Pb recoveries from the Fish Paste-2 CRM were reasonably consistent with the certified values (Table 5). For all of the studied elements the standard deviations were smaller than 8-10%.

Biochemical Assays

Superoxide dismutase (SOD) activity and concentration of malondialdehyde (MDA) were measured for kidney, liver, blood, and dorsal muscle. Tissue samples were homogenized (1:10 w/v) using a Potter Elvehjem glass homogenizer in the 50 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) and with a few phenylmethanesulfonyl fluoride (PMSF) crystals added prior to homogenization to inhibit protease. The homogenates were then centrifuged at 4°C for 15 min at 15.000 g in a SIGMA 3K-15 centrifuge. Supernatants were collected and were measured spec-

trophotometrically (Varian Cary 50 Bio). The activity of superoxide dismutase (SOD EC 1.15.1.1) was measured by the adrenaline method [3]. Light absorbance of samples was measured at 560 nm. One SOD activity unit inhibits the rate of nitro blue tetrazolium (NBT) reduction by half. The measure of NBT reduction rate corresponds to 0.0165 absorbance units per minute in a 1-cm cuvette. SOD activity was expressed as U mg⁻¹ protein. Lipid peroxidation was measured by determination of malondialdehyde (MDA) concentrations and expressed per milligram of protein (nmol mg⁻¹ protein). The absorbance was measured at 535 nm. The MDA method is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA at 45°C [16]. The homogenate protein concentration was measured for all tissues by the Bradford reagent containing Brilliant Blue G in phosphoric acid and methanol. The absorbance at 595 nm was recorded and the protein concentration was determined by comparison to a standard curve. Standard protein was bovine serum albumin (BSA). Blood results were expressed as per mg haemoglobin, the content of which was determined by Drabkin's cyanmethaemoglobin method [17], using haemoglobin standard from AQUA MED ZPAM-KOLASA SP.J. catalogue No 1010.2.

Statistical Evaluation

The results are given as arithmetic mean values (mean) and standard deviations (±SD). The statistical analyses were performed using the computer software STATISTICA 6.0. The data obtained were subjected to statistical treatment involving one-way analysis of variance. ANOVA (Duncan's test) was used to test significance of differences at the significance level of P≤0.05 (A) and P≤0.01 (a), and comparison of correlation coefficients (R²) was performed.

Results

Positive correlation was observed between fish growth rate and the experiment duration. Body weight increased by 102.0-240.7% (R²=0.860-0.981), and total body length by 61.6-122.3% (R²=0.913-0.977). Anatomical and pathohistological examination was conducted in order to eliminate potentially sick individuals. Autopsy revealed no disorders or disease symptoms. There were no blood congestions in gill lamellae, and consistency of internal organs did not indicate any disease symptoms.

The mean concentration of cadmium ranged from 0.002 to 0.024 mg kg⁻¹ w.w. Fig. 2 shows that the highest Cd levels were found in the kidney and liver of Siberian sturgeon (0.022±0.008 mg kg⁻¹ w.w.). The lowest Cd levels were in the muscle of common carp (0.001±0.001 mg kg⁻¹ w.w.), and Siberian sturgeon and rainbow trout (0.002±0.001 mg kg⁻¹ w.w.). Significant differences (P≤0.01) were obtained only between the concentrations of Cd in muscle of common carp and the remaining fish (Fig. 2d). Throughout the study Cd concentration in the blood, kidney, and liver of all the studied fish were not significantly different (P≤0.05) (Fig. 2).

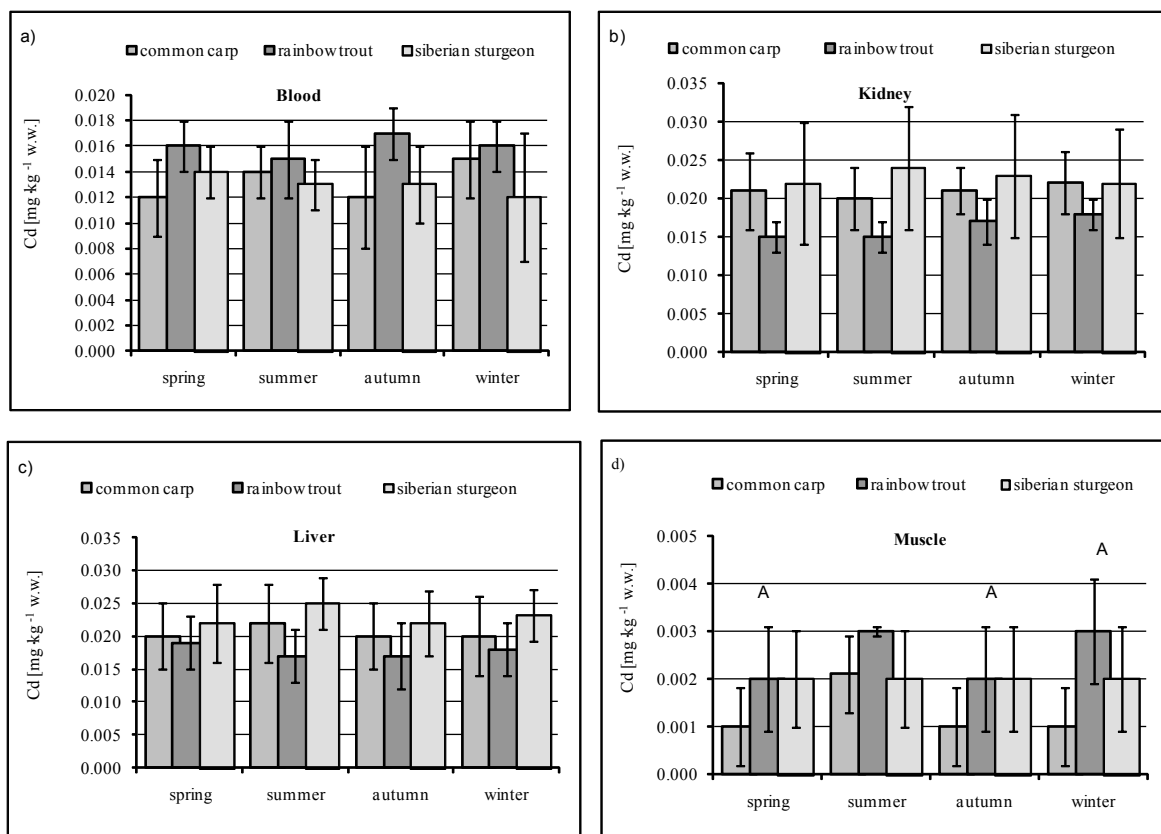


Fig. 2. Comparison of cadmium levels (mean and standard deviation) in tissue: blood (a), kidney (b), liver (c), and dorsal muscle (d) of the three examined freshwater species; a – significant difference ($p \leq 0.05$); A – significant difference ($p \leq 0.01$); means that do not share the same alphabetic sign are significantly different.

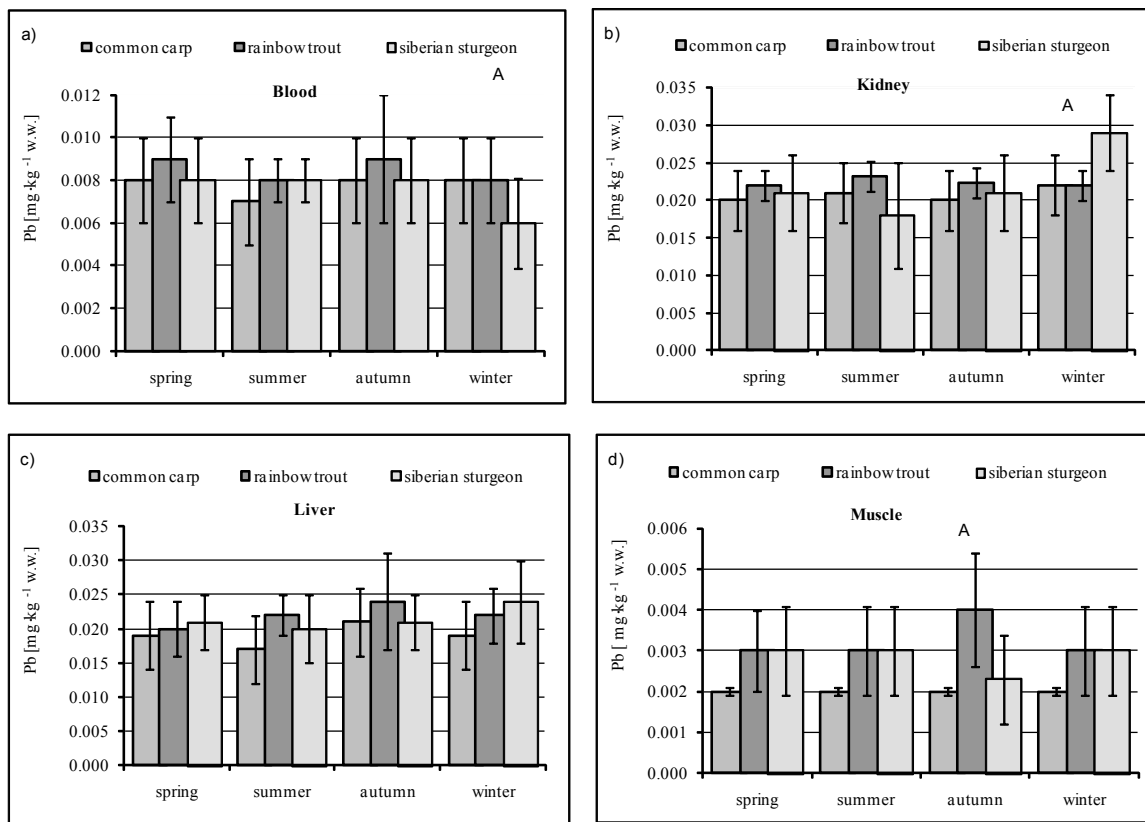


Fig. 3. Comparison of lead levels (mean and standard deviation) in tissue: blood (a), kidney (b), liver (c), and dorsal muscle (d) of the three examined freshwater species; a – significant difference ($p \leq 0.05$); A – significant difference ($p \leq 0.01$); means that do not share the same alphabetic sign are significantly different.

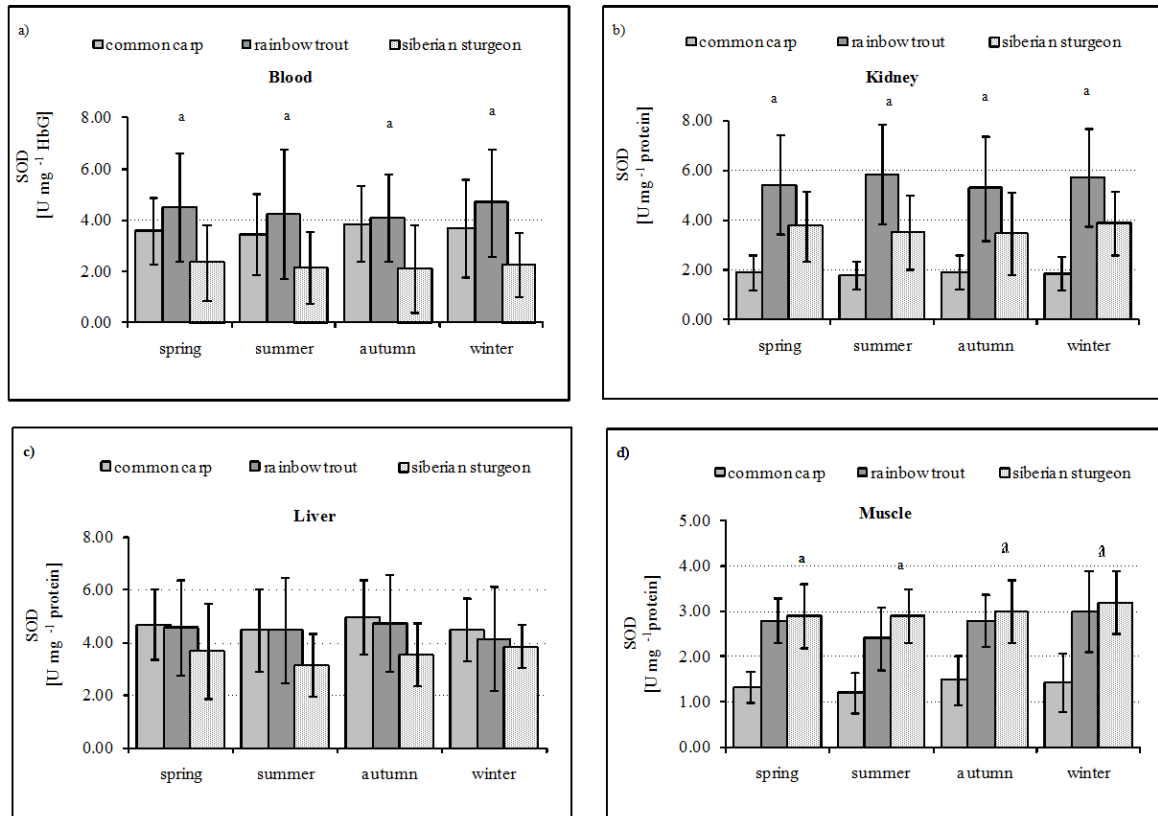


Fig. 4. Comparison of SOD activity (mean and standard deviation) in tissue: blood (a), kidney (b), liver (c), and dorsal muscle (d) of the three examined freshwater species; a – significant difference ($p \leq 0.05$); A – significant difference ($p \leq 0.01$); means that do not share the same alphabetic sign are significantly different.

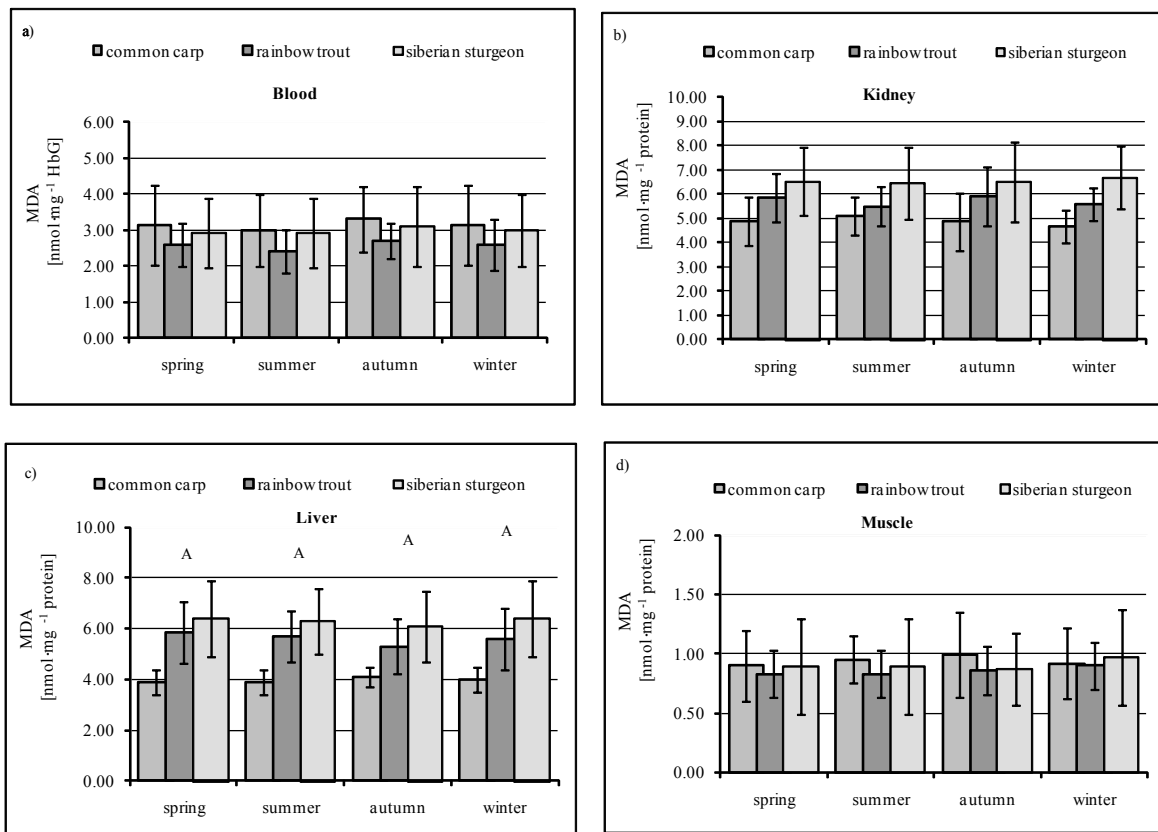


Fig. 5. Comparison of MDA levels (mean and standard deviation) in tissue: blood (a), kidney (b), liver (c), and dorsal muscle (d) of the three examined freshwater species; a – significant difference ($p \leq 0.05$); A – significant difference ($p \leq 0.01$); means that do not share the same alphabetic sign are significantly different.

Table 6. Comparison of the percentage Cd, Pb, SOD, MDA levels in the studied freshwater fish.

Research parameter	Tissues	Fish species											
		Common carp				Rainbow trout				Siberian surgeon			
		Percentage [%]											
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Cd	Muscle	2	3	2	4	4	5	4	5	3	3	3	3
	Liver	37	40	39	34	34	34	34	34	37	38	37	39
	Kidney	39	35	38	37	30	31	31	28	37	36	38	40
	Blood	22	22	21	25	32	30	31	33	23	23	22	18
Pb	Muscle	4	4	4	4	6	6	7	5	6	6	5	5
	Liver	39	36	41	37	36	39	40	40	40	41	40	38
	Kidney	41	45	39	43	41	41	38	40	40	37	40	47
	Blood	16	15	16	16	17	14	15	15	14	16	15	10
SOD	Muscle	11	11	12	12	16	14	17	17	23	25	25	24
	Liver	41	41	41	39	27	26	28	24	28	27	29	29
	Kidney	16	16	16	16	31	34	31	33	30	30	28	29
	Blood	32	32	31	33	26	26	24	26	19	18	18	18
MDA	Muscle	7	7	7	7	5	6	6	6	5	5	5	5
	Liver	30	30	31	31	39	40	36	38	38	38	37	38
	Kidney	38	39	37	37	39	37	40	38	39	39	39	39
	Blood	23	24	25	25	17	17	18	18	18	18	19	18

Table 6 presents percentage comparison of Cd concentration in the examined organs, taking into consideration the seasons and the species of the freshwater fishes. The lowest percentage Cd level was found in dorsal muscle (2-5%) in all the examined fish species. The highest percentage Cd was found in the liver (34-40%) and kidney (28-40%), and was comparable in the examined fish species (Table 6).

Lead (Pb) levels ranged from 0.002 to 0.021 mg kg⁻¹ w.w. The highest metal levels were found in the liver (0.024±0.006 mg kg⁻¹ w.w.) and kidney (0.029±0.005 mg kg⁻¹ w.w.) of Siberian sturgeon, and the lowest in the muscle of common carp and Siberian sturgeon (0.002±0.001 mg kg⁻¹ w.w.) (Fig. 3). Statistically significant (P≤0.01) differences in Pb content among species were detected for blood and kidney in winter, and for muscle in autumn (Fig. 3). In all the studied species no significant differences in percentage lead concentration were found between the fish species, examined tissues and the seasons (Table 6). Percentage lead concentration ranged within: 4-7% in muscle, 39-41% in liver, 37-47% in kidney and 10-17% in blood (Table 6). It proves that neither the seasons nor the lead accumulation influenced the fish organisms.

SOD activity in the fish ranged within 1.20 and 5.84 U mg⁻¹ protein. The highest SOD activity was detected in the kidney of rainbow trout 5.84±1.98 U mg⁻¹ protein, and the

lowest in the dorsal muscle of carp 1.20±0.44 U mg⁻¹ protein (Fig. 4). Significant interspecies differences were obtained for SOD activity in the blood, kidney, and muscle (P≤0.05) throughout the study, while SOD activity in the liver did not show any statistically significant differences (Fig. 4). In dorsal muscle of the examined fish SOD activity ranged within 11-25%. SOD activity in carp muscle (11-12%) was statistically lower compared to the activity of the trout (14-17%) and the sturgeon (23-25%). I also found that SOD activity in the carp kidney (16%) was statistically lower compared to the remaining fish (28-34%). The percentage of SOD activity in fish blood differed (Table 6). The obtained results indicate that SOD activity in the examined freshwater fish tissues is insensitive to seasonal influences.

Fig. 5 showed that malondialdehyde (MDA) levels varied from 0.83 to 6.71 nmol mg⁻¹ protein. The highest MDA levels occurred in the liver (6.71±1.30 nmol mg⁻¹ protein) and in the kidney (6.41±1.50 nmol mg⁻¹ protein) of Siberian sturgeon. The lowest MDA levels were found in the muscle (0.83±0.07 nmol mg⁻¹ protein) of rainbow trout. Carp had significantly (P≤0.01) lower liver levels of MDA than the remaining two fish species, while no significant interspecies differences were detected for the blood, kidney, and muscle (Fig. 5). Also, the degree of lipid peroxidation in the

studied fish showed no significant seasonal differences (Table 6). The degree of lipid peroxidation in the muscle ranged within 5-7%. MDA in liver oscillated around 30-40%, whereas in the kidneys it was around 37-40%, and in blood around 17-25% (Table 6). The results have indicated that the degree of lipid peroxidation in the examined freshwater fish tissues was insensitive to seasonal influences.

Discussion of Results

The importance of free radical reactions and ROS in physiological processes of living organisms and in mechanisms of toxicity by exposure to a variety of environmental pollutants stimulated an explosive increase of research and applications into the field of oxidative stress caused by ROS. The current knowledge that such processes of oxidative damage occur in aquatic organisms gave the impetus to extend environmental and ecotoxicological studies to aquatic organisms as sentinels of environmental contamination by toxic chemicals [18].

Cadmium exposure reportedly affects antioxidant defenses in fish. In this sense, it has been shown that Cd can compete with essential metals in protein-binding sites, triggering a release of Fe^{2+} and Cu^{2+} ions and causing increased generation of ROS [19]. Risso-de Faverney et al. [20] clearly related cell death to the oxidative stress caused by cadmium in trout hepatocytes. Their study has shown that only changes of cadmium concentration in the examined tissue lead to seasonal differences. Statistically significant differences were observed in the Cd concentration between the spring and winter times. Andersen et al. [21] reported low muscle concentrations of Cd in fish (roach, perch, and bream) that ranged from 0.000 mg kg^{-1} to 0.045 mg kg^{-1} w.w. However, my research has shown that the mean concentration of cadmium ranged from 0.001 to 0.022 mg kg^{-1} w.w in all the examined fish tissues.

Slight significant differences were found between Cd concentration in the summer and winter. The study results prove the influence of the season of the year on Cd concentration in fish muscle. Fish are exothermic animals and temperature significantly influences their metabolic rate. In the summer time water temperature increases, on average, 1.72-20.96°C compared to winter. A slower metabolic rate was observed in winter in comparison to spring. This could inhibit the metabolism and thus slow down Cd elimination from fish bodies. In my study, statistically significant interspecies differences were found in Cd in muscle, differently than in the study of Srebočan et al. [22], who did not observe such differences in the muscle of fish caught in ponds and rivers or of different feeding types (predatory and non-predatory).

In Danish freshwater fish, Pb seemed not to pose any problems to the consumer. The mean concentrations (0.05 mg kg^{-1} w.w.) of Pb in the muscle of predatory fish (perch and pike) ranged from 0.02 mg kg^{-1} w.w. (roach, bream) to 0.05 mg kg^{-1} w.w. (whitefish) in muscle of non-predatory fish [20]. Barak and Mason [23] did not find any difference in concentrations of metals (Cd, Pb, Hg) in muscle of roach

caught at 4 different sites on the Brett and Chelmer in rivers, England. The results of my study are comparable with the results of other authors.

Activities of antioxidant enzymes and the 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 behavioral changes, thus altering normal body homeostasis. The generation of ROS in cells impairs antioxidant defense or exceeds the ability of the antioxidant defense system to eliminate the oxidative stress. This situation may be associated with an increased influx of free radicals. Fish become more sensitive to diseases and lose adaptation capabilities to different water conditions [24]. Lipid peroxidation is a well-established mechanism of cellular injury in animals, and is used as an indicator of oxidative stress in cells and tissues. Malondialdehyde is widely used as an indicator of lipid peroxidation [25]. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in humans, other vertebrates and model systems [26, 27]. MDA reacts readily with amino groups on proteins and other biomolecules to form a variety of adducts [28], including cross-linked products [29]. MDA also forms adducts with DNA bases that are mutagenic [30] and possibly carcinogenic [31]. The thiobarbituric acid (TBARS) method is commonly used to measure MDA in biological samples [32]. However, this reaction is relatively nonspecific; both free and protein-bound MDA can react.

In my study I estimated the effect of water temperature on physiological conditions of common carp, rainbow trout, and Siberian sturgeon and found statistically significant correlations between fish age, season of the year in which the studies were conducted and any of the analyzed parameters. Water temperature in the breeding ponds ranged from 1.72-20.96°C, and exceeded the rainbow trout optimum temperature range at the end of spring and in summer. Therefore, the ponds appeared not to offer conditions good enough for rearing rainbow trout the whole year. There is a wide range of oxygen tolerance among fish. Cold-adapted fish, like rainbow trout, usually need high oxygen levels, while cyprinid species can survive from nearly full anoxia to hyperoxia [1, 2]. Hyperoxia is the opposite of anoxia/hypoxia state and stimulates the generation of free radicals. The influence of high temperature on aquatic biocenoses manifests in the increase of biological production rate and also in shortening lifecycles of aquatic organisms that die in large numbers due to the lack of synchronization with climate rhythms. Ritola et al. [33] have conducted a series of studies in which freshwater fish were exposed to hyperoxia and ozone. In one study, rainbow trout were treated with ozone or hyperoxia for 4 h, and assays were performed during a 48 h recovery period after exposure. Rearing conditions alone had no effect on lipid peroxidation indices in liver, but ozone exposure increased it after only 1 h in the gills. The authors explained that the weak effect on lipid peroxidation was due to the adequate antioxidant defenses under the given

conditions. In another experiment, these authors have shown that in vitro exposure of rainbow trout plasma and red blood cells to ozone and/or hyperoxia for 5 min resulted in marked changes in TBARS (thiobarbituric acid-reactive substances, products of lipid peroxidation) content. It is worth noting that ozone exposure causes changes beyond hyperoxia. Both treatments resulted in increased TBARS levels, although this rise was short-lived under hyperoxia compared to consistently high levels during ozone exposure. In the current study no statistically significant seasonal differences were found in SOD activity, lipid peroxidation degree (MDA) and Pb in the examined fish. However, I found interspecies and seasonal differences in Cd level in the examined fish tissues. The age of the fish had a significant influence on the study, which was done during the most intensive growth period between the 8th and 20th months of life. Wdziaczek et al. [34] studied CAT and SOD activities and lipid peroxidation in erythrocytes and livers of different fish species. They reported that younger fish showed higher antioxidant activity than older fish. My study confirms these assumptions. Also, Otto and Moon [35], who evaluated the activity of antioxidant enzymes in rainbow trout and black bullhead of two age classes (1+ and 3+), reported that glutathione reductase and SOD activities were significantly higher in hepatic and extrahepatic tissues of younger fish. Ahmad et al. [8] have shown that low lipid peroxidation reflects protective effects of oxidative enzymes. Exposure to polluted water induced tissue-specific peroxidative damage in gills, kidney, and liver of European eel (*Anguilla anguilla*), and the most affected tissue was kidney [7].

Fish appear to possess the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species [36]. Common carp and rainbow trout are important sources of human food. Gills are the first organs to be exposed to waterborne contaminants [37]. Muscle tissue is the main consumable part of a fish and bioanalysis of this tissue is essential to monitor fish quality for human consumption and health [38]. In addition, the generation of free radicals is more pronounced in muscle as a consequence of a high level of oxygen consumption [39].

Kidney plays a vital role in the maintenance of an organism's internal environment, being the key to the regulation of extracellular fluid volume and composition, as well as acid-base balance. It is also a target of toxic chemicals that can disrupt kidney function and cause temporary or permanent derangement of homeostasis [40].

Antioxidant defenses in fish are also dependant on feeding behavior and nutritional factors. In my research I found a significant effect that food eaten by the fish had on antioxidant enzyme activities and the lipid peroxidation levels, as was confirmed by Ross et al. [41].

We did not observe any influence of the season on the superoxide dismutase activity or on the degree of lipid peroxidation. SOD activity (1.20-5.84 U mg⁻¹ protein) and MDA (0.83-6.71 nmol mg⁻¹ protein) in the examined organs fluctuated around the given values throughout the research. Common carp, rainbow trout and Siberian stur-

geon were in good health condition, with the Fulton's condition factor usually above 1 (Table 1). Aller Aqua feed was enriched with vitamins E (150 mg) and A (2500 IE). This could lead to a balance of SOD activity and MDA, as was proven by small fluctuations in SOD activity throughout the study. It was also confirmed by the studies done by Parker [42], and Kohen and Nyska [43]. Studies of the antioxidant role of vitamins indicate that vitamin E is the main soluble lipid antioxidant in animals and acts as a radical scavenger [42]. Dietary vitamin E supplementation was reported to increase efficiency of flesh deposition in sea bass [44], and stabilize lipids and improved organoleptic properties of trout fillets [45]. Also, the degree of lipid peroxidation in the currently examined organs did not significantly differ among the seasons. Only interspecific differences were found.

The study revealed positive correlation (R^2 from 0.966 to 0.987) between Cd or Pb levels in fish blood or muscle and MDA levels or SOD activity. At low metal levels in the blood and dorsal muscle, higher SOD activity occurred, which reduced lipid peroxidation. Increased metal levels in the liver and kidney led to decreased SOD activity and increased lipid peroxidation. Increases in metal concentrations in the aquatic environment increased lipid peroxidation in the three fish species and simultaneously reduced SOD activity. Gabryelak et al. [46] reported significant seasonal and interspecific differences in the activities of antioxidant enzymes in erythrocytes of common carp, tench and goldfish. Reported activity was higher in spring than in autumn. However, in my study I did not estimate SOD activity in blood. Only small seasonal changes were found between the summer and winter cadmium concentration in the study fish. Palace and Klaverkamp [47] found that the activity of antioxidant enzymes varied among freshwater species from geographically proximate lakes. Lozovskaya and Lozovskii [48] reported that activity of the antioxidant defense system of interspecific sturgeon hybrid fry (Russian sturgeon with barbell sturgeon) was higher than in intergeneric hybrids (beluga with barbel sturgeon).

Conclusion

The study showed differences in Cd and Pb concentrations in the organs of common carp, rainbow trout, and Siberian sturgeon. SOD activity and MDA in common carp, rainbow trout, and Siberian sturgeon were also different in the examined organs. No difference in the degree of lipid peroxidation was found in the muscle. I only found statistically significant seasonal differences in cadmium concentrations between spring and winter.

Acknowledgements

This study was financially supported by the State Committee for Scientific Research (KBN) (grant No. N 304 026 31/0814).

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