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RESEARCH PAPER

Investigation of The Effects of Boron on Some Enzyme Activities and Lipid Peroxidation of Common Carp (*Cyprinus carpio*)

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Abstract

In this study, after the 30 days of boron exposure, the boron bioaccumulation, the catalase (CAT), carbonic anhydrase (CA) and glucose-6-phosphate dehydrogenase (G6PD) activities and a lipid peroxidation product called malondialdehyde (MDA) levels in different tissues of *Cyprinus carpio* were investigated. Boron accumulation was performed by Atomic Absorption Spestroscopy and biochemical analysis were performed by spectrophotometric method. Boron amounts of all tissues of the fishes in the aquariums containing 10 mg/L boron were higher than the amounts in control groups. It was determined that CAT and G6PD activities of the skin of the fishes in the aquarium supplemented with boron decreased significantly compared to the control group, but the other tissues were not affected significantly. CA activities of the muscle, liver, gill and brain tissues of the group exposed to 10 mg/L boron water were higher than the control group, while the activity in intestine, eye and skin tissues decreased compared to the control group. MDA levels in the muscle, liver, and gill tissues of the fishes in the boron supplemented aquariums decreased compared to the control group.

Keywords: *Cyprinus carpio*, bioaccumulation, boron, enzyme activity.

Introduction

In recent years, environmental pollution has reached terrible levels. Especially, the pollutants in waters frighten the lives of aquatic living beings as well as human health. Boron (B) is a semimetal and its utilization increases day by day. Nowadays, boron is used in different sectors such as fertilizer, drugs, rocket fuel, in detergents as cleaning and bleaching agent, in medicine, in production of soaps (Eisler, 1990; Gonzalez *et al*., 2015; Pahl *et al*. 2005). Boron mixes with water in different ways. The mixed boron affects drinking water and agricultural irrigation waters (Harari *et al*., 2012). The most important resources for drinking water are underground waters. The boron content of the underground rocks structure passes to the drinking waters by dissociation (Schoderboeck *et al*., 2010). When considering the relevant studies, the boron concentrations were observed as 0.017 - 1.9 mg/L in ground water, 0,52 – 9,6 mg/L in sea water, 0.2-1000 mg/L in streams and hot water (Argust, 1998; Tagliabue *et al*., 2014; Wolska and Brijak, 2013). World Health Organization (WHO) has defined the limit of boron in drinking water as 0.5 mg/L (WHO, 2012). Environment Protection Agency has established that boron concentration shall be lower than 1 mg/L (EPA, 2010).

Turkey ranks first in world with its boron reserve of 72%. At national level, Kütahya is one of the richest regions for boron reserves. Also, there are many boron processing plants around the province of Kütahya. This situation reinforces the potential increase of boron concentrations in waters of Kütahya region and the probability to affect the living beings by these concentrations (EMW, 2016).

Common carp (*Cyprinus carpio*) is a fish species of economic value growing up in all lakes, lagoons, reservoirs and streams in Turkey and all around the world (Memiş and Kohlmann, 2006; Rechulicz *et al*., 2014). Thus, it is one of the most studied fish species (Roohi and Imanpoor, 2015). Also, Woods (1994) reported that the range of boron concentrations can be at 0,5 - 9,6 mg/L levels in natural waters. The aim of this study is to observe the effect of 10 mg/L boron on accumulation in different tissues of *Cyprinus carpio* and on CAT, CA and G6PD activities and levels of MDA.

Materials and Methods

Fish Sampling and Experimental Procedures

In this study, common carp (*Cyprinus carpio*) fishes of 11.49±0.24 cm length, 21.77±1.29 g weight were brought from Mediterranean Aquaculture Research, Production and Training Institute (Kepez / Antalya). Boric acid (H_3BO_3), which is an inorganic form of boron, has been used in the study. At the end of one month acclimatization time, a total of 42 fishes have been randomly distributed in 6 aquariums of 100x30x40 cm dimension. 3 of these aquariums had a 10 mg/L boron concentration. The study was ended 30 days after the addition of boron into the aquariums. During this time, the physicochemical properties of aquarium waters (22˚C) were regularly controlled and aquarium water was kept at a level that will not negatively affect the health of fishes.

Fish Development

The fishes used in the experiments were fed daily with commercial feeds containing 47.5% raw protein and 6.5% fats at a 2% rate of their live weight (Tetra Discus). The food conversion efficiency, the proportional weight increase and the condition factors were calculated to observe the development of fishes.

Boron Bioaccumulation

Fish tissues (muscle, liver, gill, brain) were subjected to combustion before performing boron analyses. Combustion was performed applying 9±0.1 mL nitric acid and 3±0.1 mL perchloric acid to samples in a microwave combustion unit (CEM Mars Xpress). Then, samples were filtered and adjusted to 100 ml. Boron analyses were performed using Atomic Absorption Spectroscopy (Analytik Jena-ContrAA 300) (APHA, 1992; ASTM, 1985).

Biochemical Analysis

The muscle, liver, brain, gill, eye, skin tissues of fishes taken from aquariums were conserved at -80 ˚C until the dissection and analysis. Before the biochemical analyses, tissues were homogenized for 3 minutes at 8000 rpm in a chilled 50 µM, pH 7.4 sodium-phosphate buffer containing 0.25 M sucrose using a homogenizer. Homogenates were centrifuged 30 minutes at +4˚C, at 9500 g. Protein quantification using Bradford assay (1976), MDA Quantification using Draper and Hadley Method (1990), CAT Activity using Aebi Method (1974), CA activity using Wilbur and Anderson method (1948), G6PD activity using Beutler method (1971) were performed on the obtained supernatants.

Statistical Analyses

The data of the present study were statistically analyzed using the SPSS Package Program (IBM), version 15. The relations of fish development, enzyme activities and bioaccumulation ratios between groups were determined using the student t-Test method. The results were examined at $P<0.05$ and $P<0,001$ significance levels.

Results

The average food conversion efficiencies (unit) of the fishes used in this study were 0.55 and 0.62 in control and experimental groups respectively; the average proportional weight increases (unit) were 33.18 and 37.46 in control and experimental groups respectively; the condition factors (unit) were 1.39 and 1.42 in control and experimental groups respectively. Although the values obtained for the experimental group were higher than for control group without Boron addition, this difference was not statistically significant.

Boron accumulation levels in tissues of the fishes in the aquariums containing 10 mg/l boron water were higher than the levels in control groups, but this difference was not significant (Table 1). Boron bioaccumulation in brain tissues was higher than in other tissues in both groups.

A decrease was observed in CAT enzyme activities of muscle, intestines, gill, eye and skin tissues of fishes exposed to boron. While the CAT activities decrease observed in muscle, intestine, gill and eye tissues of fishes were not statistically important ($P>0.05$); this decrease was found to be statistically significant in skin tissue ($P<0.05$). An increase was observed in CAT enzyme activities of liver and brain tissues of fishes exposed to 10 mg/L boron water. However, these CAT enzyme activities of these tissues were not statistically significant ($P>0.05$).

Table 1. Boron bioaccumulation levels of the studied fishes in the experiments (mg/g wet weight)

	Control (n=5) gdfg(((((((8889jnon((((((98989hbkj((((((jgsphdknşfhpdıjihıorj hetg((8Grubu (Ort±SH)	10 mg/L B (n=5) (Ort±SH)
Muscle	ND	0.010±0.005
Liver	0.022±0.017	0.038±0.008
Gill	0.001±0.000	0.029±0.012
Brain	0.044±0.017	0.135±0.004

(ND: Not detected)

It was determined that the increase in CA enzyme activities in muscle tissues of fishes exposed to 10 mg/L boron water was significantly important (P<0,001). When compared to the control group, the decrease observed in intestine, eye and skin tissues of fishes exposed to boron was considered statistically insignificant (P>0.05). The increase in CA enzyme activities of liver, gill and brain tissues of fishes exposed to boron was not important neither (P>0.05).

An important decrease in G-6-PD enzyme activities of skin tissues of fishes exposed to 10 mg/L boron water was observed (P<0.001). When compared to the control group, an increase was observed in intestine, gill, brain and eye tissues in fishes exposed to boron. This increase was not considered statistically significant for intestine, gill, brain and eye tissues (P>0.05). The decrease in G-6-PD enzyme activities in liver and muscle tissues in fishes exposed to boron was not considered statistically significant neither (P>0.05).

As shown in Table 3, an important decrease in MDA levels of eye and skin tissues of fishes exposed to boron was observed. The slight decreases in MDA levels in muscle, liver, gill, intestine and brain tissues in fishes exposed to boron were not considered statistically significant (P>0.05).

Discussion

The average food conversion efficiency, the proportional weight increase and the condition factors parameters of the *Cyprinus carpio* fishes fed daily with commercial feeds at a 2% rate of their live weight and kept at a 10mg/L boron concentration were higher than for the control group, but were not of statistical significance (P>0.05). Based on this, it is possible to state that the growth and development of common carps (*C. carpio*) in waters with high concentration of boron such as 10mg/L will not be affected negatively; on the contrary, the positive effect of boron at this concentration can be mentioned. Boron is essential for normal development of living beings. There are limited data on boron effects, and especially its lethal effects on aquatic animals.

Boron accumulation levels in muscle, liver, gill and brain tissues of the fishes used in the study were higher than the levels in control groups (P>0.05). Among the tissues analyzed, Boron accumulation levels in brain tissue were found to be higher than other tissues. The accumulation order of boron in analyzed tissues was observed to be brain > liver > gills > muscle. Metal accumulation in fishes may vary according to the metal, environment concentration, effectiveness time, the species, the development stage, the metabolic activity, the physicochemical properties of water, the other metals present in the environment, the tissues and organs (Bashir *et al*., 2013; Hollis *et al*., 1999; Melgar *et al*., 1997; Sağlamtimur *et al*., 2003). In a study of Özkurt (2000), boron concentrations in Çatören and Kunduzlar Barrage Lagoons were found to be between 1,25 - 8,52 ppm. Boron bioaccumulation in muscles tissues of fishes living in these lagoons was found to be around 50-60 ppm (Özkurt, 2000). In this study, boron amount in muscle tissues of *C. carpio* exposed to boron concentration (10mg/l) higher than the boron levels of Çatören and Kunduzlar Barrage Lagoons was found to be around 10 ppb. This value obtained in our study for muscle tissue is lower compared to the values obtained by Köse *et al*. (2012) for *C. carpio.* This may be related to the limited time of one month of our study.

When analyzing the results obtained, it has been observed that 10mg/l boron concentration lead to changes in enzyme activities in different tissues of *C. carpio*, but most of these modifications were not found statistically significant. CAT and G6PDH activities of skin tissues, CA activities of muscle tissues of *C. carpio* fishes exposed to 10 mg/l boron water were significantly different compared to the control group fishes (P<0.05), but no significant difference was observed for the enzyme activities measured in the other tissues (P>0.05) (Table 2 - 3). When evaluating the studies about the effects of boron on animals, no genotoxic effect of boron has been suggested (Dieter, 1994).

Lipid peroxidation is one of the most important indicators of the oxidative damage of a cell. MDA levels are a good indicator of lipid peroxidation. The increase of MDA levels in tissues is accepted as a sign of cell damage. Although an important decrease of MDA levels was observed in eye and skin tissues of fishes exposed to 10 mg/l boron water, no

Table 2. CAT and CA activities of different tissues of the studied fishes in the experiments

	CAT (EU/mg protein)		CA (EU/mg protein)	
	Control (n=16)	Experiment (n=16)	Control (n=16)	Experiment (n=16)
Muscle	0.43±0.00	0.38±0.00	0.40±0.02	0.68±0.03**
Intestine	0.57±0.23	0.40±0.09	7.28±1.04	6.97±1.25
Liver	1.11±0.23	1.40±0.2	6.63±0.88	9.55±2.14
Gill	0.09±0.03	0.06±0.00	1.57±0.11	1.70±0.09
Brain	0.07±0.00	0.11±0.05	0.69±0.09	0.85±0.05
Eye	0.09±0.05	0.02±0.00	1.59±0.24	1.51±0.09
Skin	0.11±0.01	0.05±0.01*	3.01±0.40	2.76±0.37

*P<0.05 **P<0.001

Table 3. G6PD activities and MDA levels of different tissues of the studied fishes in the experiments

	G6PD (EU/mg protein)		MDA (nmol/ml)	
	Control (n=16)	Experiment (n=16)	Control (n=16)	Experiment (n=16)
Muscle	0.04±0.02	0.04±0.01	3.41±0.39	4.54±0.51
Intestine	0.98±0.82	5±2.47	5.01±0.4	4.04±0.24
Liver	3.46±1.31	2.77±1.28	5.25±0.68	6.20±0.50
Gill	0.47±0.04	0.52±0.04	8.71±1.22	9.74±1.41
Brain	0.28±0.03	0.34±0.02	3.42±0.34	2.58±0.31
Eye	0.48±0.09	0.51±0.07	12.79±0.98	7.23±0.52**
Skin	0.51±0.08	0.15±0.02**	6.88±0.65	4.98±0.48*

*P<0.05 **P<0.001

significant difference was determined for the other tissues. Based on this, it may be concluded that a concentration of 10 mg/l boron water does not lead to lipid peroxidation in different tissues of common carps.

As a result; it can be proposed that growth and development of common carps (*C. carpio*) in waters of 10 mg/l boron are not affected negatively, that boron bioaccumulation is not important in the tissues, and that enzyme activities do not show significant changes except for some tissues. It can be stated that boron amounts present in surface waters do not constitute a risk for common carps. However, it is difficult to state that a longer exposition to 10 mg/l boron will give the same results in common carps.

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