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Evaluation of Zinc Accumulation in Tissues of Cyprinus carpio and Oreochromis niloticus

Burcu YEŞİLBUDAK[1*], Cahit ERDEM[1]
[1]Department of Biology, Faculty of Science and Letters, Çukurova University, 01330, Adana, Turkey

*Corresponding Author:
E-mail: yesilbudak@gmail.com

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Abstract
Cyprinus carpio and *Oreochromis niloticus* were exposed to 0.5 mg L^{-1} concentration of Zn over 1, 15, 30 days and accumulation in several tissues were determined. Metal accumulation in these tissues was measured using Atomic Absorption Spectrophotometric Techniques. Statistical evaluation of the experimental data was carried out by Variance Analysis and Student Newman Keul's Procedure (SNK). Highest accumulation of zinc was in gill tissue on day 15, followed by liver and muscle tissues, whereas on day 30 the order was liver, gill and muscle tissues in both species. Tissue accumulation of zinc was higher in *C. carpio* compared with *O. niloticus.* Zn accumulation in the tissues of metal exposed fish increased with increasing exposure periods. One may conclude that the variation of zinc accumulation in tissues might be depended on metabolical, structural and functional differences of both species.

Keywords: Zinc, accumulation, *C. carpio*, *O. niloticus*

INTRODUCTION

Zinc compounds are used in wood protection, catalysts, corrosion control in drinking water systems, photographic paper, ceramics, textiles, fertilizers, pigments, batteries and as nutritional supplements or medicines [2]. As a substantial micronutrient, zinc exists in natural waters at levels from 0.002 to 0.2 mg L^{-1}; while its concentrations may rise up to 43 mg L^{-1} in polluted waters near waste water discharges [27].

Zinc was listed as priority hazardous pollutants in many countries due to its toxicity, persistence, and affinity for bioaccumulation [22]. There are a number of studies concerning increased levels of toxic metals especially in aquatic environments as a result of rapid population growth and industrialization in recent years. Heavy metals and metal compounds that form a part of the pollutants are spread in aquatic ecosystems and their acceptable levels are often expressed as micrograms or nanograms per liter [14]. However, metals pass into aquatic environments as a consequence of domestic, industrial discharges and other man-made activities [4]. As their levels in water and sediment exceed certain concentrations with time, they are transferred to upper trophic levels at more concentrated forms through the food chain [23].

Fish tissues such as gill, muscle and liver are used as indicators of heavy metal pollution in freshwater and marine environments. Gills have a wide surface area due to their lamellar structure and are in direct contact with the external environment. Liver tissue as a detoxification center is of great importance in accumulating heavy metals and is the main site in synthesizing metal binding proteins [15; 5]. Muscle is not an active tissue in accumulating heavy metals. It is, however, generally taken into account in metal accumulation studies in aquatic animals since it is the main edible tissue consumed by various organisms including human [7].

The portion of the digestive tract with the highest capacity in absorbing zinc was the upper region of the intestine whereas the stomach had the least capacity in *Pseudopleronectes americanus* (Walbaum, 1792) [18]. Zinc homeostasis is maintained in fish by regulating the excretory mechanisms and controlling gastrointestinal uptake.

Zinc, as copper, catalyzes the synthesis of enzymes that function at variety of biochemical events in low concentrations. However, increased concentrations in tissues lead to structural and functional abnormalities [21].

Environmental factors such as dissolved oxygen, temperature, hardness, salinity and the presence of other metals influence zinc toxicity in aquatic organisms (Skidmore, 1964). For example, hypoxic conditions and high temperatures increase, whereas salinity and hardness decrease its toxicity [27].

O. niloticus and *C. carpio* (Linnaeus, 1758) are widely distributed in Çukurova region and are economically cultured as an alternative protein source. Fish are generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems [24]. Hence the aim of the present study were to find out accumulation of zinc in gill, liver, muscle tissues of these two species comparatively under the same ambient conditions. Thus our objective was to detect accumulation of zinc in tissues of *C. carpio* and *O. niloticus* after exposing 0.5 mg L^{-1} Zn over 30 days. The concentration of 0.5 mg L^{-1} Zn is well below the 96 h LC_{50} values of both species [6; 10].

MATERIALS and METHODS

Two fresh water fish species, *O. niloticus* and *C. carpio* (Linnaeus, 1758) were used as experimental materials. Fish were obtained from the cultivation pools of the 6th Regional Directorate of State Water Works, Adana. Experiments were carried out in the Basic Sciences research laboratories of

Mersin University, Faculty of Aquaculture under controlled conditions.

Experiments were carried out in two series taking the species examined into consideration. Two glass aquaria 40x120x40 cm in size were used in each series. The first aquaria in each series were filled with 120 liters of 0.5 mg L^{-1} Zn whereas the second aquaria were filled with the same amount of zinc free tap water and used as the control groups. Zinc sulphate salt ($ZnSO_4.5H_2O$) was used in the preparation of the test solutions. Trisodium citrate ($C_6H_5Na_3O_7.5H_2O$) was added to stock solutions of metals in order to prevent the precipitation of the metal solutions.

The experiments were carried out in triplicate with two fish at each replicate. Taking the 1, 15 and 30 days of exposure into account 18 fish were placed in each aquarium. The experimental room was air conditioned (25±1.1 ºC) and illuminated with two fluorescent lamps (daylight 65/80 W) for 12 hours. Experimental aquaria were aerated with central air conditioning system and fish were fed once a day with commercial fish food (Çamlı Feed, İzmir - TURKEY, Pınar: Palette No: 2) in amounts 2% of their total biomass. Dissolved oxygen and temperature were measured using an oxygen meter (YSI 550A) and pH using a pH-meter (WTW 330i). Total alkalinity and total hardness were measured making use of titration methods [3]. Some physical and chemical parameter values of the experimental water were as follows; water temperature 25±1.2 ºC, total hardness 268.7±4.8 mg $CaCO_3$ L^{-1}, pH 6.91±0.1, total alkalinity 319±0.5 mg $CaCO_3$ L^{-1}, dissolved oxygen 6.46±0.6 mg O_2 L^{-1}. Zinc levels in tap water were below the detection limits and Zn levels of experimental water measured at different exposure periods were 0.490±0.12 mg Zn L^{-1}. Calibration of instrument was done by standard solutions prepared from commercial materials. Heavy metal concentrations of the tissue samples were analyzed in the laboratory following the standard methods of APHA [1].

Water in experimental and control tanks were replaced once in two days to avoid changes in concentration due to adsorption, precipitation and evaporation. Six fish were removed from each aquarium at the end of specified periods. Gill, liver and muscle tissues of two fish used in each replication were dissected and combined. Tissues were left at 150^{0}C for 48 hours and their dry weights were determined (Sartorius CP-224S). They were then transferred to test tubes, a mixture of nitric acid (Merck, 65%, S.G.: 1.40) and perchloric acid (Merck, 60%, S.G.: 1.53) (2:1; V/V) was added and were wet burned at 105^{0}C for 3 hours [13]. Tissues were transferred to polyethylene tubes and their total volume was made up to 5ml with distilled water. Zinc levels in the tissues were determined using an Atomic Absorption Spectrophotometer (GBC 999). Experimental data was analyzed statistically by a series of Analysis of Variance and SNK [19] using a SPSS 15.0 statistical program. All statistical analyses were conducted using the Office Excel 2003 software package.

RESULTS and DISCUSSION

No mortality was observed in *O. niloticus* and *C. carpio* exposed to 0.5 mg L^{-1} zinc over 30 days period. Zinc levels in gill, liver and muscle tissues of *C. carpio* and *O. niloticus* exposed to 0.5 mg L^{-1} Zn for 1, 15 and 30 days are given in Table 1 and Figure 1.

Table 1. Statistical evaluation of zinc accumulation in tissues

Exposure Days	*C. carpio* * [1]			*O. niloticus* * [1]		
	Muscle	Gill	Liver	Muscle	Gill	Liver
Control	as	at	ax	as	at	at
Day 1	bs ↑	bt ↑	as ↑	as ↔	at ↓	as ↓
Day 15	as ↑	bt ↑	ax ↑	as ↑	at ↑	as ↓
Day 30	as ↑	bt ↑	bt ↑	as ↑	as ↓	as ↓

*= SNK; Letters a, b and s, t, x show differences among exposure periods and among tissues respectively. Data shown with different letters are significant at the P<0.05 level.

[1]= Change of Zn accumulation in tissue with respect to control (↑: increase, ↑: decrease, ↔: no change)

No significant difference was observed in muscle Zn accumulations of both species at the end 30 days exposure period (P>0.05). Zinc accumulation decreased significantly with increasing exposure periods in gill tissue of *C. carpio* (P<0.05) while the measured accumulations on days 1, 15 and 30 were significantly increased comparing with Zn accumulation of the control group. There was no significant exposure period difference in muscle, gill and liver tissues of *O. niloticus* (P>0.05). Liver accumulation of Zn was only significant on day 30 compared with the shorter exposure periods in *C. carpio* (P<0.05). A 58% and 286% increases were observed accumulation of Zn in gill and liver tissues of *C. carpio* respectively on day 30 compared with control. In general gill and liver tissues of *C. carpio* accumulated more Zn than that of *O. niloticus*. Exposure to 0.5 mg L^{-1} Zn increased the metal accumulation significantly in gill and liver tissues of *C. carpio* on day 30 compared with the control (P<0.05; Table 1 and Figure 1). This difference,

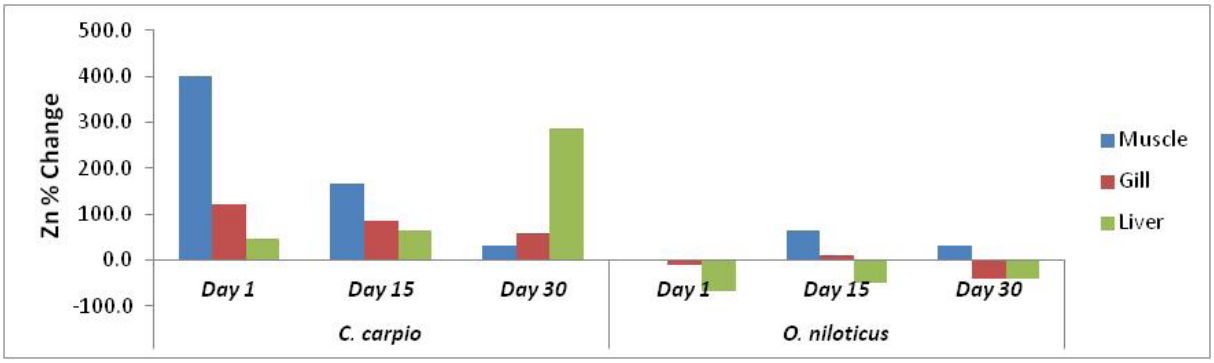

Figure 1. Change of Zn accumulation in muscle, gill and liver tissues of *C. carpio* and *O. niloticus* with respect to control (%)

however, was not significant for muscle tissue (P>0.05). Highest metal accumulation was observed in liver followed by gill and muscle at all the exposure periods tested.

There was no significant time depended accumulation in the tissues *O. niloticus* (P>0.05). Zinc accumulation was higher in gill followed by liver and muscle tissues on days 1 and 15 (P<0.05), whereas there was no statistical difference among the tissues on day 30 (P>0.05; Table 1 and Figure 1).

When the two species were compared for their tissue accumulation of zinc, gill accumulation of *C. carpio* was significantly higher than *O. niloticus* at all the exposure periods tested (P<0.05), being eightfold higher on day one. Accumulation of zinc in liver tissue was also higher in *C. carpio* than *O. niloticus* at all exposure periods (P<0.05). There was a significant difference in muscle accumulation of zinc between the two species on days 1 and 15, being higher in *C. carpio* than *O. niloticus*.

Zinc accumulation was highest in liver tissue of *Scylorhinus canicula* (L., 1758) exposed to sublethal concentrations of zinc for 3 weeks [17]. Lowest zinc levels in *O. niloticus* and *C. carpio* were detected in muscle tissues at the end of 30 days exposure period. Recently a similar result was obtained for cadmium accumulation in these two fish species indicating the lowest accumulation in muscle tissues [25]. Detection of highest metal level in liver followed by muscle tissue could be explained by transportation of metals from liver to other tissues in order to extend the carrying capacity of liver, which is the detoxification center. One may explain the high levels of Zn in liver by the bindings of Zn to metallothionein (MT) contained at highest concentration by liver [11].

Zinc concentrations in various tissues of 24 aquatic organisms was studied comparatively and zinc levels in muscle, gill and liver tissues of *C. carpio* was higher than that of *Oreochromis mossambicus* (L., 1758) [20]. The level of zinc were found to be much higher in gill tissue compared with liver and muscle tissues in *O. niloticus* and *C. carpio* exposed to 0.5 mg L^{-1} Zn for 1, 15 and 30 day periods.

Exposure to heavy metals increases mucus secretion in fish to prevent gill uptake, hence high levels of metals found in this tissue might be due to mucus bounded metals. The liver is playing a major role in detoxification and storage for heavy metals. The gill of fish has a direct contact with the Zn in the water so that they play a role in the uptake and excretion of the Zn [12].

Heavy metal accumulation in tissues of fish is dependent on exposure dose, period, water temperature, age of fish, interaction with other metals, water chemistry and metabolic activity the of fish [16; 9; 8]. It is difficult to compare the metal accumulations even between the same tissues of different species because of metabolic, structural and functional differences. Zinc accumulation was generally higher in the tissues studied in *C. carpio* than in *O. niloticus* compared to control at all exposure periods which can be explained by interspecies differences in metal metabolism of the two species studied.

Studying heavy metal accumulation helps not only to determine structural and functional disorders in metal sensitive aquatic organisms but also to evaluate the environmental effects of metal pollution and to understand their routes of uptake, biotransformation and excretion [26]. To sum up, results show that the fish tissues can be useful bio-indicators of Zn contamination in water.

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