Bioaccumulation and Depuration of Copper in the Kidney and Liver of a Freshwater Fish, *Capoeta fusca*

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ABSTRACT

Background: This study aims to investigate the patterns of bioaccumulation and depuration of copper in the selected kidney and liver of *Capoeta fusca*.

Methods: The fish were collected between September and November 2010 from a qanat in Birjand. They were exposed to two types treatments with copper (0.25 and 0.75 mg/L) for a period of 41 days. The fish under study were exposed to the above-mentioned sub-lethal concentrations separately for 14 and 21 days (accumulation period). At the end of this period, the remaining fish were kept in tap water (elimination period) for 31 and 41 days.

Results: The findings showed that the accumulation of copper in lower and higher sub-lethal concentrations was higher in kidney as the mean accumulation of copper on day 21 was 1.9±0.1 µg/g and 2.93±0.47 µg/g respectively, in 0.25 µg/g and 0.75 µg/g concentrations. On the other hand, the results also showed that the depuration level of copper in the given concentrations was higher in liver than kidney. The bioaccumulation and depuration of copper significantly increased in the kidney and liver of *C. fusca* ($P<0.01$).

Conclusion: Based on the present work, it is concluded that *C. fusca* has a potential for the rapid accumulation and depuration of copper in freshwater. Also, the results indicate that the fish *C. fusca*, as representative fish species in the East of Iran, can be a useful bioindicator organism of water contamination with copper.

Keywords: Copper, Elimination, Long-Term Exposure, Toxicity, Uptake.

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INTRODUCTION

Birjand is the center of South Khorasan province in the east of Iran. Although there are no permanent rivers in this province, there exist valuable sources of native fish population in its qanats. Qanat is a unique environment for fishes and comprises an adit which taps the groundwater and provides a permanent flow (1). In many districts of the plateau of Iran, fishes are only observed in qanats, some of them have flown there for hundreds of years (2). The *Capoeta fusca*, a cyprinid, is one of the most important fishes in qanats in the east of Iran (3). Importance of this species of fish has been recognized from the genetic conservation point of view.

Pollution of the natural environment by heavy metals is a worldwide problem because these metals are indestructible and when they exceed a certain concentration, most of them have toxic effects on living organisms (4). Heavy metals enter the aquatic environment by atmospheric deposition, by weathering from the geological matrix, or from anthropogenic sources, such as industrial discharge, sewage, agricultural waste, and mining wastes (5, 6). Fish species are widely used

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to biologically monitor variation in environmental levels of anthropogenic pollutants (7, 8). Studies have also indicated that fish are able to accumulate and retain metals from their environment and the accumulation of heavy metals in organs of fish is dependent upon exposure concentration and duration as well as other factors, such as pH, hardness, characteristics of the individual fish or species, habitat, metabolic rate of the animals, and so forth (9-12). Therefore, it is of great importance to undertake a study to determine the amount of metal pollutants in order to control and manage a suitable conservation of them.

Copper is an essential element which is carefully regulated by physiological mechanisms in many organisms (13, 6); it may also bioaccumulate and reach toxic levels (14). Similar to other trace elements, Cu toxicity depends on the species, age, diet; and a reflection of variation in efficiency of absorption (15,16). Copper level in natural unpolluted water is as low as 0.5–1 μg L^{-1} (17). However, industrial developments have contributed to a continuous increase of copper in the aquatic ecosystem (18). Few studies have been carried out on this fish species due to the fact that it has not been wide spread in different regions. Anyway, the aim of this study was to investigate the bioaccumulation and depuration of copper in kidney and liver organs of a native fish, *Capoeta fusca*, at laboratory condition, which is observed only from the east of Iran.

MATERIALS AND METHODS

From September to November 2010, *C. fusca* belonging to the family Cyprinidae, with average weight (±SD) of 17.3 (±1.9) g and average length of 12.4 (±0.6) cm were obtained from a qanat in Birjand. The fish were transported to the laboratory in polythene bags by water of qanat. Prior to the experiment, the fish, for 10 days, acclimatized to the laboratory conditions in pre-cleaned glassy aquariums with tap water. Fish were separately maintained at 21.5±1.3°C, pH 7.8±0.2; hardness 380±3.2 mg/L as $CaCO_3$; nitrite 0.07 mg/L; dissolved oxygen 6.5±0.2 mg/L; and ammonia 0.05 mg/L. The tap water had no detectable amount of copper.

In the present study, the heavy metal copper in the form of copper chloride ($CuCl_2$ $6H_2O$-Analar grade, Merck) was used. The 96 h LC_{50} concentration of copper was 7.5 mg/L for *C. fusca* as calculated by using probit analysis method. The fish were divided into seven groups of 14; the first group served as the control group, and the others as the experimental ones. Thereafter, these 14-fish samples were randomly exposed to 40 liters of water in the aquarium. Copper accumulation and depuration were studied in fish exposed to one-thirtieth and one-tenth of LC_{50} taken as lower (0.25 mg/L) and higher (0.75 mg/L) of 96 hr LC_{50} concentration of copper over 41 days of exposure. The fish were exposed to the above-mentioned sub-lethal concentrations separately for a period of 14 and 21 days (accumulation period). At the end of these periods, the remaining fish were kept in tap water (elimination period) for another period of 31 and 41 days. At each interval of 14, 21, 31 and 41 days of long-term exposure, six fish were sampled from each group for determination of copper in kidney and liver.

At the end of each exposure period, the organ samples (kidney and liver) were digested in a mixture including nitric acid (HNO_3) and perchloric acid ($HClO_4$). The organs were, then, accurately weighed into 150-mL Erlenmeyer flasks, and 10 mL nitric acid (65%) was added to each sample and the samples were left overnight to be slowly digested (19). After that, 5 mL perchloric acid (70%) was added to each sample. Digestion was performed on a hot plate (sand bath) at 200°C, for about 6h until the solutions were clear. Later, the digested samples were diluted by 25 ml distilled water.

The concentration of copper was measured by using a Shimadzu AA 680 flame furnace atomic absorption spectrophotometer and the copper concentration, in an organ, was presented as µg/g wet weight. All the experiments were conducted in 3 replications and the average of the values was reported along with standard deviations. To analyze the significant difference between the rates of copper concentration in kidney and liver, analysis of variance (ANOVA) was used. Data analysis was carried out using the statistical package Minitab (Release 14).

RESULTS

The average concentration of copper in the kidney and the liver of *C. fusca* under different exposure periods are given in tables 1 and 2. The results showed that, after 21 days, the accumulation level of copper in the kidney and the liver was 1.9±0.1 µg/g and 1.12±0.08 µg/g respectively in the 0.25 µg/g concentration while in the 0.75 µg/g concentration; it was 2.93±0.47 µg/g and 1.99±0.19 µg/g. Also, the results indicated that, after 41 days, the depuration level of copper in the kidney and the liver was 0.53±0.05 µg/g and 0.30±0.03 µg/g respectively in the 0.25 µg/g concentration whereas in the 0.75 µg/g concentration, it was 0.87±0.17 µg/g and 0.40±0.14 µg/g. The bioaccumulation level of copper in the 0.25 µg/g and 0.75 µg/g concentrations was higher in the kidney, whereas during the depuration period, the depuration level of copper in the given concentrations was higher in the liver.

The results of statistical analysis showed that the bioaccumulation and depuration levels of copper by two organs, the kidney and the liver, were significant at the higher level ($P<0.01$) in the given concentrations.

Table 1. Bioaccumulation and depuration of copper in the kidney and liver of *C. fusca* exposed to 0.25 µg/g ww concentration.

Organ	Control Group	Treated groups				*P*-value
		accumulation		depuration		
		14 days	21 days	31 days	41 days	
Kidney	0.58±0.02	1.24±0.06	1.9±0.1	0.77±0.1	0.53±0.05	P> 0.01*
Liver	0.36±0.04	0.95±0.02	1.12±0.08	1.04±0.22	0.30±0.03	P> 0.01*

* The difference between the control and the exposure groups is statistically significant at P< 0.01
Values are expressed as mean of six individuals ± SD.

Table 2. Bioaccumulation and depuration of copper in the kidney and liver of *C. fusca* exposed to 0.75 µg/g ww concentration.

Organ	Control Group	Treated groups				*P*-value
		accumulation		depuration		
		14 days	21 days	31 days	41 days	
Kidney	0.58±0.25	1.2±0.36	2.93±0.47	1.81±0.04	0.87±0.17	P> 0.01*
Liver	0.47±0.23	2.4±0.45	1.99±0.19	0.92±0.04	0.40±0.14	P> 0.01*

* The difference between the control and the exposure groups is statistically significant at $p < 0.01$
Values are expressed as mean of six individual ± SD.

DISCUSSION

The concentration of a metal in an organism is the product of equilibrium between the concentration of the metal in an organism's environment and its rate of ingestion and excretion (20). Uptake and depuration are two of the most important factors in metal metabolism. Metal accumulation in the organs of fish varies according to the rates of uptake, storage, and depuration (18). This means that metals which have high uptake and low depuration rates in the organs of fish are expected to be accumulated to higher levels (21,22).

The results of the present study showed that the bioaccumulation level of copper was higher in the kidney (Tables 1 and 2). According to Palaniappan and Karthikeyan (8), the bioaccumulation level of chromium and nickel in different organs of *Cirrhinus mrigala*in were 1.082 µg/g and 6.07 µg/g concentrations, and after 21 days were higher in the kidney than in the liver. Asagba *et al*. (23) have also reported high accumulation of the cadmium in the kidney of *Clarias gariepinus* than in any other organs. The results of a study by Akan *et al*. (24) done on different organs of *Tilapia zilli* indicated that the accumulation level of copper was higher in the kidney. Also, the results of a study done by Senthil Murugan *et al*. (25) on the bioaccumulation pattern of zinc in different organs of *Channa punctatus (Bloch)* for a period of 45 days showed that the bioaccumulation level of zinc was higher in the kidney than liver. However, according to Ghedira *et al*. (26), Subathra and Karuppasamy (18), and Gbem *et al*. (26), the accumulation level of copper in different organs of *Sparus aurata*, *Mystus vittatus*, and *Clarias gariepinus* was respectively higher in the liver.

The concentration of copper in kidney rose from 0.58 to 1.9 µg/g and from 0.58 to 2.93 µg/g in 0.25 and 0.75 µg/g sub-lethal concentrations respectively; i.e. 3 and 5-time increases compared to control group. Also, the highest level of accumulation (BCF = 3.9±0.63 for 0.25 µg/g and 7.2±0.40 for 0.75 µg/g) was found in the kidney (Table 3). The copper level in kidney of fish exposed to two different sublethal concentrations of copper was significantly higher (P<0.01) than the level found in the control groups in all exposure periods. Since kidney is the principal organ involved in the storage of metal, in all the experimental treatments, the highest level of the accumulation is observed in this organ. This might be due to the strong irrigation and the function of excretion (8). On the other hand, the increased accumulation level of copper in the kidney, over time (Figure 1) is more consistent. Topashka-Ancheva *et al*. (27) and Mazon and Fernandes (28) suggested that these increased copper contents in the kidney are possibly from selective reabsorption of essential electrolytes and glucose as well as essential metals such as copper from urine at the proximal tubules (18).

Table 3. Magnitude of bioaccumulation, Bioconcentration factor (BCF) of copper in the kidney and liver of *C. fusca* exposed to 0.25 and 0.75 µg/g ww concentration.

Concentration/Organ	Magnitude of bioaccumulation		Bioconcentration factor(BCF)
	14 days	21 days	
0.25 µg/g			
Kidney	×2.12	×3.25	3.9±0.63
Liver	×2.52	×3.11	2.6±0.26
0.75 µg/g			
Kidney	×1.86	×5.6	7.2±0.40
Liver	×6.19	×4.6	4.8±0.32

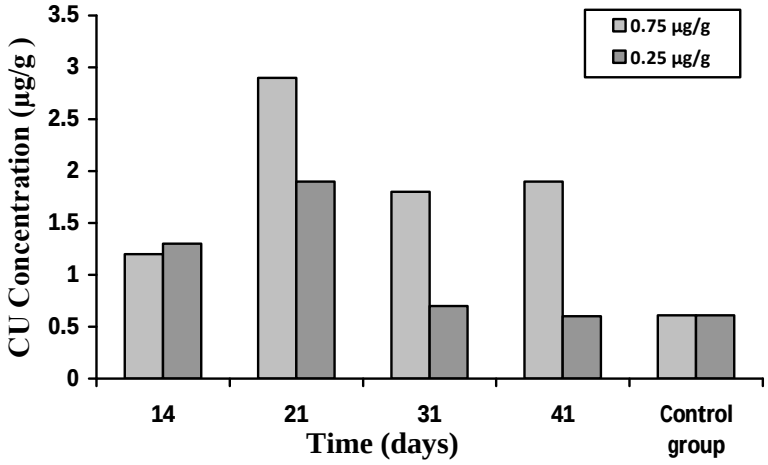

Figure 1. Accumulation and depuration of copper in the kidney of C. fusca exposed to 0.25 and 0.75 µg/g concentrations.

The concentration of copper in the liver of the experimental group exceeded approximately 3 times than that was found in the same organ of the control group (from 0.38 to 1.12 µg/g) in lower (LSL) sub-lethal concentrations, while the concentration of copper in the liver increased 4 times (from 0.47 to 1.99 µg/g) at higher (HSL) sub-lethal concentration (Figure 2). The level of copper content in the liver of treated groups was significantly ($P<0.01$) higher when compared to the control groups. Metal concentrations in the liver reflect its multifunctional role in detoxification, storage processes, and redistribution; hence, it is an active site of pathological effects induced by metal contaminants (9, 29, 8). It also acts as a better indicator of water pollution than other organs in fish. This may be attributed to the inclination of liver to accumulate contaminants of various kinds at higher levels from the environment (9, 30). Moreover, the accumulation of the tested metals in liver could be based on the greater propensity of the elements to react with the oxygen carboxylate, amino group, nitrogen, or sulphur of the mercapto group in the metallothionein protein which has the highest concentration in the liver. These complexing agents are slowly redistributed to the renal cortex.

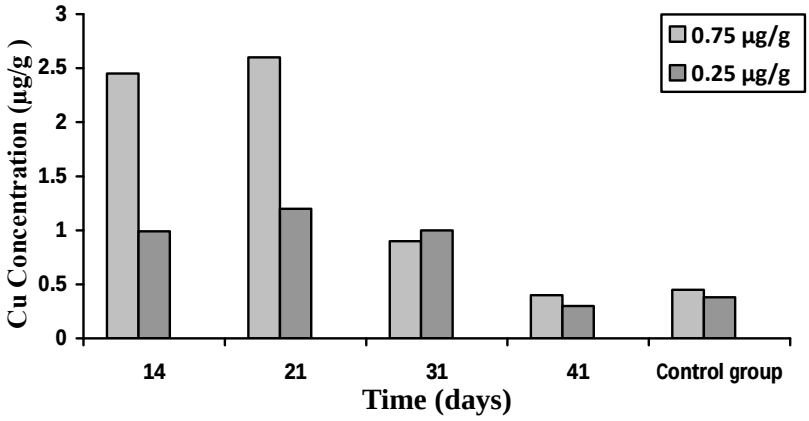

Figure 2. Accumulation and depuration of copper in the liver of C. fusca exposed to 0.25 and 0.75 µg/g concentrations.

Like accumulation, several factors influence the depuration of metals from the organs such as duration, temperature and metabolic activity of fish as well as the organ concerned (21,16). The depuration experiments were started after 21 days of absorption. In the present study, liver showed the greatest depuration of copper to two different sub-lethal concentrations. This is consistent with the findings presented by Subathra and Karuppasamy (18), as it showed the higher level of depuration of copper in liver.

CONCLUSION

The accumulation of copper in *C. fusca* was observed to be rapid and bioaccumulation increased with increasing metal concentrations in water and exposure time. In conclusion, the accumulation and depuration of copper in *C. fusca* depend on the organ, concentration, and exposure time.

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