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BIOACCUMULATION AND ELIMINATION RATE OF COBALT BY *CAPOETA FUSCA* UNDER CONTROLLED CONDITIONS

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ABSTRACT

The objective of the present study was to investigate the pattern of accumulation and elimination of cobalt on selected organs of *Capoeta fusca*, after chronic exposure. During July to September 2010, *C. fousca* was obtained from qanat in Birjand. Cobalt accumulation and elimination were studied in fish exposed to one- thirty of LC_{50} taken as 6.8 mg/L of 96 hr LC_{50} concentration of cobalt over 30 days of exposure. The cobalt was assayed using Shimadzu AA 680 atomic absorption spectrophotometery and the results were given as µg/g wet wt (from fish). This finding showed that the accumulation patterns of cobalt are in the following order: liver>muscle>gill>skin. The elimination patterns of cobalt are in the following order: skin>gill>muscle>liver. The bioaccumulation and elimination of cobalt was significantly increased in the organs of *C. fusca* ($p<0.01$). The accumulation of cobalt in *C. fusca* was observed to be rapid and bioaccumulation increasing metal concentration in the water and with exposure time. In conclusion, the results of the present study showed that the accumulation and elimination of cobalt in *C. fusca* is dependent on organ and time.

Key words: *Capoeta fusca*, exposure time, bioaccumulation and bio-elimination, cobalt toxicity.

INTRODUCTION

The term qanat, describes an underground water channel, consisting of vertical shafts connected at their bottom with a sub-horizontal tunnel (Stiros, 2006). Qanats are mostly dug in places where there is no permanent and reliable water on the surface. Birjand, a desert region, is the center of province of South Khorasan in the east of Iran and it is one of the desert regions. Though there are no any permanent rivers in the province their qanats are sources of native fish population (Omidi *et al* ., 2009). Fishes in qanats, all over Iran constitute 25 species in Coad's research which constitute 40% of the plateau fauna (Johari *et al* ., 2009). The *Capoeta fusca*, a cyprinid, is one of the most important fishes in qanats of eastern Iran. This family consists of a total of 20 species distributed in the South of China, North of India, Turkmenistan, Aral Sea, Middle East and Anatoly (Alp *et al* ., 2005).

Pollution of the natural environment by heavy metals is a worldwide problem since these metals are non-biodegradable and many of them have toxic effects on living organisms up to certain concentration (Mansouri *et al* ., 2011a; Ghrefat and Yusuf, 2006). One of the most important properties of a toxic pollutant is its ability to accumulate in the organs of an aquatic organisms (Palaniappan and Karthikeyan, 2009). Bioaccumulation of metals reflects the amount of toxin ingested by the organism, the pattern in which the metals are distributed through different organs and the extent to which the metals remained in each one of these organs (Senthil Murugan *et al* ., 2008). Therefore, it is of great importance to know the bioaccumulation potential of a pollutant (Palaniappan and Karthikeyan, 2009). Heavy metals are taken up through different organs of the fish because of their high uptake affinity. In this process, many of these heavy metals are concentrated at different levels in different organs of the body (Erdoğrul and Ates, 2006). Studies have shown that fish are able to accumulate and retain heavy metals from their environment (Vinodhini and Narayanan, 2008; Subathra and Karuppasamy, 2008; Asagba *et al* ., 2008) and it has been shown that accumulation of metals in tissues of fish is dependent upon concentration and exposure time, salinity, temperature, hardness and metabolic rate of an organism (Canli *et al* ., 1998; Canli and Atli, 2003). Fish in freshwater environments near urban and industrial locations often face pollutants due to run-off and in most cases deliberate discharges. Knowledge of the distribution of metals in tissues is useful in identifying particular organs that are sensitive and selective to heavy metal accumulation (Gbem *et al* ., 2001). Mansouri *et al* . (2011b) investigated Bioaccumulation and elimination of nickel concentrations in selected tissues of *Capoeta fusca*. Ebrahimpour *et al* . (2010b) studied the influence of water hardness on acute toxicity of copper and zinc concentration on *Capoeta fusca*. The objective of the present study was to investigate bioaccumulation and elimination pattern of cobalt in gill, liver, muscle and skin organs of a native fish, *Capoeta fusca* under laboratory conditions.

MATERIALS AND METHODS

During July to September 2010, *C. fusca* belonging to the family cyprinidae, with average weight (20.6 ±1.42 g) and length of 13.6 (±0.18) cm were obtained from qanat in Birjand. The fish was transported to the laboratory in polythene bags in same water. Prior to the experiment, fish were acclimatized to the laboratory conditions for 10 days Fish were separately maintained at 25.9±1.2°C, pH 8.3±0.5; hardness 295±18 mg/L as $CaCO_3$; nitrite 0.04±0.03 mg/L; dissolved oxygen 6.1±0.1 mg/L; ammonia 0.05±0.04 mg/L, at least for 40 days prior to the experiments in cobalt free tap water.

Cobalt was used in the form of cobalt chloride ($CoCl_2$ $6H_2O$-Analar grade, Merck). In these studies the 96 h LC_{50} of cobalt was 204 mg/L for *C. fusca* as calculated by using probit analysis method (it was calculated before experiment). Fish were divided into four groups each containing 14 fish. Group 1 served as control and the remaining were experimental ones. Thereafter, these 14-fish samples were exposed randomly to 40 liters of water in the aquarium. Cobalt accumulation and elimination were studied in fish exposed to one-thirty of LC_{50} taken as 6.8 mg/L of 96 hr LC_{50} concentration of cobalt over 30 days of exposure. The fish were exposed to the above-mentioned concentration separately for a period of 5, 10 and 15 days (accumulation period). At the end of exposure trials these trials, the remaining fish were kept in tap water for another 20, 25 and 30 days (elimination period).

At the end of each exposure period, dissections for separate organs (gills, liver, muscle and skin) were performed. Two fish were pooled in order to take two sample organs (gills, liver, muscle, and skin) as the weight of muscles and skins of two fish was 1 g and the weight of livers and gills was 0.5 g. The organ samples were digested in a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (Ebrahimpour and Mushrifah, 2010a; Mansouri *et al* ., 2011b). Organs were, then, accurately weighed into 150-mL Erlenmeyer flasks and 10 mL nitric acid (65%) was added to each sample. The samples were then left overnight for slow digestion (Ip *et al* ., 2005; Mansouri *et al* ., 2011b). Thereafter 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 or until the solutions were clear. The digested samples were diluted by 50 ml distilled water. The concentration of cobalt was measured by Shimadzu AA 680 flame furnace atomic absorption spectrophotometer. All the experiments were conducted in 3 replicates and the average of the values was reported along with standard deviations. The results, expressed in µg metal/g dry tissue (µg/g), were treated statistically using analysis of variance (ANOVA) for the comparison of several means. Statistical analyses were carried out using Minitab ver. 15.0.

RESULTS AND DISCUSSION

Table 1 summarizes the data of the average concentration of cobalt in the selected organs of *C. fusca* under different exposure periods. The accumulation patterns of cobalt are; liver > muscle > gill> skin, respectively. Also, the elimination patterns of cobalt are; skin> gill > muscle > liver, respectively. The liver accumulated the highest level of cobalt (6.39±0.26 µg/g), and then the gill (5.91±0.24 µg/g). Elimination was highest in skin level of cobalt (0.88±0.07 µg/g), and then the liver (0.98±0.17 µg/g). Examination of the levels of cobalt after 5 days of exposure (Table 1) shows that the maximum tissue load was in the liver, while after 15 days of exposure, the rate of incorporation of cobalt in liver and muscle was higher than gills.

Results of the study revealed increases of the cobalt concentrations in every tissue sampled from the tested group at the end of the experimental period (Table 2). The cobalt level in liver of fish exposed was significantly higher ($P<0.01$) than the level found the control group at exposure periods. Thus, in liver tissue its concentration increased 26 times towards control group (from 0.24 to 6.39 µg/g). The increased accumulation of cobalt in the liver over time (Fig. 1) this may be due to the movement of metals from the gills and from the other tissues to the liver for detoxification (Kalay and Canly, 2000). According to Subathra and Karuppasamy (2008) the accumulation level of copper in different organs of *Mystus vittatuswas* higher in the liver; this study was accomplished in a period of 96 hours and 28 days. Kotze *et al* . (1999) have reported a higher accumulation of copper in liver tissue than any other organ in *Oreochromis mossambicus* as well as in *Clarias gariepinus*. Target organs, such as liver has tendency to accumulate heavy metals in high values, as shown in many species of fish in different areas; in *Liza abu* and *Silurus triostegus* in Atatürk Dam Lake (Karadede *et al* . 2004), in *Scardinus erythrophthalmus* in Karataş Lake (Kır *et al* . 2006), in *Cyprinus carpio* in Beyşehir Lake (Tekin-Özan and Kır 2007), in *Barbus capito pectoralis* and *Chondrostoma nasus* in Büyük Menderes River (Koca *et al* ., 2008). Liver tissues are believed to be the main site of trace metal detoxification within fish (Kirby *et al* ., 2001). This can possibly be attributed to the tendency of the liver to accumulate pollutants of various kinds at higher levels from the environment (Licata *et al* . 2005). In the other words, metallothionein biosynthesis is induced after exposure to sublethal levels of heavy metals (Wong *et al* ., 2001). In addition, the accumulation of the tested metals in liver could be based on the greater tendency of the elements to react with the oxygen carboxylate, amino group, nitrogen or sulphur of the

mercapto group in the metallothionein protein which is at highest concentration in the liver (Al-Yousuf *et al* ., 2000).

Table 1: Accumulation and elimination of cobalt in the selected organ tissues of *C. fusca* exposed to 6.8 µg/g concentration

Organ	Accumulation			Depuration		
	5 days	10 days	15 days	20 days	25 days	30 days
Control						
Gill	0.25±0.01	0.21±0.04	0.1 ±0.03	0.22±0.01	0.12±0.05	0.25±0.01
Liver	0.46±0.08	0.25±0.04	0.24±0.04	0.30±0.02	0.19±0.01	0.25±0.01
Muscle	0.18±0.03	0.17±0.04	0.24±0.06	0.20±0.02	0.17±0.01	0.19±0.02
Skin	0.19±0.04	0.16±0.04	0.18±0.03	0.06±0.01	0.12±0.1	0.11±0.01
6.8 µg/g						
Gill	2.40±0.15	3.80±0.99	5.49±0.17	3.38±0.37	2.67±0.19	0.98±0.17
Liver	2.54±0.30	4.91±0.42	6.39±0.26	2.50±0.44	2.52±0.44	1.55±0.05
Muscle	1.33±0.15	3.36±0.32	5.91±0.24	2.84±0.66	1.55±0.43	1.16±0.22
Skin	1.36±0.20	2.71±0.39	4.6 ±0. 2	3.96±0.24	2.21±0.38	0.88±0.07

The difference between the control and exposures are statistically significant at $P < 0.01$

Table 2: Magnitude of bioaccumulation, Bioconcentration factor (BCF) of cobalt in the tissues of *C. fusca* exposed to 6.8 µg/g concentration

Organ	Magnitude of bioaccumulation			Bioconcentration factor
	5 days	10 days	15 days	
Gill	×9.6	×18	×30.5	0.80±0.02
Liver	×5.5	×19.6	×26.6	0.91±0.01
Muscle	×7.3	×19.7	×24.6	0.86±0.03
Skin	×7.1	×16.9	×25.7	0.68±0.01

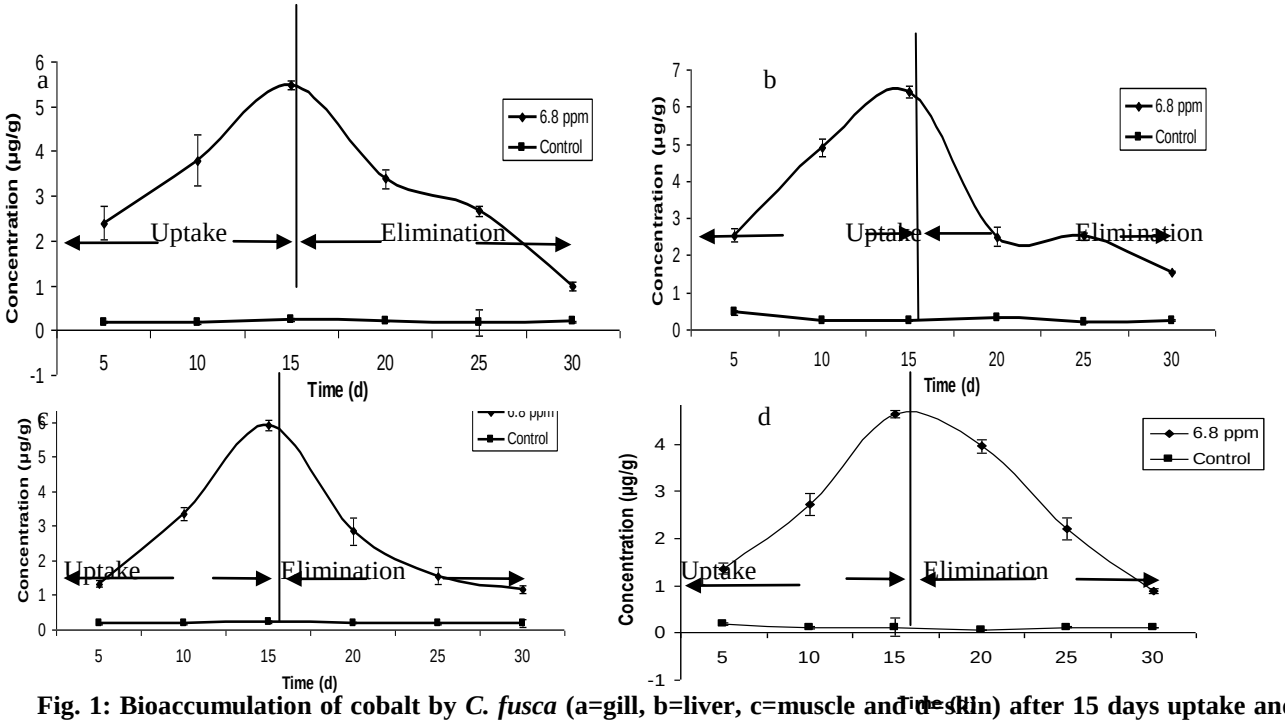

Fig. 1: Bioaccumulation of cobalt by *C. fusca* (a=gill, b=liver, c=muscle and d=skin) after 15 days uptake and 15 days elimination.

The fish gill is a multifunctional organ performing vital functions, including respiration, osmoregulation, acid-base balance and nitrogenous excretion (Oliveira-Filho *et al* ., 2010). Cobalt concentration in gill tissue of experimental group exceeded 30 times approximately that found out in the same tissue of the control group (from 0.18 to 5.49 µg/g). Also, cobalt concentration in the gill of the fish exposed 6.8 µg/g of cobalt was increased with a significant level ($P<0.01$) towards control group. Higher magnitude of bioaccumulation cobalt observed in gill tissues than other tissues in experiment group, maybe it's due to higher metabolic activities of gill or complexing with the mucus (Heath, 1987; Tekin-Özan and Kir, 2007; Prabhu Dass Batvari *et al* ., 2008), which is difficult to be removed completely from the tissue before the analysis. In other hands, the gill tissue is exposed to environmental metals to a greater extent than the other tissues and this might cause more accumulation and adsorption of the metals in or on the gill surface (Kalay and Canli, 2000). Thus, the concentrations of metals in gills reflect the concentration of metals in the waters (Ikem *et al* ., 2003).

The muscle and skin accumulated the lowest levels of cobalt (5.91±0.24 and 4.64±0.12, respectively), even after 15 days of exposure (Table 1), because these organs were not active organs in accumulating heavy metals. Cobalt concentration in muscle and skin increased 24 times and 25 times after chronic exposure (from 0.24±0.06 to 5.91±0.24 and 0.18±0.03 to 4.64±0.12, respectively). The same results were also shown by Houserova *et al* . (2006) and Mansouri *et al* . (2011b) that amount of bioaccumulation of skin tissue was lower than liver and gills. In other words, the mucogenic activity of the body skin epithelium in fish is very high when compared to gills (For accumulation) (Paul and Banerjee 1996). This increased mucogenesis may play a crucial role in preventing the metal ions from entering the body, as the coagulated mucus all over the body might be acting as a protective ion trap (Licata *et al* ., 2005), but the gill is a tissue which was active and passive exchanges occur between the animal and aquatic environment. First high levels of metals accumulate in the gill tissues by absorption and adsorption (Kargin, 1998). The depuration experiments were started after 15 days of absorption. In the present study, the skin and gill showed the greatest depuration of cobalt to sub-lethal concentration. A study by Kalay and Canli (2000), and Mansouri *et al* . (2011b) showed that the gill than liver and muscle of the fish is the first organ for quick elimination.

Conclusions: Current studies have shown different organs have different affinities toward cobalt accumulation which is directly proportional to the exposure time. Bioaccumulation of cobalt was higher in liver but it eliminated at faster rate in gills. It means that accumulation and depuration of cobalt in *C. fusca* is function of nature of tissue and time of exposure.

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