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Experimental Studies on Concentration and Depuration of Cobalt in the Selected Organs of Fresh Water Fish *Capoeta fusca*

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Abstract: The purpose of this study is surveying in bioaccumulation pattern and depuration of cobalt in the liver tissue, gills, muscle and skin of fresh water fish*Capoetafusca*. Therefore duringJuly to September 2010, the *C. fusca* with an average weight of 18.8±2.1grams and total length of 13.1±0.8 cm from Birjand qanats were collected. Cobalt accumulation and elimination were studied in fish exposed to one-tenth of LC_{50} taken as 20 mg/L of 96 hr LC_{50} concentration of cobalt over 30 days of exposure. The results obtained indicate that at the end of 30 days of exposure, the total tissue organ cobalt concentration followed the pattern liver >gill >muscle > skin and gill >liver >skin>muscle for accumulation and elimination, respectively.Results showed that cobalt accumulation in the liver was higher than the other tissues. Besides, the results showed that the elimination level of cobalt in the gills was the highest. In conclusion, the present study indicates that the accumulation and elimination of cobalt in *C. fusca* is dependent on tissue and time.

Key words: Tissue · Toxicology · Bioaccumulation · Cobalt · Fresh water fish · *Capoeta fusca*

INTRODUCTION

Birjand is located in south of Khorasan province in Iran and it is one of the desert region. There are no any permanent rivers in the province. However, there are sources of native fish population in their qanats [1]. Qanat is a water-management system used to provide a reliable supply of water to settlements or for irrigation in hot, arid and semi-arid climates; the technology is known to have developed in ancient Persia and then spread to other cultures [2]. Fishes in qanats, all over Iran constitute 25 species in Coad's research, e.i. 40% of the plateau fauna [3]. In the present research, a study was undertaken on one of the family fish of Capoeta genus. The *Capoetafusca* [4], a cyprinid, is one of the most important fishes in qanats of eastern Iran [5, 6]. This species of fish has been recognized of great importance from the genetic conservative point of view [1].

Heavy metal contamination of aquatic ecosystems has been recognized as a serious pollution problem. All heavy metals are potentially harmful to most organisms at some level of exposure and adsorption [7]. The natural aquatic systems may extensively be contaminated with heavy metals released from weathering of geological matrix, or from anthropogenic sources, such as industrial effluents and mining wastes [8, 9]. Metals such as cobalt may present environmental risk when occurring at elevated concentrations [10]. Cobalt is of relatively low abundance in the Earth's crust and in natural waters. The major anthropogenic sources of environmental cobalt include mining and processing (smelting) of cobalt-bearing ores, the use of cobalt-containing phosphate fertilizers on soil and atmospheric deposition from activities such as the burning of fossil fuels and smelting and refining of metals [11]. In the marine environment cobalt is needed by blue-green algae (cyanobacteria) and other nitrogen fixing organisms [12]. The natural concentrations of cobalt in fishes are very low and cobalt accumulation in fishes was not observed in areas where cobalt concentration in water was close to the background values [13]. Test results for marine fish suggest that at least the species tested are relatively insensitive to cobalt, with 96-h LC_{50} ranging from 52.5 to >1000 mg/L[11]. The study reported here shows that the 96-h LC_{50} values of $CoCl_2$ on *Oncorhynchusmykiss* were reported to be 1.4 mg/Lby Marr *et al.* [14];

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while Ewell *et al*. [15] reported the 96-h LC_{50} value of $CoCl_2$ on *Pimephalespromelas* as 21.8 mg/L. The 96-h LC_{50}value of $CoCl_2$ on Carassius auratus was found to be 333 mg/L respectively [16].

Cobalt is essential in the body in that it is a component of cyanocobalamin (vitamin B_{12}), constituting nearly 4.5% of its molecular weight [12]. Most animals need the element for the synthesis of the vitamin by intestinal microflora and such bacteria have also been isolated from the intestinal tract of fish [17]. Cobalt as part of vitamin B_{12} is associated with nitrogen assimilation and synthesis of hemoglobin and muscle protein. In addition, cobalt influences certain enzymes. Cobalt binds to insulin and also reduces plasma glucose levels [12, 18]. However, excessive intake of cobalt by organisms results in toxic effects [19]. The aim of this study was to investigate accumulation and depuration of cobalt in gill, liver, muscle and skin tissues of a native fish, *Capoetafusca*, under laboratory conditions.

MATERIALS AND METHODS

Birjand is the center of province of South Khorasan in the east of Iran. From July to September 2010, *C. fusca* belonging to the family cyprinidae, with average weight (±SD) of 18.8 (±2.1) g and total size of 13.1 (±0.8) cm were got 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 precleaned glassy aquariums with tap water.Fish were separately maintained at 25.7±1.3°C, pH 8.1±0.3; hardness 292±15 mg/L as $CaCO_3$; nitrite 0.04±0.03 mg/L; dissolved oxygen 6.2±0.2 mg/L; ammonia 0.05±0.02 mg/L, at least for 40 days prior to the experiments. The tap water had no detectable amount of cobalt.

In the present study, it was used the heavy metal cobalt in the form of cobaltchloride ($CoCl_26H_2O$-Analar grade, Merck). The 96 h LC_{50} concentration of cobalt was 204mg/L for *C. fusca* as calculated by using probit analysis method [20]. Fish were divided into four groups of 14; the first group served as the control group and the others as the 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-tenth of LC_{50}taken as 20 mg/L of 96 hr LC_{50} concentration of cobalt over 30 days of exposure. The study fish were exposed to the above-mentioned concentration separately for a period of 5, 10 and 15 days (accumulation period). At the end of these periods, the remaining fish were kept in tap water (elimination period) for another period of 20, 25 and 30 days. In the other hands, control group was kept in tap water for 30 days to the experiments (accumulation and elimination period).

At the end of each exposure period, there were some dissections for separate organs (gills, liver, muscle and skin). 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 gram and the weight of livers and gills was 0.5 gram. The organ samples were digested in a mixture including of nitric acid (HNO_3) and perchloric acid ($HClO_4$) [9, 21, 22]. Organs were, then, accurately weighed into 150-mL Erlenmeyer flasks, 10 ml nitric acid (65%) was added to each sample and the samples were left overnight to be slowly digested [9, 21, 23]; 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. After that, the digested samples were diluted by 50 ml distilled water. The concentration of cobalt was measured by using a Shimadzu AA 680 flame furnace atomic absorption spectrophotometer and the cobalt 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. The numbers of sample were 144. Data analysis implied evaluation of cobalt bioaccumulation magnitude between the experimental and control group organs (Table 2). To analyze the significant difference between the rates of cobalt concentration in organs, it was used the analysis of variance (ANOVA). The analyses of data were carried out using statistical package Minitab (version 15).

RESULT 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 > gill > muscle> skin, respectively. Also, the elimination patterns of cobalt are: gill> liver >skin > muscle, respectively. The liver accumulated the highest level of cobalt (19.18±2.54µg/g) and then the gill accumulated the highest level (18.51±2.35 µg/g). Also, the gill eliminated the highest level of cobalt (1.23±0.08µg/g) and then the liver eliminated the highest level (2.22±0.12 µg/g).

Table 1: Accumulation and depuration of cobalt in the selected organ tissues of *Capoeta fusca* exposed to 20 µg/g concentration

	Accumulation			Depuration		
Organ	5 days	10 days	15 days	20 days	25 days	30 days
Control						
Gill	0.25±0.01	0.21±0.04	0.18±0.03	0.22±0.01	0.12±0.05	0.25±0.01
Liver	0.46±0.08	0.25±0.0	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±0.10	0.11±0.01
Experiment						
Gill	6.21±0.73	15.08±1.59	18.51±2.35	13.52±0.24	6.30±0.09	1.23±0.08
Liver	4.28±0.47	15.25±1.34	19.18±2.54	10.56±1.14	7.55±0.82	2.22±0.12
Muscle	5.28±0.76	11.21±2.30	17.21±0.92	6.69±0.56	8.35±0.56	5.8±0.860
Skin	4.05±0.78	9.73±1.63	13.17±0.91	11.81±0.30	7.54±0.13	2.61±0.06

The values 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 20 µg/g concentration

	Magnitude of bioaccumulation (20 µg/g)			
Organ	5 days	10 days	15 days	Bioconcentration factor
Gill	×24.84	×71.66	×102.83	0.92±0.11
Liver	×9.30	×61.00	×79.91	0.95±0.12
Muscle	×29.33	×65.94	×71.70	0.85±0.04
Skin	×21.31	×60.81	×73.16	0.65±0.04

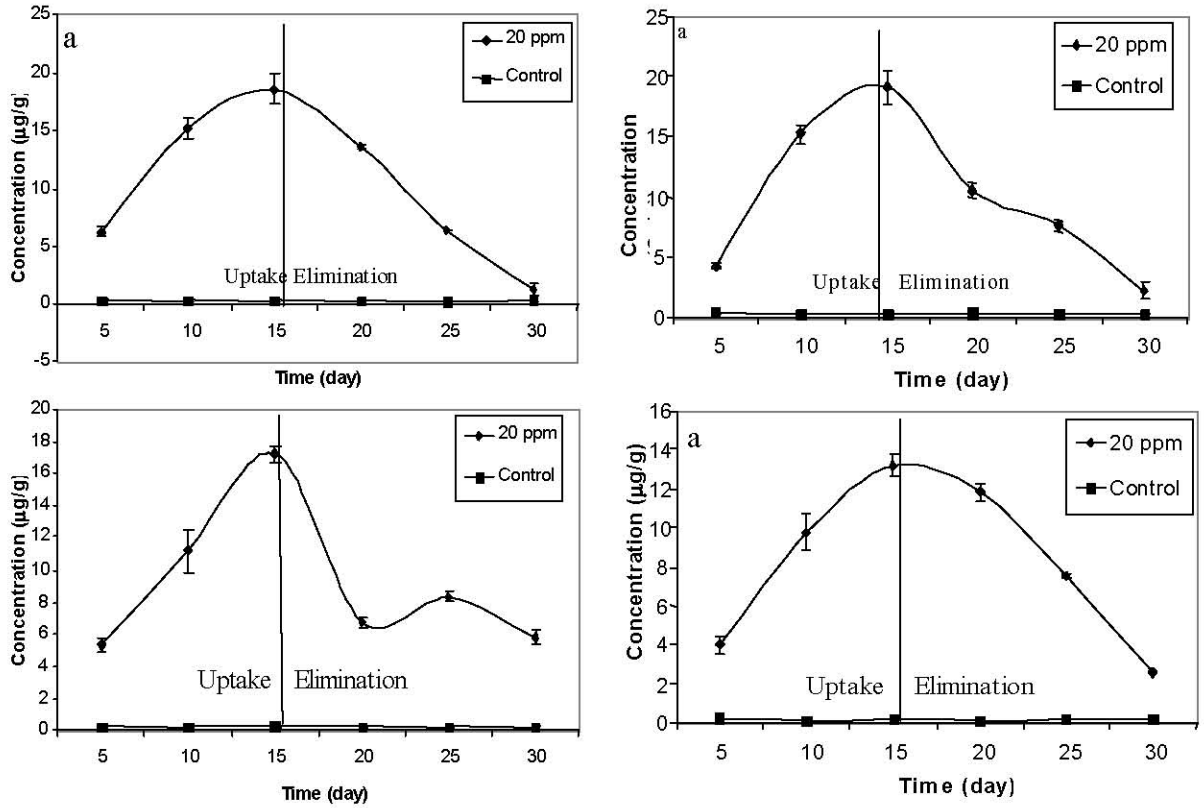

Fig. 1: Bioaccumulation of cobalt by *C. fusca* (a=gill, b=liver, c=muscle and d=skin) after 15 days uptake and 15 days elimination

The results indicate that the cobalt accumulation gradually increases during the exposure period (Fig. 1). The liver tissue was found to accumulate high concentrations of cobalt (19.18±2.54 μg/g). The concentration of cobalt in liver increased from 0.24±0.04 in control groupto 19.18±2.54 μg/g in 20 μg/g concentrationafter 15 days (Table 1), it means 79-fold increases toward control group (Table 2). Also, the higher value of the bioconcentration factor (BCF) observed in the liver (0.95±0.12) reflects the affinity of the hepatic tissues for cobalt concentration. This can possibly be attributed to the tendency of the liver to accumulate pollutants of various kinds at higher levels from the environment [24].

Next to liver, the gills accumulated the highest level (18.51±2.35 μg/g) of cobalt. The concentration of cobalt in gill increased from 0.18±0.03 to 18.51±2.35 μg/g in 20 μg/g concentration (Table 1), it means 102-fold increases toward control group (Table 2). These high cobalt levels in gill tissue can possibly due to the fact that they are the main sites for cobalt uptake, particularly in freshwater fish and due to the large surface that is in contact with environmental water and the very thin barrier separating the external and internal media of the animal [25]. However accumulated cobalt in the gill tissue of this species was lower than that in the liver. Lower amounts of cobalt in gills suggest that cobalt is excreted more rapidly and reduce the body burden of cobalt and suggest that cobalt are not accumulated in prolonged period in gill tissue.

The muscle and skin accumulated the lowest levels of cobalt (17.21±0.92 and 13.17±0.91, respectively), even after 15 d ays of exposure (Table 1), because these organs were not active organs in accumulating heavy metals [26]. These findings agree with those of [27] who found relatively low concentrations of cobalt in muscle of fish sampled. Cobalt concentration in muscle and skin increased 71-fold and 73-fold after chronic exposure (from 0.24±0.06 to 17.21±0.92 and 0.18±0.03 to 13.17±0.91, respectively). In general, similar results were reported from a number of fish species that the muscle is not an active tissue in accumulating heavy metals [28, 29, 30]. Tissues like the liver and gills are highly active in fish metabolism and therefore may accumulate metals to higher levels than other tissues like the muscle, as has been shown in this study and studies carried out with other fishes [31, 32, 33]. In the other words, the less cumulative effect of cobalt could explain why this metal was found to be less potent as a toxicant [34].

In the present investigation, the maximum levels of cobalt accumulation have been observed in liver compared to other organs in*Capoetafusca*. Marr *et al.* [14] reported a temporal pattern to cobalt toxicity in rainbow trout (*Oncorhynchus mykiss*). Cobalt concentrations that would eventually cause 100% lethality caused no lethality until at least 72 h of exposure. Suzuki *et al.* [35] reported that 75% of cobalt inyellowtail *Seriolaquinqueradiata* was present inthe blood, viscera and muscle.According to Subathra and Karuppasamy [36] the accumulation level of copper in different organs of *Mystusvittatus*was higher in the liver; this study was accomplished in a period of 96 hours and 28 days. Kotze *et al.* [37] have reported a higher accumulation of copper in liver tissue than any other organ in Oreochromis mossambicus as well as in Clarias gariepinus. In the other hands, the results of a study done by Mansouri *et al.* [21] on the bioaccumulation and elimination of nickel in different organs of *Capoetafusca* for a period of 30 days showed that the bioaccumulation level of nickel was higher in the gills than in the liver.

Like accumulation, several factors also influence the elimination of metals from the tissues, such as duration, temperature, interaction with other heavy metals and metabolic activity of animals, as well as the tissue concerned [32, 38]. The elimination routes of metals from fish are generally bile, urine, elimination from the gills and mucus [39, 40]. In the present study, the gills showed the greatest elimination of cobalt (1.23±0.08 μg/g). The reason for rapid elimination of cobalt in the gill, in comparison with other organs, may be due to the fact that gills are more in contact with the water environment and it is able to excrete cobalt while exposed to cleaner water [31].

CONCLUSION

The accumulation of cobalt varied among tissues. Results showed that cobalt accumulation in the liver was higher than the gills, muscle and skin. The accumulation of cobalt in the liver increased over time. Besides, the results showed thatthe elimination level of cobalt in the gills was the highest. The present study indicates that the accumulation and elimination of cobalt in *C. fusca* is dependent on tissue and time.

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