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Cadmium Accumulation in Several Tissues of Common Carp *Cyprinus carpio*, Linnaeus 1758 Treated by Potassium Permanganate, Cobalt Chloride and Vitamin B Complex

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During short term exposure of adult common carp to 2.5 mg/l of cadmium, maximum accumulation was recorded in the liver followed by kidney, gut, gill, testis, muscle and eye. Intramuscular injection of vitamin B complex significantly reduced the accumulation of cadmium by liver, gut, testis, muscle and eye. Potassium permanganate and cobalt chloride could not influence the accumulation of cadmium by these tissues.

Key Words: Cadmium, Potassium permanganate, Cobalt chloride, Vitamin B Complex, *Cyprinus carpio*

Introduction

Uptake of cadmium (Cd) by fish is influenced by several ions and chemicals. Zinc naturally occurs along with Cd and influences Cd accumulation in the intestine of zebra fish (*Brachydanio rerio*) but produces little impact on the accumulation of Cd either by liver or kidney of this fish (Wicklund et al. 1988, 1990). Ramamoorthy and Blumhagen (1984) found no effect of zinc on the accumulation of Cd by flag fish (*Jordanella floridae*) while Duncan and Klaverkamp (1983) suggested a decrease in uptake and/or increase in excretion of Cd by white sucker as a response of zinc. Calcium inhibits uptake of Cd by fish (Stephenson & Mackie 1988, Wicklund & Runn 1988) due to its tendency to compete with metal ions for binding sites on gill surface (Hunn 1985) and changing gill permeability leading to decrease in metal uptake (Pärt et al. 1985). In contrast, Cd forms lipophilic complexes with xanthates which facilitates penetration of Cd over the gill membranes (Gottofrey et al. 1988). Chelating agents like EDTA considerably reduces the accumulation of Cd in the intestinal tissue of fish (Sinha et al. 1992). EDTA also promotes excretion of Cd from the body of fish (Koizumi et al. 1992). Vitamin B complex has been found as an effective antidote for eliminating metals in mammalian system (Tandon et al. 1987) but its uses and efficacy as antidote for eliminating metals in fish are not well understood. Potassium permanganate ($KMnO_4$), is a strong oxidizing agent and is used commonly as a water disinfectant in fish pond. Cobalt chloride is also used in pond for enhancing the survival of hatchlings of airbreathing fish *Heteropneustes fossilis* (Khan & Mukhopadhyay 1971). Interaction of Cd with $KMnO_4$ and $CoCl_2$ in water produces ill effects on fish (Das & Kaviraj 1994a, 1994b). But we do not know if such

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interaction can influence the accumulation of Cd in fish. In this investigation we tested whether treatments of $KMnO_4$, $CoCl_2$ and vitamin B complex could influence the tissue accumulation of Cd by common carp *Cyprinus carpio*. Treatments of $KMnO_4$ and $CoCl_2$ were made by direct application in the test medium, but vitamin B complex was treated by intramuscular injection.

Materials and Methods

Experiments were conducted in the laboratory for 96 hrs in 15 l glass aquaria. Unchlorinated tap water (*p*H 7.17±0.19, DO 8.5±0.72 mg/l, carbonate alkalinity 47.5±8.29 mg/l as $CaCO_3$, bicarbonate alkalinity 192.0±8.29 mg/l as $CaCO_3$, hardness 195.0±5.0 mg/l as $CaCO_3$ and temperature 28.0±1.5°C) was used as diluent. The exposures used were 2.5 mg/l Cd (T_1), 2.5 mg/l Cd + 1.0 mg/l $KMnO_4$ (T_2), 2.5 mg/l Cd + 2.0 mg/l $CoCl_2$ (T_3), 2.5 mg/l Cd + 0.25 ml of vitamin B complex as intramuscular injection per fish (T_4), 0.0 mg/l Cd (control, T_5), 1.0 mg/l $KMnO_4$ (T_6), 2.0 mg/l $CoCl_2$ (T_7) and 0.25 ml of vitamin B complex as intramuscular injection per fish (T_8). Fish (*Cyprinus carpio*) (length, 16.70±1.33 cm and weight, 74.73+17.49 g) were obtained from local hatchery and were acclimated in the laboratory condition for 192 hrs before their use. Two fish were tested per aquarium and for each treatment at least 4 replicates were maintained following Complete Randomized Design (CRD) recommended for laboratory test (Gomez & Gomez 1984).

Sampled fish were washed in distilled water, soaked by blotting paper and digested on wet-weight basis in strong nitric acid, perchloric acid and sulphuric acid (Churnoff 1975). For estimation of Cd in tissues the sampled fish were dissected by acid washed scissors and the tissues were directly weighed on wet-weight basis. After digestions Cd was estimated in the digested sample by Atomic Absorption Spectrophotometer (AAS, Varian Tectron, AA-1475).

Data from all short term (96 hrs) laboratory bioassays were tested for significance of variance among various exposures by single factor analysis of variance on CRD design. For appropriate analysis of variance, the Chi-square (χ^2) test for homogeneity was applied. Pair comparisons between the treatments (main-plot or sub-plot) were shown by Least Significant Difference (LSD) Test (Gomez & Gomez 1984).

Results

Single factor ANOVA showed that Cd concentration in various tissues of common carp significantly varied among different exposures of Cd and control. Maximum concentration of Cd was recorded in the liver followed by kidney, gut, gill, testis, muscle and eye. Cadmium was also detected in these tissues of control fish but the concentrations were significantly much lower than the tissue of Cd-treated fish. Fish exposed to $KMnO_4$, $CoCl_2$ and vitamin B complex also showed a background concentration of Cd comparable to control ($P>0.05$). Cadmium concentration of only control has been represented in the figure since the above treatments showed no variation of tissue Cd level from control. LSD test with mean values (table 1) showed that $KMnO_4$ and $CoCl_2$ treatments had no impact on the accumulation of Cd by liver, gut, testis and eye because concentration in these tissues in the combined exposure of Cd and $KMnO_4$ (T_2) and Cd and $CoCl_2$ (T_3) were not significantly different from single exposure of Cd (T_1). But intramuscular injection of vitamin B complex significantly reduced the accumulation of Cd by these tissues in treated fish (figure 1). However, concentration of Cd in kidney and gill were alike in all groups of Cd treatments (T_1-T_4). Accumulation of Cd by muscle was decreased by the treatments of $KMnO_4$ and vitamin B complex but $CoCl_2$ had no such impact on Cd accumulation by muscle.

Table 1. *Least Significance of Difference (LSD) of mean cadmium concentration in different tissues between various treatments during short term bioassay.*

Tissue	Source of variations						LSD Values		
	T_1vsT_2	T_1vsT_3	T_1vsT_4	T_2vsT_3	T_2vsT_4	T_3vsT_4	5%	1%	0.1%
Liver	0.736	-0.268	7.283^c	-1.004	6.547^c	7.551^c	2.151	3.016	4.262
Kidney	0.241	-1.031	0.860	-1.272	0.620	1.891	2.183	3.061	4.326
Gill	-0.619	-0.548	-1.617^a	0.071	-0.998	1.069	1.610	2.251	3.181
Muscle	1.129	0.141	0.998	-0.989	-0.132	0.857	0.573	0.803	1.135
Gut	0.517	1.395	5.516^c	0.879	5.000^c	4.121^b	2.149	3.014	4.259
Testis	0.654	-0.805	1.726^b	-1.459^b	1.072^a	2.531^c	1.026	1.439	2.034
Eye	-1.058	0.399	1.101	1.457^a	2.159^c	0.702	1.088	1.525	2.156
Blood	0.580^a	0.589^a	1.267^c	0.009	0.686^a	0.678^a	0.566	0.794	1.122

$^aP < 0.05$, $^bP < 0.01$ and $^cP < 0.001$.

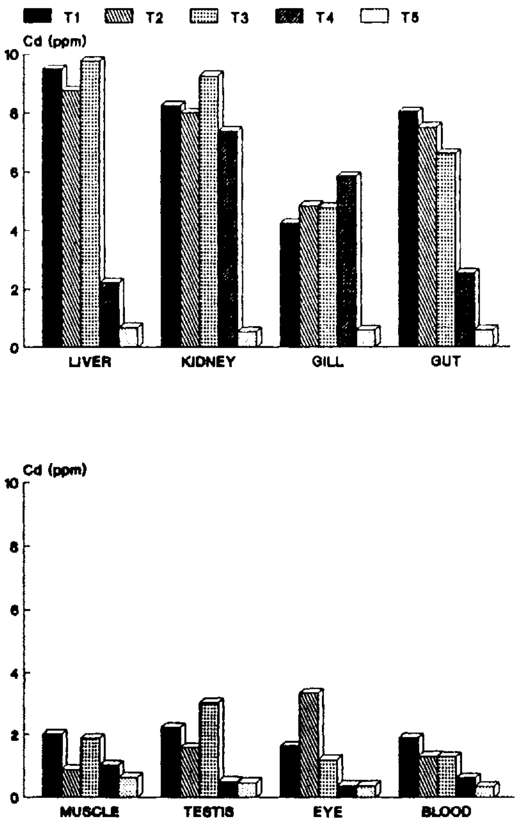

Figure 1 Concentration of cadmium in various tissues

Discussion

Liver and other soft organs of fish are important target for Cd (Dallinger et al. 1987). Franzin (1984) observed a positive correlation between age and liver Cd concentration in a natural population of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) residing in Cd contaminated lakes. Barak and Mason (1990) also found a positive correlation between length and levels of Cd in liver. Therefore, Cd if present in water, is continuously accumulated in the liver of fish. Das and Kaviraj (1990) found that accumulation of Cd in liver was related with the concentration of dissolved Cd in water. However, apart from interspecific differences, if any, in the physiological regulation of Cd uptake difference in exposure history (Duncan & Klaverkamp 1983) may result in the divergent pattern of Cd accumulation. Although kidney, liver, intestine and gill have been found to be the principal sites of Cd accumulation in fish (Benoit et al. 1976, Pohl 1990, Moore 1991), opinion differs regarding the relative capacity of Cd accumulaion by these organs (Ruparelia et al. 1987, Olsson et al. 1988). In the present 96 hrs test, all these organs accumulated more Cd than either testis, or blood or muscle. Experiments with several species of freshwater salmonids have shown that muscle and skin generally accumulate low levels of Cd (Benoit et al. 1976, Wilson et al. 1981). Cadmium is accumulated in the body of fish either through food or directly by absorption from water. No food was given to fish during our 96 hrs test. Therefore, accumulation of Cd occurred from water, either through direct transfer across the gills or through accumulation of water in the gastrointestinal (GI) tract. Since gut of all the treated fish showed a high concentration of Cd, it is assumed that a considerable proportion of Cd in the present investigation was accumulated through GI tract. Maximum levels of Cd reported for liver, kidney and gill of the trout *Salvelinus fontinalis* exposed to 3.4 µg Cd/l for 100 weeks by Benoit et al. (1976) were approximately 10, 50 and 7 µg/g dry weight respectively. With a much higher exposure (2. 5 mg/1) but for a very short duration (96 hrs), almost similar levels of Cd was detected in the liver and gill in the present investigation. Relatively higher value of Cd in kidney observed by Benoit et al. (1976) was probably due to tendency of Cd to excrete through kidney of fish (Giles 1988). It has been suggested on the basis of high concentration of Cd in kidney that this organ is a route of excretion of Cd in fish (Kumada et al. 1980). Muramoto (1980) observed an increase in depuration of Cd from gill of Cd contaminated carp.

Intramuscular injection of vitamin B complex, during the short term (96 hours) exposure of Cd, significantly reduced the Cd concentration in liver, gut, testis and blood, but not in gill and kidney. Gill and kidney are supposed to be two principal organs through which elimination of metal may occur. Relatively high concentration of Cd in these two tissues and low concentration in other tissues at T_4 indicated that vitamin B complex could not only reduce the uptake of Cd but also influence its elimination through gill and kidney. Chelating agents can promote excretion of Cd from the body of fish (Koizumi et al. 1992). Efficacy of some components of vitamin B complex to chelate and eliminate lead through excretion in mammalian system has also been established (Tandon et al. 1987). The present experiment also indicated that vitamin B complex could be effectively used as antidote to reduce accumulation and promote excretion of Cd in fish.

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