Bull. Environ. Contam. Toxicol. (1997) 59:120-124

Bulletin of Environmental Contamination and Toxicology

Effects of Chelation on the Bioconcentration of Cadmium and Copper by Carp *(Cyprinus carpio* L.)

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Received: 5 June 1996/Accepted: 11 March 1997

The discharge of heavy metals such as copper and cadmium by industry represents a serious water pollution problem due to the toxic properties of these metals and their adverse effects on water quality.

Copper is an essential trace element for humans; however, it is toxic at high concentrations. There is no evidence that cadmium is biologically essential but its toxicity for organisms is well known. Even at low concentrations it can cause toxic effects to most organisms.

Organic chelation compounds like ethylenediamine tetraacetic acid (EDTA) and histine are known as effective chelation agents to form stable metal-chelate complexes. There has been considerable speculation about the effects of organic chelation compounds on the toxicity of cadmium and copper to aquatic organisms such as fish and green algae, but, unfortunately, the results obtained are contradictory. One hypothesis is that chelates formed in the water reduce the concentration of free metal ion and then reduce the metal toxicity to aquatic organisms (Helen 1990; O' Brien et al. 1990). The alternative emphasizes that the metal-chelate complex formed in water can increase metal solubility and promote metal convection and diffusion and hence potential uptake and toxicity (Laube et al. 1980). Furthermore, literature on the effects of organic chelation compounds on the bioconcentration of cadmium and copper by aquatic organisms is rather meager.

In the present investigation, the bioconcentration process of copper, cadmium and the influence of adding EDTA or histine on this process was studied at environmentally elevant concentrations and at various times of exposure. The results were expressed as individual tissue metal burdens as well as bioconcentration factors in order to evaluate the effects of organic chelation compounds on the uptake of cadmium and copper by carp (Cyprinus carpio *L.)* which was selected as the test organism because of its position in the food chain immediately before the human consumer. The uptake and bioconcentration of cadmium and copper were estimated with and without organic chelation compounds.

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MATERIALS AND METHODS

Carp (Cyprinus carpio *L.)* used in this study were young-of-the year and were maintained in purified and de-chlorinated tap water at 14-16 °C for 1 week before the experiment. The average length and weight were 7.0 cm and 3.7g , respectively.The chemical parameters of the water used were as follows: dissolved oxygen > 7.0 mg L^{-1}; conductivity 180 $m\Omega^{-1}cm^{-1}$;total alkalinity 1.23-2.47 mmol L^{-1}; total hardness 40-65 mg L^{-1}as CaO; and content of chloride 6.8-8.2 mg $L^{-1.}$

Three glass aquaria with a 20-L capacity were used for triplicate experiments. The fish were divided into three groups of 30. Each group of fish was placed into an aquarium containing 15 L of diluent water .The pH was controlled at 7.0 Cadmium (0.5 $g.L^{-1}$) or copper (0.2 $g.L^{-1}$) were added to the test water with and without EDTA (4.378 M) for cadmium and histine (8.00 M) or EDTA (4.12 M) for copper. A volume of 15 L of test water in each aquarium was renewed every other day. The fish were fed dry food after each renewal.

Each experiment was carried out during a 40-d period. Fish were sacrificed at various time intervals (5-10 days) for each of the test groups, The sample fish were rinsed with distilled water and the surface of the fish body was dried with paper. Careful dissection was made to divide fish into various tissue categories. Muscle, gills and internal organs were removed into 25-ml PTFE beakers, respectively. After weighing, these samples were disgested with HNO_3- $HClO_4$mixture and evaporated to near dryness. The residues were dissolved in 0.2N HCl. The resultant solutions were subsequently used for analysis by atomic absorption spectrophotometry (Hitachi Z-8100). Accuracy of the present method was tested by adding standard reference and satisfactory recovery results were obtained as 93% for cadmium and 102% for copper, respectively

RESULTS AND DISSCUSSION

The application of organic chelation compounds significantly reduced the uptake of cadmium (Fig.1) and copper (Fig.2) The bioconcentration kinetics of uncomplexed cadmium (II) and copper (II) ions were linear and the equilibria were not reached at the end of 40-d exposure. Assuming C_w is the concentration in water ($g.\ ml^{-1}$); C_B is the concentration in different tissues in carp (µg.g-1); k_1 is the uptake rate constant ($ml.g^{-1}.d^{-1}$), t is time (days), the bioconcentration of uncomplexed cadmium (II) and copper (II) ions can be described by the following equation:

$$C_B = k_1 C_w \qquad (1)$$

Using linear regression we were able to derive the uptake rate constants k_1 from the slope of the linear regression equations, the bioconentration factors (BCF) were calculated using the following equation:

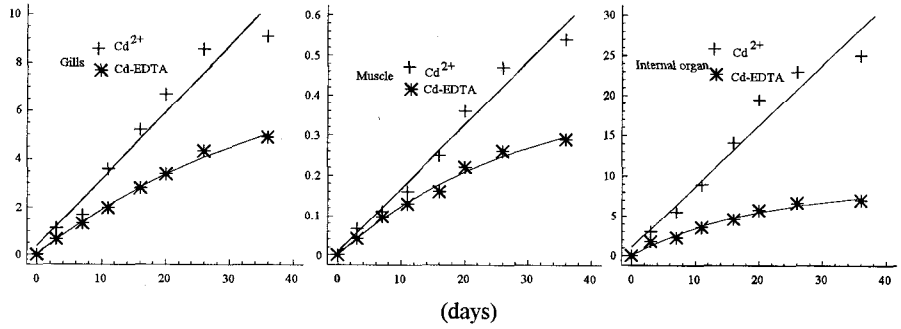

Figure 1. The influence of EDTA on the bioconcentration kinetics of cadmium in different tissues of fish.

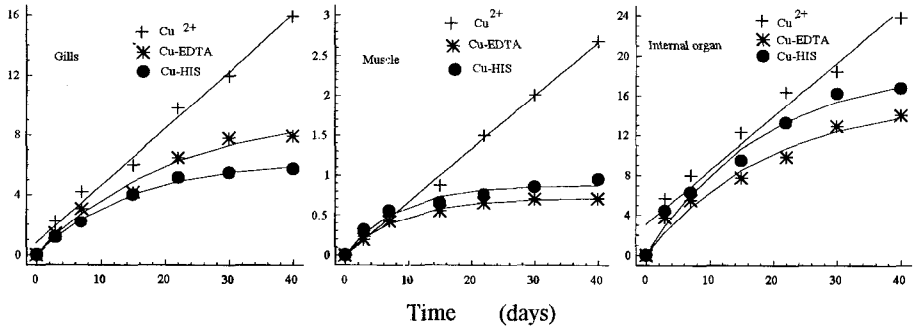

Figure 2. The influence of EDTA and Histine on the bioconcentration kinetics of copper in different tissues of fish.

$$BCF = \frac{C_{B\max}}{C_w} \qquad (2)$$

Where: C_{Bmax} is the maximum concentration of cadmium and copper in different tissues in carp (μ g.g-1)

When organic chelation compounds were added, there were two phases in the bioconcentration process: a phase during which the concentration increased slowly and finally a second phase during which the metal concentration remained more or less constant. This last phase could be compared to steady-state

The bioconcentration kinetics of copper and cadmium which complexed with organic chelation compounds can be described as a first-order process according to the following equation:

$$C_B = \frac{k_1}{k_2} C_w (1 - e^{-k_2 t}) \qquad (3)$$

Where: k_2 is the elimination rate constant (d^{-1}).

At steady-state, the bioconcentration factor (BCF) can be defined as:

$$BCF = \frac{k_1}{k_2} = \frac{C_B(t-\infty)}{C_w(t-\infty)} \qquad (4)$$

Table 1. Summary of uptake- rate constants, bioconcentration factors (BCF) and correlation coefficients for regression equations during the exposure of carp to cadmium for 40 days

Tissue	Metal Species	k_1 ($ml.g^{-1}.d^{-1}$)	BCF ($ml.g^{-1}$)	r^2
Muscle	Cd^{2+}	0.032	1.28	0.99[a]
	Cd+EDTA	0.029	0.818	0.98[b]
Gills	Cd^{2+}	0.56	22.4	0.98[a]
	Cd+EDTA	0.44	16.2	0.99[b]
Internal organ	Cd^{2+}	1.52	60.64	0.98[a]
	Cd+EDTA	0.88	17.26	0.98[b]

[a] calculated by linear regression n=7 $P< 0.01$ according to equation 1.

[b] calculated by non-linear regression n=7 $P < 0.01$ according to equation 3.

Table 2. Summary of uptake- rate constants, bioconcentration factors (BCF) and correlation coefficients for regression equations during the exposure of carp to copper for 40 days

Tissue	Metal Species	k_1 ($ml.g^{-1}.d^{-1}$)	BCF ($ml.g^{-1}$)	r^2
Muscle	Cu^{2+}	0.34	13.4	0.98[a]
	Cu-HIS	0.53	4.4	0.97[b]
	Cu-EDTA	0.40	3.6	0.99[b]
Gills	Cu^{2+}	1.9	76	0.99[a]
	Cu-HIS	2.11	31.5	0.99[b]
	Cu-EDTA	2.42	48.4	0.98[b]
Internal organ	Cu^{2+}	2.71	108	0.98[a]
	Cu-HIS	4.83	96.5	0.98[b]
	Cu-EDTA	3.98	79.5	0.97[b]

[a] calculated by linear regression n=7 $P< 0.01$ according to equation 1.

[b] calculated by non-linear regression n=7 $P < 0.01$ according to equation 3.

The uptake-rate constants (k_1) of uncomplexed ions and the complexed metals listed in Tables 1 and 2 were calculated using linear regression and non-linear regression according to equations 1 and 3, respectively. BCFs were calculated by equations 2 and 4, respectively. Tables 1 and 2 also show that the internal organ had the greatest ability to bioconcentrate copper and cadmium. The sequences of BCF for copper and cadmium in different tissues were also for the internal organs > gills > muscle. This sequence was not changed by adding organic chelation compounds. But, for a given tissue, this study showed significant differences in the bioconcentration kinetics of cadmium and copper, when organic chelation compounds were added. The bioconcentration of free ions were at least two times higher than the metals complexed with organic chelation compounds. This

indicates that the application of organic chelation compounds (EDTA and HIS) could reduce cadmium and copper uptake greatly. Previous studies (Sunda and Zamuda 1982) demonstrated that organic chelation compounds can form stable complexes which can hardly be accumulated in organisms, thereby reducing the toxicity of these metals to organisms. For fish, the diffusion process of metals in gills and skin is the main pathway for the bioconcentration of metal ions, so the charge of ions, molecular size and the stability of complexes can modify greatly the bioconcentration process of heavy metals. In the present study, considering these factors, compared with uncomplexed cadmium and copper ions, the complexed metals have low abilities to diffuse through gills and skin of fish. The uptake of cadmium and copper in the presence of organic chelation compounds was less, probably because of the formation of stable metal-chelate complexes which are poorly absorbed through diffusion in fish.

Acknowledgments. This research was financially supported by the National Natural Science Foundation of P. R. China.

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