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Accumulation of Cadmium in the Gill, Liver, Kidney, Spleen, Muscle and Brain Tissues of *Cyprinus carpio*

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Abstract

Accumulation of cadmium in the gill, liver, kidney, spleen, muscle and brain tissues of *Cyprinus carpio* was studied over 1, 3, 15 and 30 days after exposing the animals to 0.1, 0.2, 0.4 and 0.8 ppm of Cd. Experiments were carried out at 25±1°C and 12 hour regimes of light/dark photoperiod was adapted. ICP-AES spectrophotometric methods were used in determining the tissue levels of cadmium. No mortality was observed during the 30 days of experiments at any Cd concentrations tested. Cadmium levels were significantly higher at all the tissues studied compared with the control levels. Kidney tissue was found to be the target organ in accumulating cadmium in *C. carpio* at the selected concentrations and exposure periods. The following relationship was found among the tissues in accumulating cadmium; Kidney>Gill>Liver>Spleen>Muscle>Brain. It can be concluded that the variation between the tissues in accumulating cadmium might result from the metabolical, structural and functional differences between the tissues studied.

Keywords: *Cadmium,* Cyprinus carpio, *tissue accumulation.*

Cyprinus carpio'nun Solungaç, Karaciğer, Böbrek, Dalak, Kas ve Beyin Dokularında Kadmiyum Birikimi.

Özet

Araştırmada kadmiyumun 0,1, 0,2, 0,4 and 0,8 ppm derişimlerinin 1, 3, 15 ve 30 gün sürelerle etkisinde bırakılan *Cyprinus carpio*'nun solungaç, karaciğer, böbrek, dalak, kas ve beyin dokularındaki kadmiyum birikiminin belirlenmesi amaçlanmıştır. Deneyler 25±1°C sıcaklıkta ve 12 saat ışık/karanlık periyodunda yürütülmüştür. Kadmiyumun doku düzeylerinin belirlenmesinde ICP-AES spektrofotometrik yöntemler kullanılmıştır. Otuz günlük deney süresince denenen hiç bir kadmiyum derişiminde mortalite gözlenmemiştir. Tüm dokulardaki kadmiyum düzeyleri kontrole oranla istatistiksel olarak önemli derecede yüksektir. *C. carpio*'da denenen derişim ve sürelerde böbrek dokusunun kadmiyum için hedef organ olduğu saptanmıştır. Kadmiyum biriktirme bakımından dokular arasında aşağıdaki ilişki bulunmuştur; Böbrek>Solungaç>Karaciğer>Dalak>Kas>Beyin dokular arasında gözlenen kadmiyum birikimindeki ayrımın metabolik, işlevsel ve yapısal farklılıklardan kaynaklanması olasıdır.

Anahtar Kelimeler: Cyprinus carpio, *doku birikimi, kadmiyum.*

INTRODUCTION

Together with the natural processes, such as volcanic activitiy, soil erosion and weathering of rocks, anthropogenic inputs mainly through domestic, industrial and agricultural activities increase the levels of toxic substances in aquatic environments which have an adverse effects on living organisms. It is important to know the form in which these toxic substances exist in nature, their bioavailability, bioconcentration and the concen-tration in which they become toxic.

Cadmium is a toxic metal even in very low concentrations with no functional role in biological systems (Almeida et al. 2001). It is widely used in dye, plastic, glass and ceramic industries and also in the production of storage batteries, insecticides and super phosphates (Ragan and Mast 1990).

When depuration, storage and detoxification

mechanisms in fish could not compensate for the cadmium uptake, the metal then accumulates in the tissues. While tissue accumulation of cadmium varies with age, length, weight, sexual and developmental stage of the species in concern (Suresh et al. 1993, Zyadah 1999, Rashed 2001), it also differs with some environmental factors such as water temperature, alkalinity, water hardness and pH (Hodson 1988, Douben 1989, Erdem 1990).

Studies carried out under laboratory conditions showed that cadmium causes cytological and histophatological changes in gill and liver cells (Heath 1995), structural deformations in skeleton structures, malfunction of respiration and osmoregulation systems (Sorensen 1991, Novelli et al. 1999) and changes in behaviour of the species exposed (Khunyakari et al. 2001).

Cadmium taken into organism through water, body surface and gills is carried to the metabolically active organs such as liver, kidney and spleen through the circulatory system and accumulate depending on the exposure concentration and period. Studies carried out on various fish species under natural and laboratory conditions showed that cadmium accumulates mainly in tissues such as kidney, liver, spleen and gill (Pelgrom et al. 1995, Melgar et al. 1997).

Cyprinus carpio (mirror carp) is an economically important fish species which is widely used to enrich the inland waters fish population. It is an important link in the food chain and is being consumed as a protein source. The species is under the effect of domestic, industrial and agricultural activities hence, it is important to study the tissue accumulation of toxicants both in terms of fish and human population health and in determining the pollution status of the environment.

The accumulation of cadmium, a metal having no role in any biological function, in the gill, liver, kidney, spleen, muscle and brain tissues of *C. carpio* was studied after exposing the animals to 0.0 (control), 0.1, 0.2, 0.4, and 0.8 ppm Cd for 1, 3, 15 and 30 days.

MATERIALS AND METHODS

The *Cyprinus carpio* (L. 1758) used in the controlled laboratory conditions were placed in 12 glass aquaria 40x120x40 cm in depth and left an adaptation period of a month. The mean length and weight of the animals were 15.3±0.8 cm and 62.4±0.5 g, respectively at the end of this adaptation period. Twelve hours of a light/dark regime was adapted during the adaptation period and experiments, and the water temparature was kept at 25±1°C. Some chemical parameters of the water used in the experiments are as follows;

pH: 8.19±0.06

Dissolved oxygen: 7.02±0.27 mg/L

Total alkalinity: 326±0.50 mg/L $CaCO_3$

Total hardness: 230±0.75 mg/L $CaCO_3$

Aeration in the aquaria was from a central aeration system. Fish were fed with a Cd free commercial fish feed (Pınar; Bream Feed, Pellet No: 2) once a day in amounts 2% of total body weight. Cadmium solutions were prepared using water soluable $CdCl_2.H_20$ salt. Water in the control and experimental aquaria were changed every 2 days to prevent concentration changes due to evaporation, precipitation and adsorption.

The accumulation of cadmium in the tissues of *C. carpio* was studied after exposing the animals to 0.1, 0.2, 0.4 and 0.8 ppm Cd over 1, 3, 15 and 30 days periods. Four series of experiments were conducted taking the exposure periods into account and 5 glass aquaria, 40x120x40 cm in height, were used in each series. The first aquarium in each series was filled with Cd free tap water for control purposes and 120 L of 0.1, 0.2, 0.4 ve 0.8 ppm Cd solutions were added to the remaining four aquaria. Experiments were run in triplicate with three fish in each replication, hence nine fish were placed in each aquarium of one series. A total number of 180 fish were used during the experiments.

The fish taken from the aquaria at the end of each sampling period were anesthetized using 75 mg/L MS222 (Ethyl Ester 3 -Aminobenzoik Acid) to prevent sampling stress (Ruparelia et al. 1990). The tissue samples were dissected separately from the three fish in each replication and were placed in a petri dishes. Tissue samples were dried to a constant weight at 150°C for 72 hours. Tissues were taken then placed in experimental tubes after determining their dry weights and 3 mL of nitric acid (HNO_3, Merck, %65) and perchloric acid ($HClO_4$, Merck, %60) mixture (2:1 v/v) was then added in the tubes (Muramoto 1983) and boiled at 120°C for 60 minutes. The samples were transferred to polyethylene tubes and their volumes were increased to 5 mL using distelled water. Tissue cadmium analyses was carried out using Inductively Coupled Plasma Atomic Emission Spectropho-

tometric methods (Perkin Emler Optima, 3100-XL ICP AES) (Okamoto et al. 1997). Students Newman Keul's procedure was used in statistical comparison of the data (Sokal and Rohlf 1969).

RESULTS

The mean and standard errors of the metal accumulated in gill, liver, kidney, spleen, muscle and brain tissues of *C. carpio* after exposing the animals to 0.1, 0.2, 0.4 and 0.8 ppm Cd over 1, 3, 15 and 30 days are given in Tables 1-6, respectively.

No mortality was observed during the 30 days of experiments in any of the Cd solutions tested, while behavioral differences such as a decrease in swimming performance, food uptake, increased operculum movement and grouping at the surface were observed at the begining of the experiments. These behavioral changes returned to normal with increased exposure periods.

Cadmium levels in the tissues of the control fish were below detection limit of the Atomic Emission Spectrophotometer. Tissue accumulations of cadmium were significantly high compared with the control fish in all the tissues studied.

No difference was observed in gill and liver accumulation of cadmium after 1 and 3 days of exposure at all the concentrations of cadmium except 0.8 ppm Cd, while increases in concentration and exposure period elevated metal accumulation in these tissues (Tables 1 and 2).

Metal accumulation in kidney tissue showed a two-fold increase at 0.8 ppm Cd compared with 0.1 ppm Cd at all the exposure periods studied, whereas there was a 26 fold increase on day 30 compared with day 1 at this concentration (Table 3)

The accumulation of cadmium in the spleen tissue of *C. carpio* increased with increasing Cd concentrations and exposure periods (Table 4). A two-fold and a four-fold increase in cadmium accumulation were observed in 0.8 ppm Cd when compared with 0.1 ppm Cd on the 1st and 30th days of exposure, respectively.

Muscle accumulation of cadmium was 2.5 times higher on day 30 compared with day 1 in all the Cd concentrations tested (Table 5). Taking the exposure periods into consideration there was nearly a 35% increase in 0.8 ppm Cd compared with 0.1 Cd on days 3 and 15 and a 70% increase on days 1 and 30 in muscle tissue.

On days 1 and 3 no statistical difference was found in the brain accumulation of cadmium between the cadmium concentrations tested at the given exposure periods and between the exposure periods at given Cd concentration (Table 6). Accumulation of cadmium was not significant between the Cd concentrations at all the exposure periods tested except for 0.8 ppm Cd on days 15 and 30. Brain accumulation of cadmium increased significiantly on days 15 and 30 and continued to increase at the lowest and the highest Cd concentrations.

No clear difference among the tissues was observed in metal accumulation after three days exposure to cadmium. On prolonged exposure, however, maximum cadmium accumulation was detected in kidney followed by gill, liver, spleen, muscle and brain tissues. Kidney accumulation of cadmium was 55.83% of cadmium accumulated by all the tissues studied whereas this value was 0.065% for the brain tissue.

DISCUSSION

No mortality was observed in *C. carpio* exposed to 0.1, 0.2, 0.4 and 0.8 ppm Cd over a 30 day period. Similar results were obtained by De Smet and Blust (2001) after exposing *C. carpio* to 20 μmol Cu for 29 days. This can be explained by the increased synthesis of metal binding proteins such as metallothioneins and the tripeptide glutathione, the increase in the numbers of cytoplasmic granules which estherize and store free metals and also short diffusion distance between water and blood.

The liver is shown to be the primary tissue in accumulating copper at sublethal concentrations of the metal in *Oncorhynchus mykiss* Walbaum, 1792 (Dethloff et al.1999), *Tilapia nilotica* L., 1758 (Cicik and Erdem 1992) and *C. carpio* (Kargın 1990). The cadmium accumulation, however, was higher in the kidney rather than the liver tissue in *Oreochromis niloticus* L., 1758 (Sağlamtimur et al. 2003) *Anguilla rostrata* Lesueur, 1817 (Gill et al. 1992) and *T. nilotica* (Kalay 1992). The same was true in the present study carried out with *C. carpio*. Cadmium increased cytoplasmic vesicles and granules of kidney tubule cells in *Lates calcarifer* Bloch, 1790 resulting edema and it was suggested that cadmium alters the membrane permeability of the kidney tubule cells (Thophon et al. 2003). Since cadmium has no known biochemical function in biological systems, the metal is probably pumped directly to the kidneys for excretion where it effects membrane permeability, enters the kidney cells and forms Cd-

Table 1. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the gill tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	2.60 ± 0.24 bs	2.58 ± 0.16 bs	9.26 ± 0.02 bt	18.15 ± 1.97 bt
0.2	9	3.88 ± 0.52 bs	3.30 ± 0.14 bs	13.48 ± 2.10 bt	23.16 ± 1.39 cx
0.4	9	3.44 ± 0.54 bs	11.12 ± 1.59 cs	33.93 ± 1.40 ct	41.71 ± 2.17 dx
0.8	9	5.38 ± 0.40 cs	12.33 ± 1.50 ct	39.95 ± 4.43 dx	36.32 ± 1.29 ey

⋆ SNK= Letters a, b, c, d, e show differences among control and Cd concentrations; and s, t, x, y among tissues. Data shown with different letters are significantly different at the $P<0.05$ level.
ND= Not detectable. X ± sx: Mean ± standart error.

Table 2. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the liver tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	1.16 ± 0.24 bs	2.76 ± 0.14 bs	14.30 ± 1.89 bt	16.31 ± 1.02 bt
0.2	9	1.68 ± 0.03 cs	2.62 ± 0.20 bs	12.63 ± 2.78 bt	19.43 ± 1.56 cx
0.4	9	3.24 ± 0.12 ds	4.79 ± 0.29 cs	18.66 ± 1.49 bt	25.48 ± 0.48 dx
0.8	9	3.37 ± 0.14 ds	9.20 ± 1.28 dt	23.24 ± 2.55 cx	46.65 ± 1.61 ey

Table 3. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the kidney tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	3.81 ± 0.16 bs	4.33 ± 0.32 bs	44.34 ± 3.11 bt	68.81 ± 4.40 bx
0.2	9	4.32 ± 0.14 bs	4.93 ± 0.05 bcs	77.74 ± 5.30 ct	92.77 ± 3.64 cx
0.4	9	5.52 ± 0.17 cs	5.64 ± 0.35 bcs	84.54 ± 6.53 ct	128.27 ± 4.13 dx
0.8	9	6.36 ± 0.19 ds	6.42 ± 0.56 cs	99.60 ± 0.43 dt	193.67 ± 6.33 ex

Table 4. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the spleen tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	2.23 ± 0.12 bs	2.29 ± 0.08 bs	5.38 ± 0.21 bt	6.32 ± 0.15 bx
0.2	9	2.87 ± 0.03 cs	2.99 ± 0.02 cs	7.96 ± 0.07 ct	9.59 ± 0.36 cx
0.4	9	4.11 ± 0.16 ds	4.22 ± 0.13 ds	9.88 ± 0.19 dt	23.27 ± 0.80 dx
0.8	9	4.66 ± 0.14 es	4.88 ± 0.12 et	17.34 ± 0.90 et	27.96 ± 0.63 ex

Table 5. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the muscle tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	1.47 ± 0.32 bs	1.94 ± 0.16 bs	2.50 ± 0.15 bst	3.01 ± 0.05 bt
0.2	9	1.93 ± 0.05 bcs	2.00 ± 0.01 bs	2.70 ± 0.15 bct	3.84 ± 0.05 cx
0.4	9	2.66 ± 0.10 cs	2.61 ± 0.04 bs	3.04 ± 0.10 cs	4.09 ± 0.10 ct
0.8	9	2.59 ± 0.24 cs	2.69 ± 0.24 bs	3.47 ± 0.06 ds	4.88 ± 0.22 dt

Table 6. Effects of concentration and exposure period on the accumulation of cadmium (mg Cd/g d.w.) in the brain tissue of *C. carpio*.

Metal (ppm Cd)	N	Exposure period (days)			
		1	3	15	30
		X ± sx ⋆	X ± sx ⋆	X ± sx ⋆	X ± sx ⋆
0.0	9	ND a	ND a	ND a	ND a
0.1	9	0.15 ± 0.04 bs	0.09 ± 0.05 bs	0.55 ± 0.06 bt	0.83 ± 0.03 bx
0.2	9	0.18 ± 0.09 bs	0.26 ± 0.05 bs	0.71 ± 0.06 bt	0.84 ± 0.10 bt
0.4	9	0.21 ± 0.05 bs	0.20 ± 0.07 bs	0.71 ± 0.12 bt	1.06 ± 0.10 bt
0.8	9	0.42 ± 0.04 bs	0.41 ± 0.07 bs	1.25 ± 0.20 ct	1.93 ± 0.24 cx

thionein by inducing metallothionein synthesis.

The cadmium accumulation in the spleen tissue as in muscle and brain tissues was low in *C. carpio*. In a study carried out with *T. nilotica* exposed to similar Cd concentrations spleen accumulation was found to be low up to 30 days which increased on prolonged exposure and became the third tissue in accumulating cadmium following kidney and liver (Kalay 1996). It was stated that cadmium accumulation induces metallothionein synthesis in spleen tissue (Cherian and Goyer 1978). So the increase in spleen accumulation of this metal with time might indicate stimulation of metallothionein synthesis.

Muscle accumulation of cadmium in *Oreochromis aureus* Steindachner, 1864 was low compared with other tissues when exposed to the metal singularly or in combination with other metals (Allen 1995, Odzak and Zvonaric 1995). As in the present study, low levels of cadmium were detected in the muscle tissue of *C. carpio* exposed to sublethal concentrations of cadmium over 29 days (De Smet and Blust 2001). It was suggested that sublethal concentrations of cadmium in *Scyliorhinus canicula* L., 1758 causes haemodilution by forcing the water to pass from muscle to blood (Tort and Torres 1988). Since muscle is not an active tissue in accumulating metals and water loss from this tissue causes haemodilution under the effect of metals might explain the low levels of cadmium accumulated in this tissue.

The brain is one of the most important organs in animals controlling vital functions. As muscle and bone tissues it is not an active organ in accumulating cadmium. Metal accumulation in this tissue was shown to be lower than metabolically active tissues such as gill, liver and kidney (Thomas et al. 1983). Cadmium accumulation in muscle, bone and brain tissues of *T. nilotica* was studied after exposing the animals to 0.1, 0.5 and 1.0 ppm Cd over 15, 30 and 60 days periods and it was shown that brain accumulation of Cd increased with increasing concentrations and exposure periods reaching rather high levels after 60 days of exposure (Kalay and Karataş 1999). Muscle accumulation of copper was higher than that of brain in *Lepomis macrochirus* Rafinesque, 1819 exposed to sublethal concentrations of the metal (Felts and Heath 1984). Similar results were obtained for the cadmium accumulation in muscle and brain tissues from the present study carried out with *C. carpio*. The increase in the brain accumulation of cadmium on prolonged exposure might be due to the loss of function of the blood-brain barrier under the effects of metal.

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