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Influence of Iranian Natural Zeolite on Accumulation of Cadmium in *Cyprinus carpio* Tissues Following Exposure to Low Concentration of Cadmium

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ABSTRACT
The study was undertaken to illustrate comparative accumulation of cadmium in different organs and the effect of ion-exchanging agent zeolite on reduction of cadmium toxicity in common carp after 15 and 30 days post exposure to low concentration of cadmium. The experiment were carried out with 45 fish were obtained from local fish farm. Fish were randomly distributed in three indoor fiberglass tanks supplied with well water. Group I was used as a control group, while the second group exposed to 30 ppb (30 μg L^{-1}) cadmium as $CdCl_2$ and group III was exposed to 30 μg L^{-1} cadmium plus 10 mg L^{-1} natural zeolite (clinoptilolite) for 30 days. Five fish were randomly removed from each tank at the days 15 and 30 post exposure and organ samples including kidney, liver, gill and spleen were provided. Water quality parameter and the level of cadmium in water were measured two times weekly throughout the exposure. The cadmium accumulation in different organs and water were monitored using atomic absorption spectrophotometer. The present study illustrated that addition of zeohte to cadmium contaminated water; significantly ($p \le 0.05$) reduces the metal level in examinant tissues after 30 days. Following 30 days exposure the cadmium accumulation and bioconcentration factors were in the order kidney ≈ gill > liver ≈ spleen in cadmium exposed group (groupII). In kidney, liver and gill the cadmium concentration increased as the duration time so that, after 30 days the level of cadmium in kidney, liver, gill and spleen was significantly ($p \le 0.05$) higher than was in 15th day.

Key words: Cadmium, *Cyprinus carpio,* natural Iranian zeolite, clinoptilolite, bioconcentration factors

INTRODUCTION
Heavy metals like zinc, copper, lead, chromium, nickel and cadmium are discharged in the water bodies without extracting the pollutant (Singh *et al.*, 2008). Cadmium is one of the most toxic heavy metals. Its toxicity has been widely studied and reported. This metal is a serious environmental and occupational contaminant and may represent a serious health to man and animals (WHO, 1992).

Cadmium discharged in large quantities from battery and inverter manufactures, dyeing, printing and electroplating units. It tends to accumulate in tissues of biotic, flora-fauna and has deleterious effect on fish (Das and Kaviraj, 1990; Sherry and Abidi, 2002; Ashram *et al.*, 2003). The main effect of the increase in concentration of lead and cadmium in aquatic systems to teleost (Tao *et al.*, 1999) and invertebrate aquatic animals (Gundacker, 1999) is the accumulation of lead and cadmium in tissues and organs of these organisms. Metallothioneins (MTs) are low molecular mass, cysteine-rich, metal-binding polypeptides, which occur in a large number of phylogenically diverse organisms, including fish (Roesijadi, 1992; George and Langeston, 1994). MTs are implicated in regulation of essential element, copper and zinc. Additionally, a role in detoxification of non-essential metals such as cadmium has been attributed to this metal-binding protein (Nordberg, 1998). Kidney, liver and gill, which are the main target organs for cadmium in carp (Kuroshima, 1992; Cinier *et al.,* 1997). Fish accumulated cadmium from polluted environment resulting in accumulation in their tissues. Common carp have the ability to accumulate and concentrate cadmium to levels several orders of magnitude above those found in their environment (Cinier *et al.*, 1997). Cadmium in low concentration can affect the lysozyme activity, WBC count and phagocytic index in common carp (Ghiasi *et al.*, 2010). The natural zeolite clinoptilolite has a cation-exchange capacity (about 2.3 meq g^{-1}). Na^+ ion of zeolite will displace the Ca^{++} ion and Mg^{++} ion in water .Chnoptilolite is probably the most abundant zeolite, world wide and it formed chiefly from the alteration of sihcic volcanic ash by pore water during diagenesis. The exchangeable cations of a zeolite are only loosely bonded to the tetrahedral framework and can be removed or exchanged easily by washing with a strong solution of another ion (Mumpton, 1999; Cinar and Beler-Baykal, 2005). The present investigation was designed to study the effects of natural zeolite on cadmium level in different tissues of common carp following exposure to low concentration of cadmium.

MATERIALS AND METHODS

Common carp were obtained from a local fish farm in Iran were acclimated in holding tank for 1 week, then 45 apparently healthy fish, mean weight 700 g were randomly divided into three groups (I-III) 1000 L indoor fiberglass tanks supplied with cadmium free well water (hardness 320.6 mg L^{-1} as $CaCO_3$, dissolved oxygen 8 mg L^{-1}, pH 7, temperature 14°C±3), without water flow and with continuous aeration.

Group I was without cadmium and zeolite as control, group II exposed to 30 ppb cadmium as $CdCl_2$ (Merck) for 30 days and group III exposed to 30 ppb cadmium in addition 10g L^{-1} zeolite. Weight percentages of SiO_2, Al_2O_3, Fe_2O_3, TiO_2, CaO, MgO, K_2O, Na_2O, P_2O_5 and LOI measured in natural Iranian zeolite obtained from Semnan mine applied in this study were 64.4, 12.8, 1.31, 0.31, 2.37, 1.15, 1.13, 2.64, 0.21 and 13.47, respectively (Asilian *et al.*, 2004). The fish were starved during the whole examinations period. Water quality was measured throughout the exposure two times weekly. Five fish from each tank were sampled for tissues material containing kidney, liver, gill, which are the main target organs for cadmium in carp (Kuroshima, 1992; Cinier *et al.*, 1997) and spleen at day 15 and 30 post exposure. The determinations of cadmium in different organs were carried out with a SHIMADZU AA-670 atomic absorption spectrophotometer under recommended conditions for cadmium ion. Two grams of each sample was decomposed in 5 mL perchloric acid (Merck) and 20 mL nitric acid (Merck) under heating until white fumes evolved. The hydiolyzate was removed from the heat and allow standing until cooled. Then 10 mL of hydrochloric acid (9%) was added to dissolve salts and deionized water was added to make 50 mL, which was subjected to measurement (Eaton *et al.*, 2005). Sampling stages of this research was conducted during three months from beginning of October to the end of December 2008.

Statistical analyses: The control and exposure fish data were analyzed using Duncan mean comparing test and data were significant at $p \leq 0.05$ (Chung, 1981; Atar and Ates, 2009).

RESULTS AND DISCUSSION

Cadmium in concentration 30 ppb was not lethal for common carp over the 30 days experimental period. The present study illustrated that after 15 days exposure (30 μg L^{-1}) cadmium content in kidney was significantly higher than other organs but the difference between cadmium levels in other tissues was not significant. In kidney, liver, gill and spleen the cadmium concentration increased as the duration time so that, after 30 days the level of cadmium in kidney, liver, gill and spleen was significantly ($p \leq 0.05$) higher than was in 15th day (Table 1). Following 30 days exposure the cadmium accumulation were in the order kidney ≈ gill > live ≈ spleen in cadmium exposed group (group II), also after 30 days the cadmium accumulation in target tissues in cadmium and zeolite exposure group (group III) was significantly ($p \leq 0.05$) lower, when compare with similar tissue in cadmium exposure group (Table 1). In the day 30th, cadmium level in water with zeolite is lower than water without zeolite in the day 15th.

Cadmium bioconcentration factors: Bioconcentration Factors (BF) are often used to compare body burden of an organism with the degree of contamination in the water (Taylor, 1983). In the present study Bioconcentration Factor (BF) was in the order kidney ≈ gill > liver ≈ spleen after 15 and 30 days (Table 2).

No fish died during this experiment in spite of high cadmium concentration in tissues. Cadmium concentrations increased in the different organs as a function time. After 15 days at a toxicity of 30 μg L^{-1} the cadmium concentration in kidney was about 1.4-fold higher than in gill, 2-fold higher liver and 2.6-fold higher than spleen while after 30 days exposure the cadmium concentration in kidney was 1.8-fold higher than in liver and spleen. This cadmium distribution, illustrating a decrease in order of accumulation from kidney ≈ gill > liver≈ spleen. MT is involved in both metal regulation and detoxification tasks. These processes are time, age, sex and species dependent (Olsson, 1996). Previously kidney was reported as the main target of cadmium

Table 1: Cadmium contents (ppb) in different tissues and water 15 and 30 days following exposure to cadmium in low concentration (30 ppb) in group II and cadmium (30 ppb) plus zeolite 10 g L^{-1} in group III

Exposure	Kidney	Liver	Gill	Spleen	Water
15 days					
Cadmium (II)	80.4550±14.1656[a]	38.4050±17.1433[b]	57.6540±10.7610[b]	30.9500±4.3575[b]	17.9
Cadmium+Zeolite (III)	98.4200±26.7875	72.9160±23.0586	43.7088±20.0236	66.0378±18.728	5.2
30 days					
Cadmium (II)	121.9506±24.6846[a]	68.2980±9.4479[b]	129.4540±15.9899[a]	66.0738±18.7280[b]	12.8
Cadmium+Zeolite (III)	80.3400±16.6143	49.6700±6.8411	63.8250±20.0236	20.6188±2.9422	3

Values with different letter(s) having significant means at 5%, Values are mentoned as Mean±SD

Table 2: Bioconcentration factors (BCFs) in kidney, liver, gill and spleen of common carp 15 and 30 days after exposure to 30 mg L^{-1} cadmium

BCF	Kidney	Liver	Gill	Spleen
Day 15	4.49±0.79	2.24±1.13	3.01±0.72	1.72±0.24
Day 30	9.50±1.90	5.32±0.73	10.11±1.24	5.15±1.46

Values are mentioned as Mean±SD

(De Smet *et al.*, 2001). Cinier *et al.* (1999) was reported, in common carp, after 127 days Cd exposure (53 μg L^{-1}) the cadmium concentration in kidney was 4-fold higher than in liver. Melgar *et al.* (1997) were reported that following exposure the rainbow trout to 50 μg L^{-1} for 21 days the order of cadmium accumulation in the assayed tissues was kidney> live> gill. These findings are relatively in agreement with our results. Somewhat discrepancy between our results and previous reports are probably because of experimental period, cadmium dose, age and species.

In present study fish exposed to cadmium showed a progressive rise in cadmium content in time dependent manner. As shown in Table 1, cadmium accumulation in gill was increased sharply reaching from 57.6540±10.7610 at the day 15 to 129.4540±15.9899 at the day 30 but rate of increasing in other target tissues are steadily. This result is in contrast with Handy (1993) who reported that in rainbow trout, branchial contamination increased steadily. This difference in toxicity is also reflected in ability of rainbow trout to rapidly clear dietary compared to waterborne Cd (Harrison and Klaverkamp, 1989) Dietary exposure to Cd is much less toxic than exposure via the waterborne route (Handy, 1992). The gut represents a formidable mucosal barrier to toxic metals (Harrison and Klaverkamp, 1989). According to De Smet *et al.* (2001) after cadmium exposure, (Cd, Zn)-MT levels increased maximally approximately two fold in gills and 4.5 fold in kidney. MT is involved in both metal regulation and detoxification tasks. These processes are time, age, sex and species dependent (Olsson, 1996). As for (Cd, Zn)-MT concentrations, MT synthesis correlated with cadmium concentrations in gill and kidney, but low and not significant correlation was found in liver. In fish, gills are considered to be the dominant site for contaminant uptake because of their anatomical and/or physiological properties that maximized absorption efficiency from water (Hayton and Barron, 1990). However, it was evident from our study that, in general, either Kidney or gill was the site of maximum accumulation for the cadmium while liver and spleen was the over all site of least cadmium accumulation. The capacity for metallothionein is greatest in tissues that are active in metal uptake, storage and excretion. In aquatic animals, metallothioneins have been identified in the small intestine (Glover and Hogstrand, 2002; Shears and Fletcher, 1979), liver (Thiele, 1992), kidney (Ciner *et al.*, 1998) and gill of fish (Shears and Fletcher, 1985) the same organs were contaminated in this study. This induction results in relatively high concentration of metallothionein-bound metals in these organs and in cases such as Cd, results in a slower turnover of the metal. Binding of metals to metallothionein enhances bioaccumulation in gill as well as in the liver and kidney. As shown in Table 1, zeolite can decrease the cadmium level in water from 17.9 at the day 15 to 3 ppb at the day 30. Bioconcentration Factors (BCFs) are used to relate pollutant residues in aquatic organisms to the pollutant concentration in ambient waters. The Bioconcentration Factor (BCF) is related to biomagnification effects. Many chemical compounds, especially those with a hydrophobic component partition easily into the lipids and lipid membranes of organisms and bio-accumulate. If the compounds are not metabolized as fast as they are consumed, there can be significant magnification of potential toxicological effects up the food chain. As shown in Table 2, BF illustrated an increase with exposure time an inverse relationship was observed between the bio accumulation factor and cadmium level in water.

The results shows addition of zeolite to cadmium contaminated water significantly reduced the cadmium level in fish tissues. Present result is agree with James and Sampath (1999) who reported zeolite is able to reduce cadmium toxicity in water and fresh water fish, *Oreochromis mossambicus*. Zolite has extra frame work ion (Na^{+}) and framework ions (Al^{3+} and Si^{4+}) which are easily exchangeable and non-exchangeable respectively. The ionic radii of Na+, Al^{3+} and Si^{4+} are 0.95,0.50

and 0.54 A°; the ionic radius of Cd^{2+} is 0.97 A° (Huheey, 1983) which is suitably, matched to the ionic radius of Na^{+}(0.95 A°) and therefore both ions could be easily exchange with each other than Al^{3+} and Si^{4+}.

CONCLUSION

The results obtained in the present study, recommended that, application of 10 g z L^{-1} to cadmium polluted water could reduce the cadmium toxicity in common carp but finding the best level of zeolite requires more investigation. Zeolite is cheap, cause no side effects should be considered as suitable agents to improve the water quality in fish ponds.

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