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Influence of montmorillonite on cadmium accumulation in carp, *Ca a a a*

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A B S R A C

The present study was designed to test the influence of montmorillonite (MMT) on cadmium accumulation in different tissues of *Ca a a a* . 180 normal healthy *C. a a* were randomly divided into four groups. They were fed with normal diet, normal diet supplemented with MMT (0.5%), high-cadmium (cadmium chloride, 120 mg/kg) diet and high-cadmium diet supplemented with MMT (0.5%). After 60 days, cadmium concentration in several tissues were measured by eletrothermic atomic absorption spectrophotometry, metallothionein contents were determined using the Cd-chelex assay and histopathological alterations of kidney were observed. MMT inhibited cadmium accumulation in kidney, intestine, liver, spleen, blood, gills, spermary, ovary, brain, bone and muscles of *C. a a* . Metallothionein levels in liver, intestine and muscle decreased and pathological changes of kidney were reduced by MMT addition. Thus, MMT protected tissues of *C. a a* from cadmium accumulation and damage.

1. Introduction

Cadmium, a nondegradable cumulative pollutant, is one of the most deleterious xenobiotic in aquatic ecosystems. Freshwater fish are particularly vulnerable to Cd exposure (Nriagu et al., 1998; Xu and Bai, 2007) by gills and gastrointestinal tract. The harmful effects of Cd ions are renal damage, hypertension, proteinuria, kidney stone formation and testicular atrophy (ATSDR, 1999). Since fish are an important food resource and major ecosystem component and Cd may accumulate in the body throughout the food chain, it poses a serious threat to human health. Therefore, the need for strict discharge limits on Cd led to the investigation of efficient and economically beneficent treatment methods for Cd removal.

Considerable efforts have been made developing low cost removal technologies that can effectively immobilize dissolved toxic metals. Physical and chemical processes have been extensively studied to remove Cd pollutants from wastewaters at high concentrations (Tee and Khan, 1988; Chang et al., 1997; Quinaia et al., 2006). Some of these processes are adsorption, coagulation, flotation, biosorption, precipitation, ultra filtration and electrochemical methods (Al-Asheh and Duvnjak, 1997; Pino et al., 2006; Herrero et al., 2007). Adsorption onto a low cost particulate media, such as a clay mineral, offers an attractive and inexpensive remediation option (Babel and Kurniawan, 2003). In this study, montmorillonite (MMT) was added in the feed to assess its influence on Cd accumulation in the organs of *C. a a* .

2. Methods

2.1. MMT e a a

MMT used in the current work was a hydrothermal product of volcano sedimentary rocks from the Inner Mongolia Autonomous Region, China. Besides MMT, there were minor amounts of quartz and volcanic glass present. The raw material was dried in oven over night at 80 °C and then milled to <300 meshes. The milled material was dispersed in water to form a 10% dispersion and kept for about 10 min during stirring. Particles >1 μm were separated by sedimentation while the dispersion was centrifuged to get refined MMT. The refined MMT was dried at 80 °C followed by another milling to <300 meshes.

2.2. E e e a de a d fi ea

The freshwater wild carp (*C. a a*) weighing 20.68 ± 1.08 g were obtained from a local supplier (Hangzhou, Zhejiang province of China) and reared in aerated laboratory tanks at 22–25 °C for 2 weeks prior to experiments. They were fed commercial dry pellets for *C. a a* at 1.5%–2.5% of body weight two times a day during the experimental period. The parameters of the test water were as follows: pH 7.0–7.5, dissolved oxygen 5.5–7.0 mg/l. After acclimatization, a total of 180 *C. a a* were randomly allocated to four dietary treatments (Table 1) for 60 days, each of which have three replicates of 15 fish/tank. Since significant decreases in protein, lipid, and glycogen concentrations in the carcass caused a reduction in calculated whole-body energy content in fish fed with Cd concentration of 125 mg/kg (Berntssen and Lundebye, 2001), Cd exposure (120 mg/kg) in feed was selected. Diets

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Table 1
Setup for *Ca a a a* rearing

Group	Feed supplements	Number of fish
Control	Basal diet	15×3
Trial I	Basal diet+0.5% MMT	15×3
Trial II	Basal diet+120 mg/kg Cd	15×3
Trial III	Basal diet+0.5% MMT+120 mg/kg Cd	15×3

were formulated to meet nutrient requirements suggested by the NRC (1993) for crucian carp and the content of Cd in basic diet was 0.19 mg/kg. To avoid waterborne Cd contaimination, the unfed feed and feces were removed every 2 h with a siphone tube.

The experimental system was a closed recirculation system consisting of 12 self-cleaning aquarium (50 cm wide×100 cm long×50 cm high), sedimentation tanks, a biological filter and a UV filter to prevent cross contamination of micro-organisms between treatments. The system was installed in an environment-controlled laboratory maintained at 25 °C, with a photoperiod of 12 h light and 12 h darkness. The culture system was also provided with continuous aeration through an air compressor and heaters to keep water temperature.

2.3. Sa e a ce d

Feed intake was recorded/group for the feeding study. At the start and the end of the study, body weight of each animal was recorded to determine average daily gain (ADG) and feed conversion ratio (FCR) for each group. The indicators were calculated as: ADG=100(ln(Final body weight)−ln(Initial body weight))/time (days); FCR=dry feed intake (g)/wet weight gain (g); Percent survival (%)=Final fish number/initial fish number×100.

2.4. Cad de e a

The fish were quickly anesthetized with 50 mg/l MS222 (tricane methane sulphonate) for 2–3 min (this concentration is not an overdose of MS222). The kidney, intestine, liver, spleen, blood, gills, spermary, ovary, brain, bone and muscle were dissected out, placed in tubes, dried and weighed, and digested in 5 ml concentrated nitric acid (APHA, 1998). Samples were analyzed for Cd accumulation by eletrothermic atomic absorption spectrophotometry with Zeeman correction, using a graphite furnace tube (Pierron et al., 2008). The results of the accumulation experiments were reported as μg Cd/g dw.

2.5. H a ca d d e

Ten fish were randomly collected after 2 months of exposure. They were quickly anesthetized as described previously. Kidney was dissected out and fixed in Bouin's fluid for 24 h, washed several times in 70% alcoho1 and then dehydrated in a graded series of ethanol and embedded in paraffin. They were sectioned at 3–5 μm thickness, and sections were stained with hematoxylin and eosin, and observed under an Olympus Vanox 1ight microscope.

2.6. Me a e ea e e

After anesthetization, the liver, intestine, and muscle were dissected quickly, and the intestine were longitudinally cut open, rinsed with cold physiological saline before being frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

Aliquots of liver, intestine and muscle samples were homogenized in 3 volumes Tris–HCl buffer (10 mM Tris–HCl, 86 mM NaCl, pH 7.4) at 4 °C with an Ultra-turrax T3 homogenizer (IKA, Labor Technique, Shaufer, Germany). Tissue homogenates were centrifuged at 16,000 for 20 min to isolate the cytosolic fraction (supernatant). Total metallothionein concentrations in the cytosolic fraction were determined using the Cd-chelex assay (Reynders et al., 2008).

2.7. S a ca e a a

The Student -test was used for growth data, Tissue levels of Cd were compared between groups by a one-way ANOVA. The post-ANOVA test of Neumann–Keuls was used to determine specific changes in tissue Cd levels among groups of carps. A two-way-factor ANOVA was used for metallothionein studies. A value of <0.05 was considered to be significant. Results are given as mean±S.E..

3. Results and discussion

3.1. E ec d e a MMT C. a a

Traditionally, MMT has been incorporated in animal diets as a technological additive (lubricant or agglomerant) to improve feed manufacture (Hu et al., 2007). A role as enhancer of the nutritive value of diets in animals has been recently proposed. Animal feed containing MMT (10–30 g/kg) has been shown to promote growth of chickens and swine (Venglovsky et al., 1999; Tauqir and Nawaz, 2001). In present study, there was not a significant difference ($P>0.05$) in ADG and FCR between MMT (trial I) and the control group (Table 2). However, compared to the control group, the addition of 120 mg/kg cadmium to the diet (trial II) resulted in a 20.2% ($P<0.05$) decrease in ADG and 26.1% increase in FCR. Compared to trial II, the addition of MMT to the diet (Trial III) resulted in an 11% ($P<0.05$) increase in ADG and a 13% ($P<0.05$) decrease in FCR. Thus, fish fed with Cd and MMT showed better growth compared to fish fed with Cd only. It is suggested that MMT slows down the negative effect of Cd on growth of *C. a a* .

3.2. E ec d e a MMT Cd c ce a e C. a a

Exposure of *C. a a* to dietary Cd resulted in a significant Cd accumulation in the tissues in the orders kidney, intestine and liver (Fig. 1A). In *C. a a* fed with MMT there was a reduction in Cd contents of these tissues compared with control group (Fig. 1A). The reason may be that there was some Cd in basic diets. The Cd concentrates increased in the order kidney>the intestine>the liver>the spleen of carps fed with Cd (Fig. 1B), indicating accumulation or permanent damage of these tissues. Addition of MMT to the diet with Cd resulted in decrease by 21.6% ($P<0.05$) in the kidney, 31.0% ($P<0.05$) in the intestine and 42.2% ($P<0.05$) in the liver (Fig. 1B). Thus, MMT efficiently reduced Cd accumulation in tissues of *C. a a* . The possible mechanism may be that MMT can adsorb Cd via ion exchange reactions (Barbier et al., 2000).

3.3. H a ca d d e

To verify the function of MMT, we evaluated the effect of MMT on tissue injuries induced by Cd. Since the kidney was the most

Table 2
Growth performances and feed utilization of *C.a a*

	Group			
	Control	Trial I	Trial II	Trial III
Initial weight (g)	20.53±0.29	20.58±0.26	20.72±0.30	21.09±0.32
Final weight (g)	27.34±0.33^{a}	27.59±0.33^{a}	26.11±0.25^{c}	27.01±0.30^{b}
ADG (g)	0.114±0.002^{a}	0.112±0.003^{a}	0.091±0.001^{c}	0.101±0.002^{b}
FCR	3.60±0.16^{c}	3.53±0.01^{c}	4.54±0.09^{a}	3.95±0.04^{b}
Survival rate (%)	100	100	100	100

ADG means average daily gain and FCR means feed conversion ratio.
Results were presented as means±S.E. of triplicate observations.
Means in the same row with different letters were significantly different ($P<0.05$).

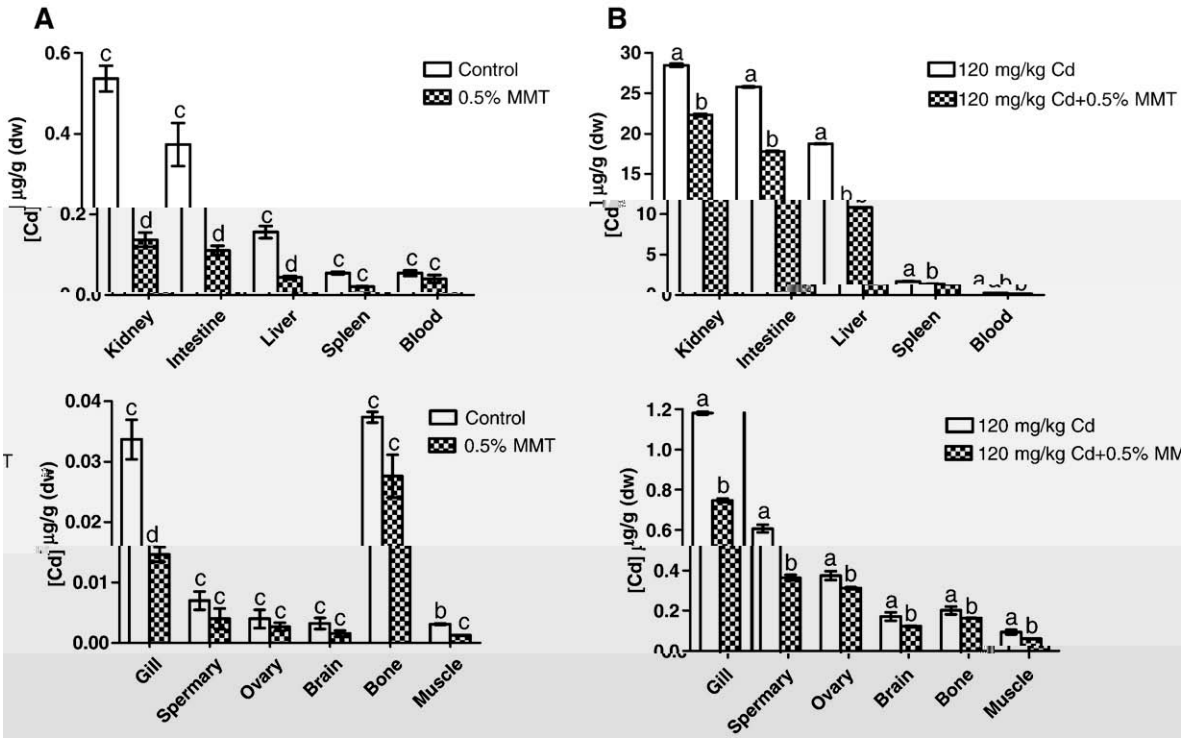

Fig. 1. Cadmium concentration (mean±S.E.) in kidney, intestine, liver, spleen, blood, gills, spermary, ovary, brain, bone and muscle of *C. ca a* exposed for 60 days to four experimental conditions. Control: fish fed with basal diet; Trial I: fish fed with basal diet+MMT (0.5%); Trial II: fish fed with basal diet+Cd (120 mg/kg); Trial III: fish fed with basal diet+Cd (120 mg/kg)+MMT (0.5%). For each time and organ, columns designated with different letters (a, b, c, d) are significantly different (LSD test, $P<0.05$).

accumulation site, it was selected to test the damage. Cd induced wide lumen and mild swelling as previous researches (Wangsongsak et al., 2007). The photomicrograph of the kidney of *C. a a* in control group showed the distal tubules were low columnar epithelium with basally round nucleus and eosinophilic cytoplasm. Parenchyma cells were regular in arrangement with clear cell outline (Fig. 2A). The kidney of the fish fed with MMT showed normal appearance similar to the control (Fig. 2B). When the fish was fed with Cd only, proximal tubules of kidney exhibited wide lumen and mild swelling. Mildly swollen proximal tubular epithelial cells with dilated nuclei were observed (Fig. 2C). However, when adding MMT to Cd containing feed, the kidney showed the normal structure (Fig. 2D). MMT can protect

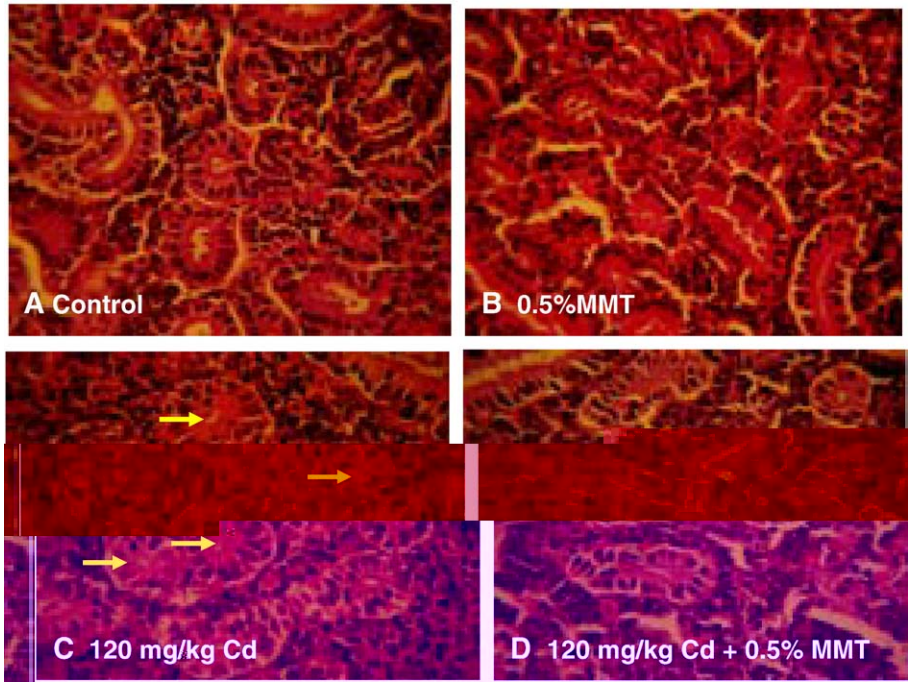

Fig. 2. Transverse section of *C. a a* kidney. A: Kidney of control fish showing normal shaped proximal tubule and tubular cells with nuclei; B: kidney of fish fed with MMT (0.5%) showing the same as control; C: kidney of fish fed with 120 mg/kg Cd exhibiting widen lumen (arrowhead), mild swelling of proximal tubule and distal tubule; D: Kidney of fish fed with 120 mg/kg Cd and 0.5% MMT showing similar performance as control.

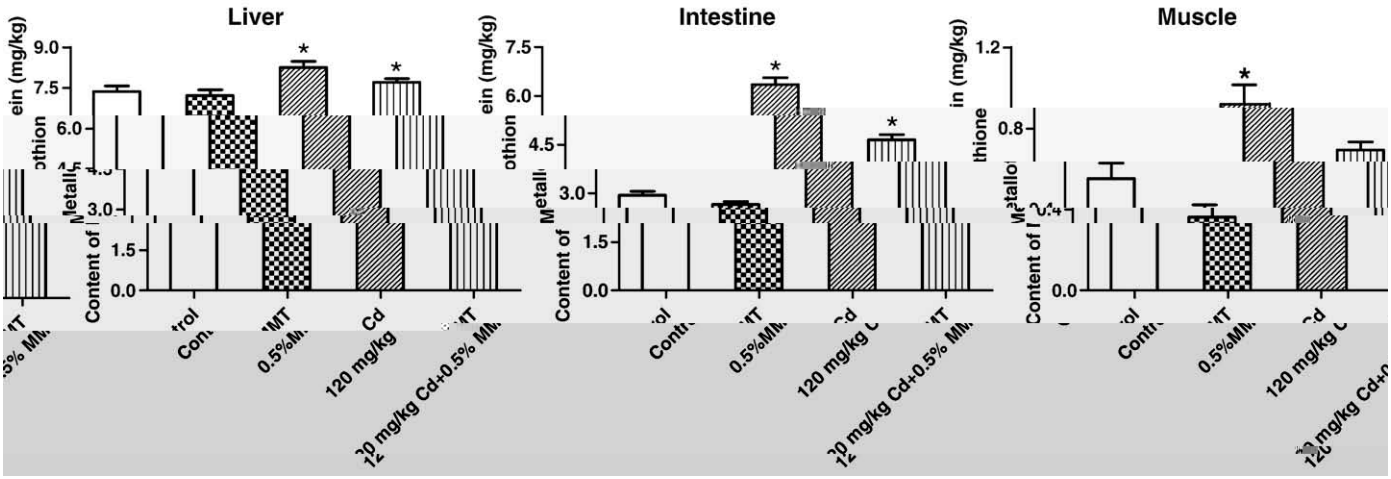

Fig. 3. Metallothionein concentration in liver, intestine and muscle of *C. a a* . Date represent the mean and standard deviation. An (*) denotes a value significantly less than the corresponding control value ($P<0.05$).

the kidney from damages caused by Cd and MMT probably play a role in reducing the accumulation of Cd in the kidney.

3.4. Me a e

Metallothionein, a tissue protein with high affinity for Cd, forms chelates with Cd . This complex formation represents an important mechanism for detoxification, or transport of Cd. Metallothionein has been applied in both laboratory and field studies (De Smet et al., 2001; Hansen et al., 2006) as a biomarker of metal exposure. In our study, metallothionein was measured in liver, intestine and muscle tissues of *C. a a* to provide evidence for MMT effect on the Cd level. There was no change of the metallothionein level in the fish supplied with MMT (Fig. 3). Metallothionein levels were significantly higher in fish fed with Cd compared to the control. The addition of MMT to the diet with Cd decreased metallothionein content (Fig. 3). One possible explanation is that the methallothionein concentrations were sufficient high to detoxify Cd. Two fundamental properties of metallothionein are their high kinetic reactivity and their high affinity to bind metalions (Stillman, 1995), therefore complexing Cd and rendering it non-toxic. This result also indicated that MMT reduced the content of Cd in tissues of *C. a a* .

4. Summary

There are numerous direct and indirect effects of MMT and cadmium which can modify the absorption, distribution and excretion of cadmium in *C. a a* . To our knowledge the present report is the first to show that MMT, when co-administered with cadmium, could reduce cadmium accumulation in the tissues of *C. a a* .

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