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Heavy Metal Accumulation and Tissue Damage in Goldfish *Carassius auratus*

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Cadmium (Cd), lead (Pb) and zinc (Zn), entering into aquatic system by industrial and consumer waste, can cause contamination of the aquatic environment. These heavy metals at concentrations far below lethal levels may still cause serious damage to the physiological processes or tissues in fish exposed to them (Hemalatha et al. 1997). Although Cd and Pb do not have a physiological role in living organisms, when entering into the food chain as a result of bioaccumulation, they can induce health problems in organisms. Zn is an essential element for plants, animals and humans, but if present in high concentrations it can also be highly toxic.

Goldfish (*Carassius auratus*) is a common species in the Yellow River and widely reared as an important food fish in north of China. With a high level of tolerance against heavy metals, goldfish is an ideal bioindicator. Since gill, liver and kidney are the sensitive targets to heavy metals, the accumulation and histological lesions in these organs were studied. There are many reports describing effects of accumulation of heavy metals in fish tissues and organs (Allen 1995; Pelgrom et al. 1995; Kargin et al. 1999; Cogun et al. 2003). However, there is little known about Cd, Pb, Zn accumulation and tissue damage in *C. auratus*. Since heavy metals contamination is rarely limited to a single compound, studies on their interrelationships are of great significance. In this paper, we examined Cd, Pb and Zn accumulations and the histological lesions in gill, liver and kidney of *C. auratus* exposed to various heavy metal concentrations and periods.

MATERIALS AND METHODS

Goldfish were obtained from a commercial dealer. These fish were reared in glass aquaria containing unchlorinated tap water and fed with commercial fish food for the duration of the experiment. Experiments were conducted in three series to determine the accumulation levels in organs of goldfish when exposed to heavy metals for 9, 18 and 36d respectively. In each series, we used 10 aquaria similar in size (50cm × 20cm × 30cm) for 10 groups, each in two replicates: control; 0.5ppm and 5.0ppm Cd (diluted with $CdCl_2 \cdot 2.5H_2O$); 0.5ppm and 5.0ppm Pb (diluted with $Pb(NO_3)_2$); 0.5ppm and 5.0ppm Zn (diluted with $ZnSO_4 \cdot 7H_2O$); 0.5ppm Cd + 0.5ppm Pb; 0.5ppm Pb + 0.5ppm Zn; 0.5ppm Cd +0.5ppm Zn. A combination of 0.5ppm of all three heavy metals resulted in 100% mortality by 7 days, so this

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exposure regimen was not pursued further. The concentrations of these three heavy metals were set referring to the 96hr lethal concentrations (Cd: 34.8ppm, Pb: 276.0ppm Zn: 27.6ppm) and their concentrations in the upper of Yellow River (Cd: 0.002ppm, Pb: 0.0139ppm, Zn: 0.038ppm). $CdCl_2 \cdot 2.5H_2O$, Pb $(NO_3)_2$ and $ZnSO_4 \cdot 7H_2O$ were obtained from Shanghai Jinshan Chemical Company and were analysis purity. After a 15d acclimation period, 10 goldfish (weight: 1.9±0.3g; body length: 3.8±0.3cm) were put into each aquarium and fed under the same conditions (water volume: 20L, water temperature: 20±1℃, water depth: 20cm, and natural photoperiod). The water in the aquaria was changed every two days with the same former concentrations of Cd, Pb and Zn. During the experiment period, there was no mortality among the experimental fish.

At the end of each exposure time of 9, 18 and 36d, 10 goldfish from each group were taken out and euthanized by an overdose (500mg/L) of tricaine methanesulfonate (MS-222) (American Veterinary Medical Association 2001), and then gill, liver and kidney were removed for accumulation detection and histological observation. The samples for accumulation detection were dried at 120℃ for 48hr, weighed and incinerated at 550℃ for 8~10hr, then dissolved by nitric acid and diluted with 10ml of distilled water and measured by flame atomic absorption spectrometry (AAS), Cd at 228.8nm, Pb at 283.3nm, Zn at 213.9nm respectively. Calibrations were performed using standard concentrations (Cd and Zn: 0.2μg/mL; Pb: 1.0μg/mL; obtained from Shanghai Chemical Company) for AAS. Recoveries and standard deviations of these three heavy metals were all > 91% and < 7% (n=15). The detection limits were 0.02μg/mL for Cd and Zn, 0.1μg/mL for Pb. The samples for histological observation were fixed in Bouin Fluid for 4hr, embedded in paraffin, sectioned into 7μm slices and stained with hematoxylin-eosin. As there were no mortality and apparent gross lesions among any groups, the slices were photographed only at the 36d exposure sample.

The results were expressed as means±standard errors (SEs). One-way ANOVA and two-tailed Student's t-test were used for statistical analysis. $P<0.05$ accepted as statistically significant, $P<0.01$ as greatly significant.

RESULTS AND DISCUSSION

The accumulations of Cd, Pb and Zn in gill, liver and kidney were expressed in μg/g dry weight (Table 1-3). Compared with the controls, there were significantly ($P<0.01$) increased accumulations of Zn in gill, liver and kidney at 0.5 and 5.0ppm groups respectively (Table 3). For all three heavy metals and organs concerned, the differences between 0.5ppm and 5.0ppm groups were greatly significant ($P<0.01$) at the same exposure time, which suggested that heavy metal accumulation in the organs was dependent upon exposure dose. The accumulation levels of the three heavy metals in gill, liver and kidney increased with treatment time prolonging ($P<0.01$), implying that heavy metal accumulation in the organs was dependent upon exposure time.

Similar to most previous reports (Tulasi et al., 1992; Allen 1995; Liu et al. 2001), our results showed that Cd and Pb accumulations in liver and kidney at 0.5ppm

Table 1. Cd accumulation levels (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5 and 5.0ppm concentrations.

Organs	Concentrations	9d	18d	36d
Gill	Control	ND	ND	ND
	0.5ppm Cd	1.61±0.12	2.75±0.30	4.48±0.54
	5.0ppm Cd	4.37±0.38 a	12.92±0.77 a	28.87±2.98 a
Liver	Control	ND	ND	ND
	0.5ppm Cd	14.49±0.97 b	23.80±1.13 b	25.27±0.69 b
	5.0ppm Cd	23.32±2.18 ab	27.24±1.00 ab	73.18±1.93 ab
Kidney	Control	ND	ND	ND
	0.5ppm Cd	16.38±1.22 b	27.66±1.83 b	38.54±1.46 bc
	5.0ppm Cd	29.34±2.61 abc	34.83±1.95 abc	86.76±4.17 abc

ND=Not detectable. a indicates significant difference between 5.0ppm and 0.5ppm Cd groups at the $P<0.01$ level; b indicates significant difference between liver or kidney and gill at the $P<0.01$ level; c indicates significant difference between kidney and liver at the $P<0.01$ level.

Table 2. Pb accumulation levels (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5 and 5.0ppm concentrations.

Organs	Concentrations	9d	18d	36d
Gill	Control	ND	ND	ND
	0.5ppm Pb	4.24±0.52	6.30±0.44	7.08±0.90
	5.0ppm Pb	15.62±0.69 a	23.42±2.23 a	28.06±2.30 a
Liver	Control	ND	ND	ND
	0.5ppm Pb	5.13±0.57 b	8.83±0.44 b	10.81±0.71 b
	5.0ppm Pb	28.02±1.94 ab	35.07±1.48 ab	53.77±0.96 ab
Kidney	Control	ND	ND	ND
	0.5ppm Pb	13.18±1.04 bc	17.83±1.46 bc	22.65±1.69 bc
	5.0ppm Pb	33.84±3.06 ab	42.73±3.81 abc	61.89±5.22 abc

ND=Not detectable. a indicates significant difference between 5.0ppm and 0.5ppm Pb groups at the $P<0.01$ level; b indicates significant difference between liver or kidney and gill at the $P<0.01$ level; c indicates significant difference between kidney and liver at the $P<0.01$ level.

and 5.0ppm groups were greatly higher than those in gill ($P<0.01$). This may be because that liver (an important storage, redistribution, transformation and detoxification organ) and kidney (a key wastes metabolism, water and electrolyte balance and acid-base concentration regulation organ) can accumulate much more

Table 3. Zn accumulation levels (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5 and 5.0ppm concentrations.

Organs	Concentrations	9d	18d	36d
Gill	Control	18.54±0.74	23.65±1.95	26.59±2.38
	0.5ppm Zn	28.80±2.86 d	54.08±2.93 d	57.33±2.12 d
	5.0ppm Zn	94.89±3.75 ad	98.03±4.27 ad	294.89±5.09 ad
Liver	Control	10.54±0.67	11.66±0.92	23.53±2.03
	0.5ppm Zn	16.80±0.77 bd	26.70±1.86 bd	53.44±1.42 bd
	5.0ppm Zn	52.53±2.12 abd	75.30±2.74 abd	107.46±3.55 abd
Kidney	Control	8.41±0.55	12.37±0.88	24.92±1.85
	0.5ppm Zn	15.63±0.61 bd	28.46±2.07 bd	52.13±1.6 bd
	5.0ppm Zn	55.68±2.52 abd	82.44±3.42 abcd	134.82±5.77 abcd

a indicates significant difference between 5.0ppm and 0.5ppm Zn groups at the $P<0.01$ level; b indicates significant difference between liver or kidney and gill at the $P<0.01$ level; c indicates significant difference between kidney and liver at the $P<0.01$ level; d indicates significant difference between treatment group and control at the $P<0.01$ level.

Cd and Pb which are not involved in any physiological function. However, the accumulations of Zn in liver and kidney were significantly lower than those in gill ($P<0.01$) both at 0.5ppm and 5.0ppm groups. This may be due to the fact that Zn is an essential and important metal in metabolic activity for the organisms.

Pb and Zn increased Cd uptake in gill, liver and kidney at 9 and 36d exposure time. However, Cd accumulation at 18d exposure time was increased in gill and decreased in liver (Figure 1). Zn increased Pb uptake significantly ($P<0.05$) in all three organs when goldfish were exposed to Pb+Zn mixture for 9, 18 and 36d (Figure 2). While Cd significantly increased Pb uptake ($P<0.05$) at 9 and 36d exposure to Pb+Cd mixture, it decreased Pb uptake at 18d exposure time. Cd and Pb increased the accumulation of Zn significantly ($P<0.05$-0.01) in gill, liver and kidney at 9d exposure time (Figure 3). At 18d of exposure to Zn+Cd mixture, Zn uptake greatly ($P<0.01$) increased in gill and kidney and decreased ($P<0.05$) in liver. At 36d exposure time, Cd and Pb had no significant effect on Zn uptake in all three organs. It was obviously related to the organ type, exposure dose, exposure time, and the characteristic of the heavy metals. The reports of Allen (1995) showed that Pb barely affected Cd uptake in gill of *Oreochromis aureus*, while Cd increased Pb uptake in gill and liver. However, the studies of Liu et al. (2001) showed that Cd had no effects on Pb accumulations in gill, liver and kidney, neither did Pb on Cd accumulation in liver of goldfish. The reports of Cicik et al. (2004) showed that Pb increased Cd accumulation in liver and decreased it in gill, and Cd also increased Pb accumulation in liver and gill of *Oreochromis niloticus*. The studies of Wicklund et al. (1988) showed that Zn could prevent Cd uptake in *Brachydanio rerio*.

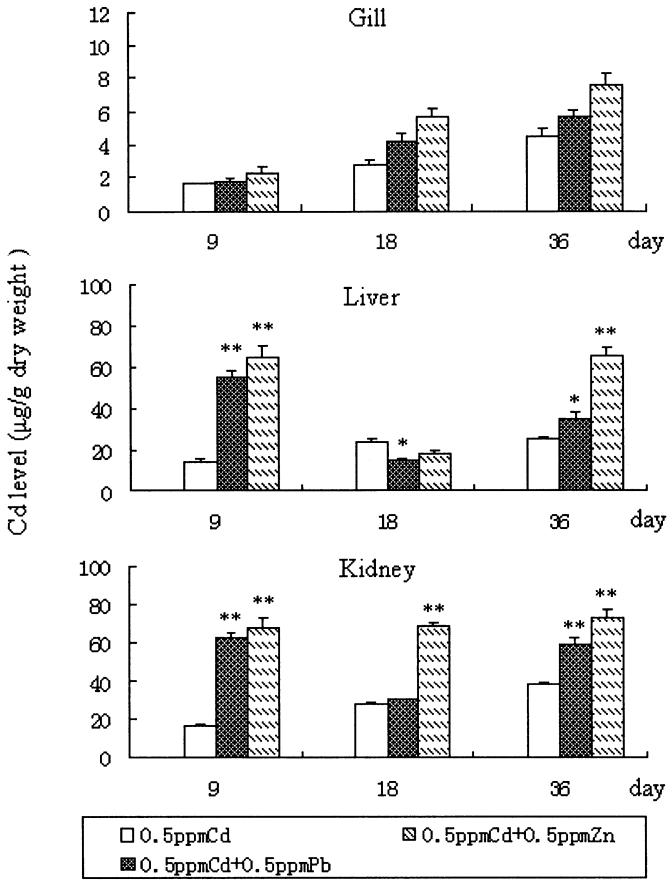

Figure 1. Effects of Pb or Zn on the accumulation of Cd (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5ppm concentration. The data of control are too low to be detected. * $P<0.05$, ** $P<0.01$ compared with 0.5ppm Cd.

Compared with the controls, the accumulations of Zn in gill, liver and kidney of goldfish exposed to Cd+Zn and Pb+Zn mixtures were significantly increased ($P<0.01$) at 9, 18 and 36d exposure time (Figure 3).

Our results showed that gill and kidney were both damaged by Cd, Pb and Zn at 36d exposure time. Due to the similar histological changes of gill and kidney in each group, we singled out four photos given in Figure 4 and 5.

In gill, epithelium exhibited hypertrophy, pyknosis and vague borders between the epithelial cells (Figure 4). The thickened respiratory epithelium due to the hyperplasia of epithelial cells increased the diffusion distance between the

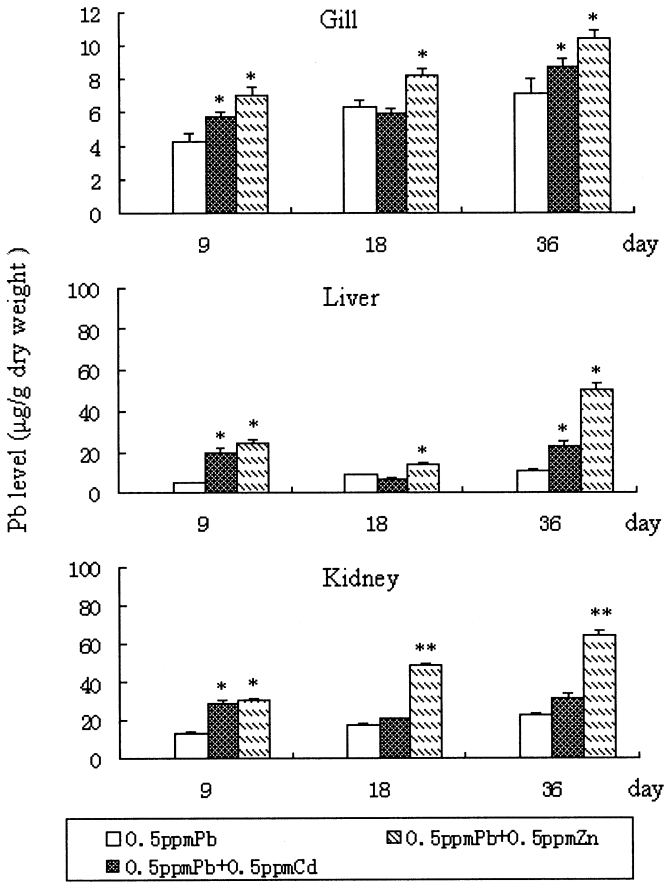

Figure 2. Effects of Cd or Zn on the accumulation of Pb (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5ppm concentration. The data of control are too low to be detected. * $P<0.05$, ** $P<0.01$ compared with 0.5ppm Pb.

ambient and vascular components. Vasodilation in the secondary lamellae of gills and periodic fluctuations in the mucous cell density are also observed at various stages of $ZnCl_2$ exposure (Hemalatha et al. 1997). In general, the alterations in gill include periodic deformation of lamellar elements, haemorrhages due to necrosis and sloughing off of the respiratory epithelium (Zheng et al. 1997), hyperplasia, intercellular vacuolization and occasional fusion of secondary lamellae, resulting in increased thickness of primary and secondary lamellae.

In kidney, histological lesions affected by heavy metals included swelling, atrophy, necrosis, vacuolation, degranulation, exocytosis, nuclear damage and plasma alterations in interstitial tissues and in tubules (Hemalatha et al. 1997; Bernet et al.

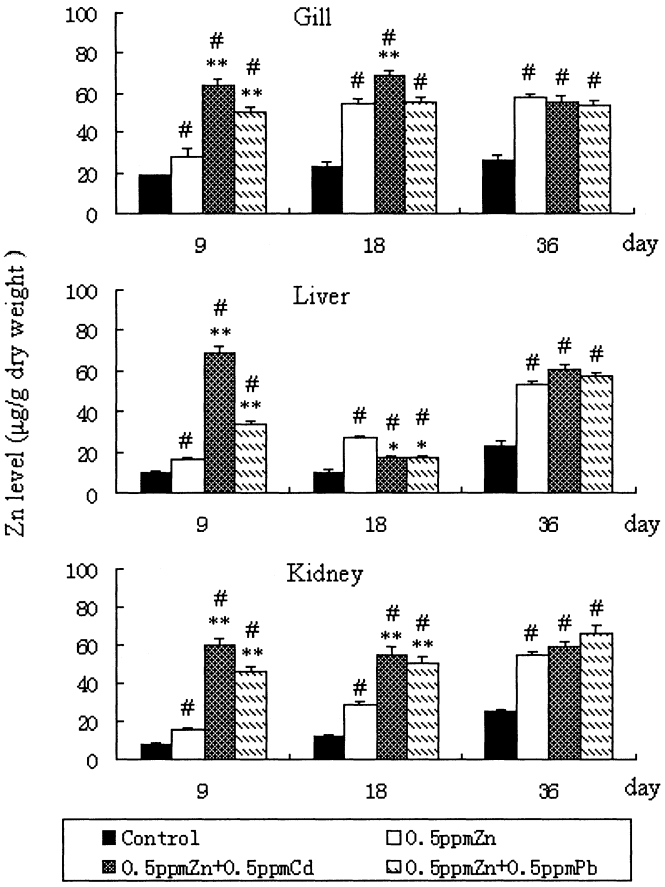

Figure 3. Effects of Cd or Pb on the accumulation of Zn (μg/g dry weight, means±SEs, n=10) in gill, liver and kidney of *C. auratus* at 0.5ppm concentration. # $P<0.01$ compared with control; * $P<0.05$, ** $P<0.01$ compared with 0.5ppm Zn.

2004). In our experiments, the renal epithelium showed disappearance of microvilli, presence of pyknotic nuclei, irregular arrangement of renal tubule-wall cells, and the loss of hemic tissue between tubules also happened (Figure 5). The disappearance of microvilli may be due to the atrophy and degranulation of the epithelia, thus induced the irregular arrangement of the renal tubule-wall cells.

Gill and kidney were both damaged by Cd, Pb and Zn at 36d exposure time, which suggested that histological lesions in these two organs were dependent upon the exposure time and the characteristic of these three heavy metals.

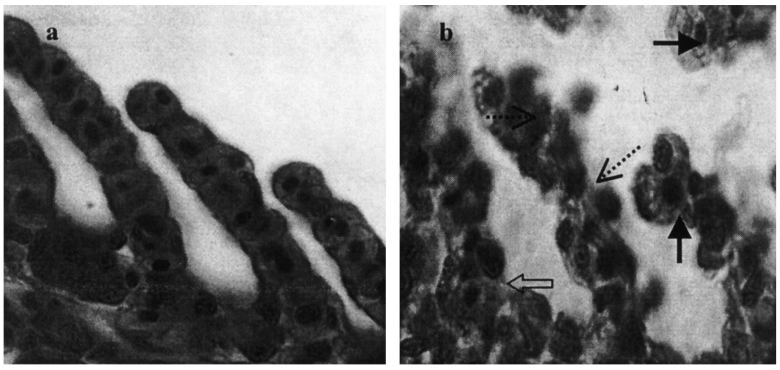

Figure 4. (a) normal gill; (b) the gill treated with Cd at 0.5ppm for 36d. Solid arrow shows hypertrophy; hollow arrow shows vague border; discontinuous arrow shows pyknosis. Magnification 200x.

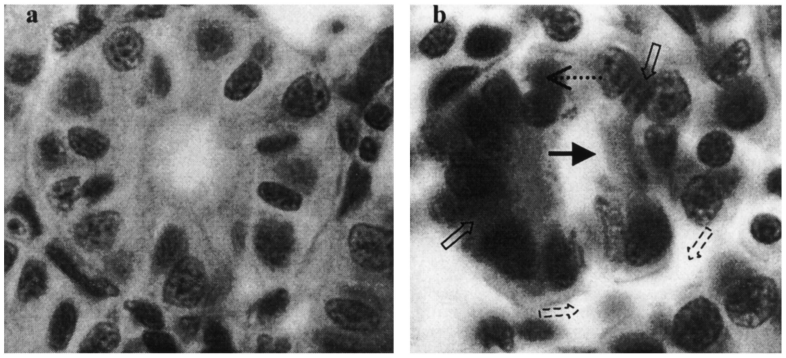

Figure 5. (a) normal kidney; (b) the kidney treated with Cd at 0.5ppm for 36d. Solid arrow shows microvilli disappeared; hollow arrow shows the renal tubule-wall cells arranged irregularly; discontinuous arrow shows pyknotic neclei appeared; discontinuous hollow arrow shows lost hemic tissue. Magnification 400x.

In conclusion, our results confirmed that heavy metal accumulation in *C. auratus* was dependent upon the exposure dose, exposure time and organ type. Histological lesions in gill and kidney observed in this paper had relation to the exposure time and the characteristic of heavy metals.

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