

Cadmium accumulation and elimination in the tissues of *Oreochromis* sp.

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Abstract

The accumulation and elimination of Cd on *Oreochromis* sp. tissues were studied in exposure phase and recovery phase. In the exposure phase, the mean rate of Cd accumulation in the *Oreochromis* sp. tissues was in the order liver >> gill >> muscle. Cd accumulation in these tissues increased with increases in Cd concentrations in the water or as time proceeded. In the recovery phase, the order elimination in the tissues was also gill > muscle > liver. Short-term depuration (10 days), quick decrease of Cd in gill and muscle of *Oreochromis* sp. were observed.

Keywords. Cadmium, accumulation, depuration, *Oreochromis* sp.

1. INTRODUCTION

Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases [1-3]. Heavy metal accumulation in the organs of fish is dependent upon the exposure time and exposure dose as well as other factors, such as temperature, age, interaction with other metals, water chemistry, and metabolic activity of the fish [4]. The major routes of metal uptake in fish are through the gills, the digestive system and the skin. It is well known that heavy metal easily accumulates in bones, gills, kidneys, liver of fish. The elimination of metals from the tissues depends on time, age, metabolic activity, interacting agents, and the biological half-life of the metals [5-8].

Cadmium (Cd) is known as one of the most dangerous environmental and industrial pollutants. It has no biological function and accumulates mainly in metabolically active tissue even at low concentrations, which in turn may cause tissue damage [9].

Therefore, the importance of studies of Cd bioaccumulation in fish, which represent a valuable source of food for humans, in the context of environmental pollution, seems unquestionable. This study focused on the accumulation and elimination of Cd in liver, muscle and gill of *Oreochromis* sp. cultured in water contaminated Cd.

2. MATERIALS AND METHODS

2.1. Chemicals

CdCl₂, H₂O₂, HNO₃ were purchased from Merck, Darmstadt, Germany.

2.2. Fish and conditional experiment

Oreochromis sp. were collected from Minh Luan fish farms, No.9, Dong village, Long Dinh commune, Chau Thanh district, Tien Giang province, Mekong Delta, Viet Nam. Similar sized fish, 20±1 cm in length and 300±1 g in weight, were used for the experiments. Fish were transported in polyethylene bags filling with oxygen. Then were kept in a 500 L tank (semi-static system, 25 % daily water renewal, dissolved oxygen (DO) = 6.5±0.7 mg/L, pH = 6.3, temperature = 28±4 °C. The experiments were carried out under controlled laboratory conditions set at 30±1 °C) and illumination for 10 h. Fish were allowed to acclimate for 12 days before the exposure studies began.

2.3. Subchronic exposure and recovery experiments

Oreochromis sp. were divided into seven treatments which were exposed to control water (control treatment) and water contaminated with

different concentration of cadmium (0.66 mg/L, 1.0 mg/L and 2.0 mg/L equal to 1/10, 1/20 and 1/30 of 96-h LC₅₀ for Cd) for one exposure period of 20 days and one depuration period of 10 days. The 96-h LC₅₀ of Cd for *Oreochromis* sp. was found to be 19.2 mg Cd/L [9]. Seventy-five fishes were randomly assigned to each treatment; each treatment consisted of three tanks with twenty-five fish per tank (n = 3). Water samples were collected twice a week for analysis of Cd concentration using inductively coupled plasma-mass spectrometry. After 20 days of exposure, fish experienced a depuration period of 10 days in control water.

2.4. Tissue sampling

At 4, 12, and 20 days of exposure and 10 days of depuration period, fish were washed with bi-distilled water. The following tissues were collected from randomly harvested fish from each group: muscle, gill and liver. These tissues were dried in oven at 105°C for 10 h before analysis of Cd concentration.

2.5. Cadmium analysis

Prior to determination, tissues of weight 1 g were subjected to preliminary mineralization in presence of 6 mL of a 2:1 v/v mixture of HNO₃ (65 %) and H₂O₂ (30 %) in the closed vessel. The samples were then heated to 85 °C for 30 min. The so obtained clear liquid was diluted with deionized water to 25 mL and then assayed for concentration of Cd using ICP-MS 7700 (JP 13052271, Agilent, USA). The method had a LOD of 1 ng Cd/mL.

2.6. The accumulation factor and the elimination rate

The accumulation factor (AF) is often used to compare the body burden of an organism with the degree of contamination in the water. The following equation was used in the present study:

$$AF = \frac{Cd_{exp} - Cd_{control}}{Cd_{water}}$$

Where, [Cd]_{exp}, [Cd]_{control}, and [Cd]_{water} are the Cd concentration in the experimental group, control group, and water, respectively [10,11].

The elimination rate (%) is the percentage decrease in the initial value after 10 days in depuration phase. The following equation was used in the present study:

$$\frac{[Cd]_{deputation\ phase} - [Cd]_{exposure\ phase}}{[Cd]_{exposure\ phase}} \times 100$$

Where [Cd]_{deputation phase}, [Cd]_{exposure phase} are the Cd concentration in the 10 days of depuration phase, 20 days of exposure phase, respectively.

2.7. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0. Data were expressed by mean ± SD. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance of the differences between mean was assessed by Duncan's test, p < 0.05 was considered significance.

3. RESULTS AND DISCUSSION

3.1. Accumulation of Cd

Metal accumulation in the organs of fish is dependent upon the exposure time and exposure dose as well as other factors, such as temperature, age, interaction with other metals, water chemistry, and metabolic activity of the fish [4]. The accumulation of Cd in tissues of *Oreochromis* sp. is presented in figure 1. Cd accumulation in these tissues increased with increases in Cd concentrations in the water or as time proceeded. The groups exposed to Cd had significantly higher accumulation of Cd in tissues than the control group (p < 0.05). The order of Cd accumulation in the tissues during exposure was liver >> gill >> muscle.

Accumulation of Cd in liver of *Oreochromis* sp. significantly increased with increases in Cd in the water and time proceed (Fig. 1). Long-term exposure (20 days) to Cd at 0.66, 1.0 and 2.0 mg/L, concentration of Cd in fish liver reached 1.84±0.17; 2.06±0.04 and 2.53±0.05 mg/kg dry weight, respectively. These values were approximately 14, 17 and 21-fold higher than those of the control group.

The gills of fish serve as an interface between the water and blood, notably for continuous diffusion of oxygen, the maintenance of acid-base and the major sites of heavy metals uptake due to direct contact with the water [12]. Long-term exposure to Cd at 0.66, 1.0 and 2.0 mg/L, Cd accumulation in gill were 0.82±0.04; 0.99±0.03 and 1.44±0.03 mg/kg dry weight, respectively. These values were approximately 5, 8, and 10-fold higher than those of the control group.

In case of muscle, lowest accumulation of Cd was observed but it increased linearly with increases in Cd in water. Accumulation of Cd in muscle of *Oreochromis* sp. significantly increased compared to

control group. After 20 days exposures to Cd at 0.66, 1.0 and 2.0 mg/L, concentration of Cd in fish muscle reached 0.29±0.02, 0.32±0.02, and 0.39±0.02 mg/kg dry weight, respectively. These values were approximately 3.6, 4, and 5 -fold higher than those of the control group.

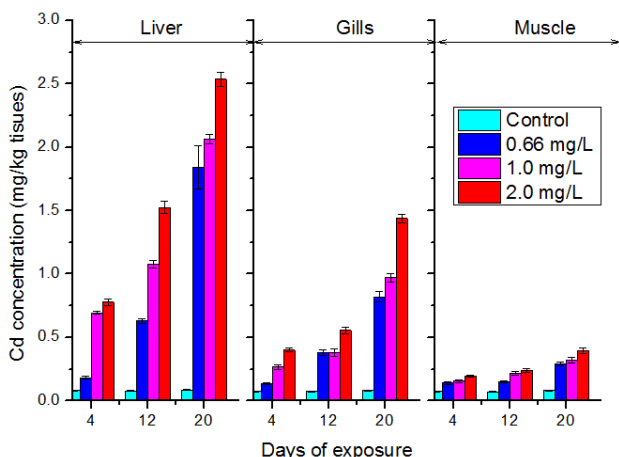


Figure 1: The accumulation of Cd in fish tissue in exposure phase (mean ± SD)

Similarly, several previous studies documented that metal accumulation in the liver tissue was higher than in muscle [13-16]. This is probably due to higher metabolic activities of liver compared to those of muscle [17]. Liver plays an important role in storage, redistribution, detoxification and transformation of pollutants [18]. In the liver, the elements react with the oxygen carboxylate, amino group, nitrogen and/or sulphur of the mercapto group in the metallothionein protein. These results are consistent with the hypothesis that accumulation in muscle becomes important only when the maximum storage capacity of the liver has been reached.

A significant linear correlation between Cd concentration in different fish tissues and this of the waterborne, the linear regression equation and correlation coefficients are presented in table 1.

Table 1: The linear correlation between Cd concentration in fish tissues and in water

Tissue	Equation	r ²
Liver	y = 0.426 x + 1.00	0.92
Gill	y = 0.265 x + 0.276	0.79
Muscle	y = 0.350 x + 0.036	0.95

The accumulation factor (AF) of Cd in fish tissues was showed in figure 2. The AF of fish tissues was increased with time proceeds whereas it

was decreased with increases in Cd concentration in water. The order of AF values was liver >> gill >> muscle.

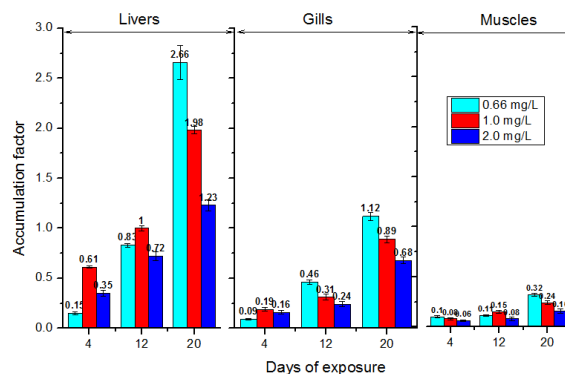


Figure 2: The AF of Cd in *Oreochromis* sp. tissues

3.2. Cadmium elimination

Elimination of accumulated Cd from organs during exposure of 20 days depended mainly on tissue. The order of Cd elimination in the tissues during recovery period was gill > muscle > liver. At the end of recovery period (day 10), sharp decrease of Cd was observed in the gill (33.7-54.3 %) and in the muscle 1 (27.8-48.8 %). Cd elimination from liver was slightly slower 26.3-34.3 %, probably due to their role in the removal of this element from body.

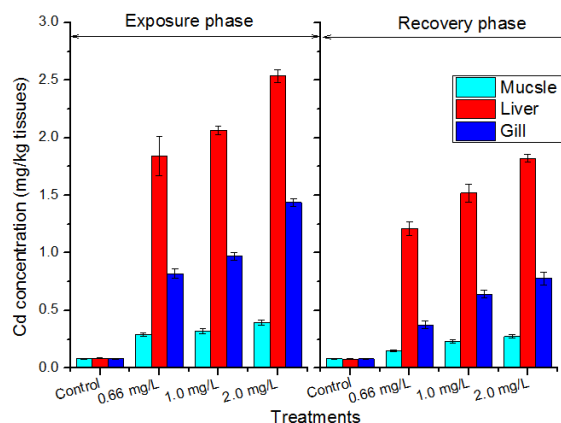


Figure 3: Cd accumulation at day 20 and elimination at day 10 in the tissues of *Oreochromis* sp.

Elimination of heavy metals depends on the biological half-life of the metals and aquatic species. Viarengo et al. (1985) reported that elimination of accumulated Cu in *Mytilus galloprovincialis* was significantly faster in comparison with Cd [19]. Slow elimination of Cd was observed in the clams *Macoma balthica* [20, 21]. Elimination of accumulated Cd in the liver and kidney of Rainbow trout was approximately more than a year [22].

4. CONCLUSION

This study demonstrated that liver of *Oreochromis* sp. is a target organ for Cd, which implies that it is also the critical organs for toxic symptom. The accumulation factor of *Oreochromis* sp. increased with time proceeds whereas it was decreased with increases in Cd in water. Short-term depuration (10 days), quick decrease of Cd in gill and muscle of *Oreochromis* sp. were observed. The rate of elimination Cd from *Oreochromis* sp. fish tissues was gill > muscle > liver.

REFERENCES

- Jørgensen LA., Pedersen B. *Trace metals in fish used for time trend analysis and as environmental indicators*, Mar. Pollut. Bull., **28**, 24-32 (1994).
- Fowler SW., Readman JW., Oregoni B., Villeneuve JP., McKay K. *Petroleum hydrocarbons and trace metals in nearshore Gulf sediments and biota before and after the 1991 war: An assessment of temporal and spatial trends*, Mar. Pollut. Bull., **27**, 171-182 (1993).
- Khan AT, Weis JS. *Bioaccumulation of heavy metals in two populations of mummichog (Fundulus heteroclitus)*, Bull. Environ. Contam. Toxicol., 1-5 (1993).
- Campana O., Sarasquete C., Blasco J. *Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish Halobatrachus didactylus*, Ecotoxicol. Environ. Saf., **55**, 116-125 (2003).
- Larsson A., Haux C. and Sjöbeck ML. *Fish physiology and metal pollution: results and experiences from laboratory and field studies*, Ecotoxicol. Environ. Saf., **9**, 250-281 (1985).
- Douben PET. *Metabolic rate and uptake and loss of cadmium from food by the fish Noemacheilus barbatulus L. (stone loach)*, Environ. Pollut., **59**, 177-202 (1989).
- Woo PTK, Yoke MS and Wong MK. *The effects of short-term acute cadmium exposure on blue tilapia, Oreochromis aureus*, Environ. Biol. Fish, **37**, 67-74 (1993).
- Heath AG. *Water Pollution and Fish Physiology*, CRC press, Boca Raton, FL, US (1995).
- Nguyen Quoc Thang, Nguyen Thi Kim Phuong, Le Van Tan, *Endocrine stress response in Oreochromis sp. from exposure to waterborne cadmium: the plasma cortisol analysis*, Toxicol. Environ. Chem., **99(2)**, 285-293 (2017).
- Seong-Gil Kim, Seok-Woo Jang, Young-Ju Lee and Seong-Soo Kim. *Cu Accumulation and Elimination in the Tissues of the Olive Flounder Paralichthys olivaceus*, Fish Aquat. Sci., **14(3)**, (210-217 (2011).
- Amrollarhi Biuki Narges, Savari Ahmad, Mortazavi Mohammadsedigh, Zolgharnein Hossein, Salamat Negin, Amrollarhi Biuki Zohreh. *Non-essential metals (Cd&Pb) accumulation and elimination in liver tissue of juvenile milkfish after sublethal exposure*, Indian Journal of Geo-marine Sciences, **41(5)**, 412-417 (2012).
- Claiborne JB, Edwards SL, Morrison-Shetlar AI. *Acid-base regulation in fishes: cellular and molecular mechanisms*, J. Exp. Zool., **293**, 302-319 (2002).
- Canli M, Atli G. *The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species*, Environ. Pollut., **121**, 129-136 (2003).
- El-Moselhy KM, Othman AI, Abd El-Azem H, El-Metwally MEA. *Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt* Egyptian Journal of Basic and Applied Sciences, **1**, 97-105 (2014).
- Jovičić K et al. *Mapping differential elemental accumulation in fish tissues: assessment of metal and trace element concentrations in wels catfish (Silurus glanis) from the Danube River by ICP-MS*, Environmental Science and Pollution Research, **22**, 3820-3827 (2015).
- Elzbieta Kondera, Katarzyna Ługowska, Piotr Sarnowski, *High affinity of cadmium and copper to head kidney of common carp (Cyprinus carpio L.)*, Fish Physiol. Biochem., **40**, 9-22 (2014).
- Agah H., Leermakers M., Elskens M., Fatemi SMR., Baeyens W. *Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf*, Environ Monit Assess, **157**, 499 (2008).
- Jeziarska B., Witeska M. *The metal uptake and accumulation in fish living in polluted waters*. In: Twardowska I et al. (eds) *Soil and water pollution monitoring, protection and remediation*, Springer, New York, 3-23 (2006).
- Viarengo A., Palmero S., Zanicchi G., Capelli R., Vaissiere R., Orunesu M. *Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cell of Mytilus galloprovincialis*, Lam. Mar. Environ. Res., **16**, 23-26 (1985).
- McLeese DW, Ray S. *Uptake and excretion of cadmium, CdEDTA, and zinc by Macoma balthica*, Bull. Environ. Contam. Toxicol., **32**, 85-92 (1984).
- Riisgard H.U., Kiorboe T., Mohlenberg F., Drabae, I, Pfeiffer Madsen P. *Accumulation, elimination and chemical speciation of mercury in the bivalves Mytilus edulis and Macoma balthica*, Mar. Biol., **86**, 55-62 (1985).
- Larson A., Haux C., Sjöbeck M. *Fish physiology and metal pollution: Results and experiences from laboratory and field studies*, Ecotoxicol. Environ. Safety, **9**, 250-281 (1985).

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