Accumulation, depuration and risk assessment of cadmium (Cd) and lead (Pb) in clam (Corbicula fluminea) (O. F. Müller, 1774) under laboratory conditions

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Abstract

The present study investigated the accumulation and depuration of two heavy metals Cd and Pb in the freshwater clam (Corbicula fluminea) under laboratory conditions. Treated clams were exposed to single and combined treatments of Cd and Pb at the concentration of 5 mg L⁻¹ and 10.5 mg L⁻¹ for 10 days, respectively. The animals were then placed in metal free water for the following 5 days for depuration experiment. Heavy metal concentrations were determined by an inductively coupled plasma optical emission spectrometer (ICP OES). Results showed that the clams were able to accumulate Cd and Pb at the maximum rate of 0.26 and 0.37 (µg g-1 day-1), respectively; and depurate at the rate of 0.32 and 0.08 (µg g⁻¹ day⁻¹), respectively. At the end of uptake period, the concentrations of Cd and Pb in the soft tissues of single and combined exposures were 1.8 and 1.1 µg g⁻¹ wet weights (WW) and 1.2 and 0.8 µg g⁻¹ WW, respectively. The clams also showed rapid depuration of Cd and Pb. At the end of depuration, the concentrations of Cd and Pb in the soft tissues were only 0.2 and 0.8 µg g⁻¹ WW in single and 0.17 and 0.4 µg g⁻¹ WW in combined exposure, respectively. The results of the present study showed that there was a competition between Cd and Pb in the tissues of the clams. The total target hazard quotients and the hazard index indicated that people might experience adverse health effects through consuming contaminated C. fluminea.

Keywords: Biomonitoring, Heavy metal, Bivalves, Human health risk

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Introduction

Heavy metals from natural weathering and human activities are harmful to marine ecosystems, with inevitable adverse health effects consequences. Human activities such as industrial complexes and residential areas have increased metal inputs to the freshwater environment. Heavy metals are toxic to when they are organisms aquatic present above critical levels. Bioaccumulation and bio-magnification of metals in aquatic animals is an environmental problem and human health concerns (Hoang et al., 2007).

Aquatic species have been studied to determine their potential as a biomonitoring organism, and mollusks become a popular choice for heavy metal monitoring (Yap et al., 2003) their feeding. because filter studies However. previous have that mollusks reported depuration mechanism to reduce heavy metal toxicity in their body (Yap et al., 2004). This mechanism might diminish the effectiveness of mollusks as biomonitoring organism, as the concentration of heavy metal in the mollusks may not accurately reflect the concentration in the environment.

During the past few decades, the fast economic and industrial growth in Asian countries has caused a negative impact on the environmental quality. The contamination of heavy metals has been extensively reported in Vietnam waters (Hoang *et al.*, 2007; Nguyen *et al.*, 2016a; Nguyen *et al.*, 2016b), in China environments (Wang *et al.*, 2015; Xu *et al.*, 2017) and in other Asian countries (Lee *et al.*, 2013). Among

metals, cadmium (Cd) and lead (Pb) are of particular concerns because of their widely used in industrial processes and resulting in widespread contamination in freshwater ecosystems (Xu et al., 2017). However, little is known about the health impact the heavy metals in contaminated food. In Vietnam very few published data on heavy metal contamination in aquatic animal is available.

Cadmium is one of the most toxic heavy metal that poses serious risks to animals. aquatic The toxicity mechanism of Cd is related to the capacity for association with the thiol groups of proteins (Zheng et al., 2016). Cadmium has caused deleterious effects in terms of nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic. Recent reports suggested that cell mediated immunity is most affected and phagocytosis, natural killer cells activity and host resistance towards experimental infections are markedly impaired in Cd toxicity (Guo et al., 2017). Lead metal is widely used in industrial processes and could be responsible for death or sub lethal changes in reproduction, growth and behavior of the aquatic animals. Lead toxicity in living cells including ionic mechanism and oxidative stress (Ahmed et al., 2011). Ramsdorf et al. (2009) reported that Pb acetate induces micronucleus, chromosomal aberrations and causes DNA damage showing a significant increase of tailed nucleoids in the erythrocytes of fishes.

The accumulation and toxic effects of Cd and Pb on aquatic organisms have been extensively studied (Paul *et*

al., 2014; Alsop et al., 2016; Zheng et al., 2016; Guo et al., 2017). However, previous studies have typically focused only on single metal exposure. The accumulation mechanism and toxicity of a complex combine exposure have not been evaluated to the same extent. In natural environment, aquatic animals may simultaneously expose to multiple heavy metals. Thereby, this study used the Asian clam Corbicula fluminea which is abundant in many rivers and lakes of Vietnam as a model for accumulation and depuration of Cd and Pb in a single and combined exposure laboratory under condition. accumulation and depuration rates were investigated. In addition. the concentrations of Cd and Pb in clams were used to evaluate the health hazards.

Materials and methods

Freshwater clams Corbicula fluminea (Fig. 1) were collected from the Dau Tieng Reservoir, and transported alive to the laboratory. The clams were introduced into 20 L aquatic aquariums containing dechlorinated tap water, 5 cm sand layer as substrate, with sufficient aeration. The clams were kept at a density of below 100 individuals per 20 L and acclimatized for 1 week under a 12:12 light: dark photoperiod experiments. temperature, pH and dissolved oxygen concentration were 27±1°C, 7.3±0.4 and 7.7 ± 0.5 mg L⁻¹, respectively. The incubation water was totally renewed every 3 days. The clams were fed daily with green alga Scenedesmus sp. at a concentration of 2×10³ cell mL⁻¹. Wet weight and shell length of the clams ranged within 3.18±0.39 g and 1.97±0.42 cm per individual, respectively.

After acclimation, clams were placed in 4 aquariums (20 L) containing 5 L of dechlorinated tap water, 2 cm sand layer as a substrate with constant aeration. Light intensity and water temperature were the same as above described.

Singe exposed experiment: For uptake period, the stock solutions 1000 mg L⁻¹ of Cd and Pb (Titrisol, Merck, Germany) were separately added into the aquariums to a final concentration of 5 mg L⁻¹ at a single dose for 10 days. Then the exposed clams were relocated in heavy metal free water for 5 days for depuration periods.

Combined exposed experiment: stock solution of Cd and Pb were added to the same aquarium at a final concentration of 6 mg L⁻¹ and 4.5 mg L⁻¹, respectively at a single dose for 10 days. These doses and duration course are often used for exposure under laboratory conditions (Yap et al., 2004; Rashid et al., 2009). Then the exposed clams were relocated in aquarium containing distilled water as heavy metal-free for another 5 days of depuration. Prior to the metal exposure, ten clams were collected and used as control samples. At day 1, 3, 5, 10 of exposure period and 11 and 15 of depuration period, ten clams were sampled for heavy metal determination. Soft clam tissues were removed immediately, dry on GF/C glass-fiber filter papers and kept at-20°C period to analysis.



Figure 1: Photos of *Corbicula fluminea*. Scale bar: 1 mm.

Heavy metals in clam's tissues were extracted as previously reported by Yap *et al.* (2003) with minor modifications. Briefly, soft clams tissues were homogenized in 2 mL of concentrated nitric acid (75%). After sonication for 3 min, the samples were completely digested with 2 mL of the same solution for 12 h at 80°C. The digested samples were then centrifuged at 4000 rpm for 10 min under room temperature. The supernatant contained metals were kept at -20°C period to analysis.

Heavy metals in clam's tissues were detected according to the method of Silva et al. (2016) with minor modifications. Briefly, an inductively coupled plasma optical emission spectrometer (ICP OES) with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with a solid state detector, a cyclonic spray chamber, and a concentric nebulizer were used for Cd and Cu detection. The ICP OES condition used was as follows: RF power: 1.3 kW; gas: argon; plasma flow: 15 L min⁻¹; auxiliary flow: 1.5 L min⁻¹; nebulizer flow: 0.75 L min⁻¹; instrument stabilization delay: 15 s; pump rate: 15 rpm; sample uptake delay: 70 s; number of replicates: 3; read time: 5 s; read: peak height; rinse time: 30 s. The data are presented in µg g⁻¹. wet weight (WW). The recoveries of Cd and Pb were 80% and 83%, respectively. The limits of detection (LOD) of Cd and Pb were 0.01 µg g⁻¹ WW and 0.06 µg g⁻¹ WW, respectively.

The first bioconcentration factor (BCF) was calculated in relation to metal concentration in seawater according to Yap *et al.* (2003):

$$BCF = \frac{Ce - Ci}{Cs}$$

Where Ce was the metal concentration in tissues at the end of exposure experiment (μg g⁻¹), Ci was the initial metal concentration in tissue before exposure experiment (μg g⁻¹), Cs was the experimental metal concentration in test water.

The rate of metal accumulation (RA) was calculated as follows:

$$RA = \frac{Metal\ level\ _{exposed} - Metal\ level\ _{control}}{Day\ of\ metal\ exposure}$$

The rate of metal depuration (RD) was calculated according to:

$$RD = \frac{\text{Metal level}_{\text{end of exposure}} - \text{Metal level}_{\text{end of depuration}}}{\text{Day of metal depuration}}$$

The health risk index (HRI) for Cd and Pb by consumption of clam was calculated by following equation (Mahmood and Malik, 2014):

$$HRI = \frac{DIM \times Metal\ level}{body\ mass}$$

Where DIM represents the daily intake of metals. Body mass of an Asian adult is 60 kg. The value of 300 g d⁻¹ has been widely used for health risk assessment (Chorus and Bartram, 1999)

especially many reported from China (Zhang *et al.*, 2012; Jia *et al.*, 2014). A represents reference oral dose (RFD) is an estimated per day exposure of metal to the human body that has no hazardous effect during life time. Rfd value for Pb and Cd is 0.004, 0.001 (mg kg⁻¹, bw day⁻¹), respectively was used for health risk assessment (USEPA IRIS, 2006).

The concentrations of heavy metal in tested animals were presented as the mean±SD. They were tested for significant group differences using one-way analysis of variance (ANOVA). The *p*-values less than 0.05 were considered statistically significant.

Results

There was no clam mortality during the experiments. The control samples did

contain any detectable concentration. The accumulation and depuration of Cd and Cu in the soft tissues of the clams were showed in Fig. 2. For both metals, the metal levels were found to increase gradually during the accumulation period but they decreased during the depuration period (p<0.05). In single exposed experiment, the concentration of Cd and Pb reached at a maximum level (1.78 and 1.05 mg kg⁻¹ WW, respectively) at the end of accumulation (Day 10) (Fig. 2A). When the clams exposed to both Cd and Pb the of concentration each metal accumulated was lower than the metal level in single exposed, but total concentration of heavy metals in the clams were higher (Fig. 2B).

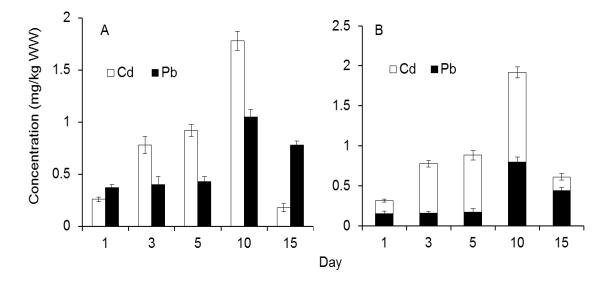


Figure 2: The concentration of Cd and Pb in the clam during uptake and depuration periods. (A) single exposed and (B) combined exposed.

In the depuration period, Cd was quickly eliminated, but Pb was slowly eliminated from the clams tissues. At the end of depuration the concentration of Cd and Pb in single and combined exposed was 0.18, 0.78 and 0.17, 0.44 mg kg⁻¹ WW, respectively (Fig. 2A, B).

The bioconcentration factor, rates of accumulation and depuration of Cu and Pb was showed in Table 1. Exposures to Cu and Pb singly showed the highest BCF values at the end of the accumulation period (Day 10), as shown in Table 1. When the clams exposed to both metals, the BCF value

of each metal was lower, but total BCF value of both metals in the clams was higher. The rates of metal accumulation in the soft tissues gradually decreased general, during exposed. In accumulation rates and depuration rate of Cd were higher than Pb. The studied clams have a higher capability to accumulate Cd (highest rate 0.33 mg kg⁻¹ day⁻¹) than Pb (highest rate 0.18 mg kg⁻¹ day⁻¹) in laboratory condition. This conclusion was also supported by the BCF values (Table 1).

Table 1: Bioconcentration factor, rates of accumulation and depuration of Cu and Pb.

	BCF				RA, RD			
Day	Single		Combined		Single		Combined	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
1	0.04 ± 0.005	0.05 ± 0.006	0.024±0.006	0.023 ± 0.005	0.26 ± 0.03	0.37 ± 0.02	0.155±0.02	0.16±0.02
3	0.13±0.016	0.052 ± 0.008	0.11 ± 0.02	0.022 ± 0.007	0.26 ± 0.02	0.13 ± 0.01	0.05 ± 0.01	0.20 ± 0.03
5	0.18 ± 0.012	0.049 ± 0.007	0.13 ± 0.01	0.021±0.006	0.184±0.03	0.086±0.01	0.035±0.01	0.142 ± 0.01
10	0.33 ± 0.018	0.18 ± 0.03	0.22 ± 0.03	0.16 ± 0.02	0.178 ± 0.01	0.105±0.03	0.08 ± 0.008	0.112±0.01
15	-	-	-	-	0.32±0.04	0.054±0.01	0.072±0.02	0.19±0.03

The health risk index (HRI) for heavy metals by consumption of exposed the clams for adults was calculated and values are given in Fig. 3. The maximum HRI of Cd and Pb at day 10 were 0.011 and 0.0063 (mg kg⁻¹ day⁻¹), respectively. The results of the present study demonstrated that the HRI of Cd was always exceeds the reference oral dose of 0.001 mg kg⁻¹ day⁻¹, and the HRI of Pb in day 10 and day 15 exceeds the reference oral dose of 0.004 mg kg⁻¹ day⁻¹ (Fig. 3). The results of the present study suggested that C. fluminea can represent a health risk to consumers even though they were kept

for long time (5 days) in clear environment.

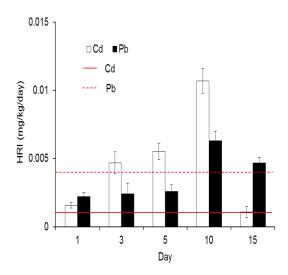


Figure 3: Health risk index of the studied clam for Cd and Pb.

Discussion

Heavy metals bioaccumulation aquatic animals can pose a health risk to the humans (El-Moselhy et al., 2014). Because bivalves are among the common food consumed by Vietnamese people (Pham et al., 2016) the research posed particular attention to the animal. Water environment has been reported to contaminate with Pb and Cd, especially in Mekong Delta (Dao et al., 2017). Cd is not an essential element; it can accumulate in human body and may cause kidney dysfunction, skeletal damage, and reproductive impairment (Zheng et al., 2016; Guo et al., 2017). Lead is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity and other adverse health effects such deficits behavioral in vertebrates. decreases in survival and growth rates, learning disabilities, causes metabolism (Jaishankar et al., 2014; Paul et al., 2014; Zheng et al., 2016). In the present study, the concentration of Cd and Pb in the clams were above the proposed level of USEPA (USEPA IRIS, 2006), even though they were kept in clear water for five days of depuration. This evidences suggested that C. fluminea living in metal contaminated sites can pose health risks to humans.

The freshwater clam *C. fluminea* is among the most common bivalves presented in Vietnamese water. And they are the most frequently consumed by local people. They are filter feeders ingesting both phytoplankton and sediment detritus (Pham *et al.*, 2017). It is well known that heavy metals

accumulated in water column can be for verv toxic aquatic animals. especially for young and eggs which are very sensitive to the pollution. In aquatic animals, the target organs such as liver, gonads, kidney and gills are metabolically active tissues accumulate heavy metals of higher levels (El-Moselhy et al., Jaishankar et al., 2014). Previous studies have reported that different contents of heavy metals in aquatic animals might be a result of different ecological needs, metabolism feeding patterns (Ayse, 2003). The concentration of Cd and Pb in tested clams contained from 0.18-1.78 mg kg WW, 0.37–1.05 mg kg⁻¹ WW, respectively, which were the same content in different important mussels, clams and oysters from Apulian Regio (Miedico et al., 2013), but lower or higher than metal contents detected in under mussel exposed laboratory condition (Yap et al., 2004; Rashid et al., 2009).

The rate of accumulation and BCF indicateds the ability of the clams to accumulate metals to a certain level. The ratios of accumulation depuration recorded in the present study were lower than those recorded in the green-lipped mussel Perna viridis under laboratory condition (Yap et al., 2004). The results of the present study are also comparable to those reported in the same Asian clam species in the field from Florida and North Carolina (Lewbart et al., 2010). Helmstetler and Cowles (2008) reported that heavy metals contents in mussel living in polluted sites were higher than those living in clear water. Other studied showed that long time exposed resulted in higher concentration of metals in aquatics animals. Perhaps, heavy metals contents in different species may depend on dose and kind of exposed, routes of exposure, and duration course of the experiment but also depend on target organs and state of organisms as well as specific-species (Rashid *et al.*, 2009; Lewbart *et al.*, 2010).

In laboratory studies, at the end of depuration C. fluminea accumulated about 0.17-0.78 mg kg⁻¹ WW of Cd and Pb, a high bioaccumulation capacity. Of course, these levels were dependent on the concentrations of metals in the environmental water and the duration of the exposure. During the depuration period, a more rapid loss of Cd than Pb was observed. The RD ratios values showed the ability of the clams to retain the accumulated Pb during the depuration period. Probably, Pb was bound to the immobilized compartment and could not easily lost due to its metallothionein binding (Yap et al., 2003). Ghosh and Adhikari (2006) reported that there competition between calcium (Ca) and other metal ions for influencing metal uptake and toxicity in the mrigal carp The Cirrhinus mrigala. authors reported that in binary mixtures of Pb, Cd and Cr with calcium, the calcium compound was found to consistently reduce the toxic effect as well as accumulation of Pb, Cd and Cr compounds. Increased Ca levels showed lower transfer of Pb, Cd and Cr from water to the gills. Many studies have reported the single metal toxicity and accumulation in aquatic organisms (Yap et al., 2004; Rashid et al., 2009; Miedico et al., 2013). However, the combined toxicity of heavy metal mixtures in aquatic animals is not existent. The present results showed is that there an accumulated competition between Cd and Pb in the tissues of the clams. In a separated exposed the clams to Cd and Pb, the concentration of each metal was higher than both metals in combined exposed. In both cases, the concentration of Cd was higher than Pb. However, the depuration rate of Pb was lower than Cd. In aquatic ecosystems, animals may simultaneously expose to multiple metals. Therefore. heavy the accumulation mechanisms of and toxicity of multiple heavy metals in animals aquatic are strongly recommended in future studies.

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