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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 aquatic organisms when they are 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 bio-monitoring organism, and mollusks become a popular choice for heavy metal monitoring (Yap *et al*., 2003) because of their filter feeding. However, previous studies have reported that mollusks have a depuration mechanism to reduce heavy metal toxicity in their body (Yap *et al*., 2004). This mechanism might diminish the effectiveness of mollusks as bio-monitoring 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 aquatic animals. 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*

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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 under laboratory condition. The 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 prior to experiments. Water 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 2×10^{3} 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:

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

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):

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

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 not contain any detectable metal 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 concentration of 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).

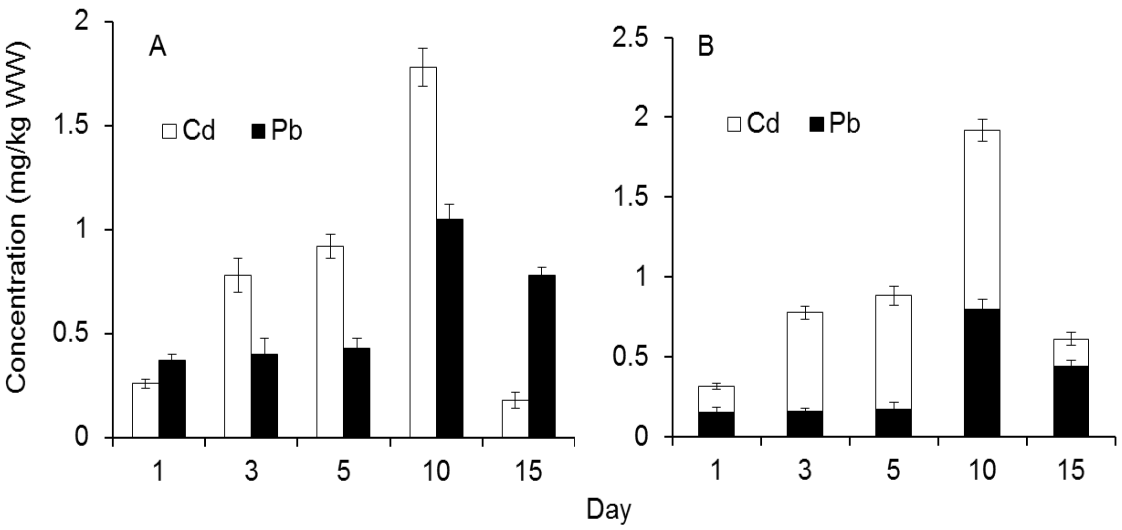

Figure 2: The concentration of Cd and Pb in the clam during uptake and depuration periods. (A) single exposed and (B) combined exposed.

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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 during exposed. In general, the 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.

Day	BCF				RA, RD			
	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.

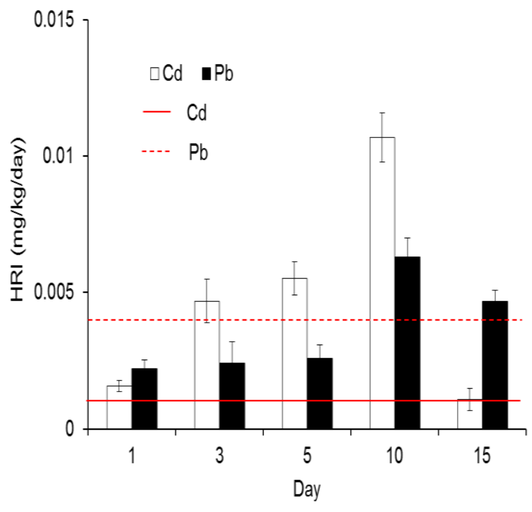

Figure 3: Health risk index of the studied clam for Cd and Pb.

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Discussion

Heavy metals bioaccumulation in 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 as behavioral deficits in vertebrates, decreases in survival and growth rates, causes learning disabilities, and 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 very toxic for 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 and accumulate heavy metals of higher levels (El-Moselhy *et al.*, 2014; 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 and feeding patterns (Ayse, 2003). The concentration of Cd and Pb in tested clams contained from 0.18–1.78 mg kg^{-1} 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 other mussel under exposed in 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 and 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 is a competition between calcium (Ca) and other metal ions for influencing metal uptake and toxicity in the mrigal carp *Cirrhinus mrigala*. The 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 that there is 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 heavy metals. Therefore, the mechanisms of accumulation and toxicity of multiple heavy metals in aquatic animals are strongly recommended in future studies.

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References

Ahmed, M.K., Parvin, E., Arif, M., Islam, M.M., Akter, M.S. and Khan, M.S., 2011. Genetic damage induced by lead chloride in different tissues of fresh water climbing perch *Anabas testudineus* (Bloch). *Environmental Monitoring and Assessment*, 182(**1**), 197–204.

Alsop, D., Ng, T.Y.T., Chowdhury, M.J. and Wood, C.M., 2016. Interactions of waterborne and dietborne Pb in rainbow trout, *Oncorhynchus mykiss*: Bioaccumulation, physiological responses, and chronic toxicity. *Aquatic Toxicology*, 177(**Supplement C**), 343-354.

Ayse, B.Y., 2003. Levels of heavy metals (Fe, Cu, Ni, Cr, Pb, and Zn) in tissue of *Mugil cephalus* and *Trachurus mediter-raneus* from Iskenderun Bay, Turkey. *Environmental Research*, 92(**3**), 277–281.

Chorus, I. and Bartram, J., 1999. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. Published on behalf of WHO, Spon Press, London. 416.

Dao, T.S., Le, V.N., Bui, B.T., Dinh, K.V., Wiegand, C., Nguyen, T.-S., Dao, C.T., Nguyen, V.D., To, T.H., Nguyen, L.S.P., Vo, T.G. and Vo, T.M.C., 2017. Sensitivity of a tropical micro-crustacean (*Daphnia lumholtzi*) to trace metals tested in natural water of the Mekong River. *Science of the Total Environment*, 574(**1**), 1360-1370.

El-Moselhy, K.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A., 2014. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egyptian *Journal of Basic and Applied Sciences*, 1(**2**), 97-105.

Ghosh, L. and Adhikari, S., 2006. Accumulation of heavy metals in freshwater fish-an assessment of **toxic** interactions with calcium. *American Journal of Food Technology*, 1, 139-148.

Guo, S.N., Zheng, J.L., Yuan, S.S., Zhu, Q.L. and Wu, C.W., 2017. Immunosuppressive effects and associated compensatory responses in zebrafish after full life-cycle exposure to environmentally relevant concentrations of cadmium. *Aquatic Toxicology*, 188(**Supplement C**), 64-71.

Helmstetler, H. and Cowles, D.L., 2008. Population characteristics of native freshwater mussels in the mid-Columbia and Clearwater Rivers, Washington State. *Northwest Science*, 82(**3**), 211-221.

Hoang, T.T.T., Vy, N,N,H. and Tu, T.C.L., 2007. Anthropogenic input of selected heavy metals (Cu, Cr, Pb, Zn and Cd) in the aquatic sediments of Hochiminh City, Vietnam. *Water Air Soil Pollution*, 182(**1**), 73-81.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(**2**), 60–72.

Jia, J., Luo, W., Lu, Y. and Giesy, J.P., 2014. Bioaccumulation of microcystins (MCs) in four fish species from Lake Taihu, China: Assessment of risks to humans. *Science of the Total Environment*, 487(**15**), 224-232.

Lee, P.-K., Youm, S.-J. and Jo, H.Y. 2013. Heavy metal concentrations and contamination levels from Asian dust and identification of sources: A case-study. *Chemosphere,* 91(**7**), 1018-1025.

Lewbart, G.A., Christian, L.S., Harms, C.A.V. and Wettere, A.J.,

2010. A comparison of heavy metal concentrations and health assessment in Asian clams *Corbicula fluminea* from Florida and North Carolina. *Journal of Aquatic Animal Health*, 22(**2**), 73-77.

Mahmood, A. and Malik, R.N. 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, 7(**1**), 91-99.

Miedico, O., Pompa, C., Tarallo, M. and Chiaravalle, A.E. 2013. Assessment of heavy metals in bivalves molluscs of Apulian region: a 3-years control activity of a EU Laboratory. *E3S Web of Conferences*, 1, 11006.

Nguyen, V.T., Ozaki, A., Nguyen, T.H., Nguyen, D.A., Tran, T.Y. and Kurosawa, K., 2016a. Arsenic and heavy metal contamination in soils under different land use in an estuary in Northern Vietnam. *International Journal of Environmental Research and Public Health*, 13(**11**),1091.

Nguyen, T.T.H., Zhang, W., Li. Z., Li, J., Ge, C., Liu, J., Bai, X., Feng, H. and Yu, L., 2016b. Assessment of heavy metal pollution in Red River surface sediments, Vietnam. *Marine Pollution Bulletin*, 113(**1**), 513-519.

Paul, N., Chakraborty, S. and Sengupta, M., 2014. Lead toxicity on non-specific immune mechanisms of freshwater fish *Channa punctatus*. *Aquatic Toxicology*, 152(**Supplement C**), 105-112.

Pham, T.L., Shimizu, K., Kanazawa, A., Gao, Y., Dao, T.S. and Utsumi, M., 2016. Microcystin accumulation and biochemical responses in the edible clam *Corbicula leana* P. exposed to cyanobacterial crude extract. *Journal of Environmental Sciences*, 44(**2016**), 120-130.

Pham, T.L., Shimizu, K., Dao, T.S. and Motoo, U., 2017. First report on free and covalently bound microcystins in fish and bivalves from Vietnam: Assessment of risks to humans. *Environmental Toxicology Chemistry*, 36(**11**), 2953-2957.

Ramsdorf, W.A., Ferraro, M.V.M., Oliveira-Ribeiro, C.A., Costa, J.R.M. and Cestari, M.M., 2009. Genotoxic evaluation of different doses of inorganic lead (PbII) in *Hoplias malabaricus*. *Environmental Monitoring and Assessment*, 158(**1**), 77-85.

Rashid, W.A., Wan, V.L. and Abdullah, M.H., 2009. Accumulation and depuration of heavy metals in the hard clam (*Meretrix meretrix*) under laboratory conditions. *Tropical Life Science Research*, 20(**1**), 17–24.

Roméo, M., Siau, Y., Sidoumou, Z.N. and Gnassia-Barelli, M., 1999. Heavy metal distribution in different fish species from the Mauritania coast. *Science of the Total Environment*, 232(**3**), 169-175.

Silva, E., Viana, Z.C.V., Onofre, C.R.E., Korn, M.G.A. and Santos, V.L.C.S., 2016. Distribution of trace elements in tissues of shrimp species *Litopenaeus vannamei* (Boone,

1931) from Bahia, Brazil. *Brazilian Journal of Biology*, 76(**1**), 194-204.

USEPA IRIS, 2006. United States, environmental protection agency, Integrated Risk Information System. <http://www.epa. gov/iris/substS>.

Wang, Z., Sun, R., Zhang, H. and Chen, L., 2015. Analysis and assessment of heavy metal contamination in surface water and sediments: a case study from Luan River, Northern China. *Frontiers of Environmental Science and Engineering*, 9(**2**), 240-249.

Xu, Y., Wu, Y., Han, J. and Li, P., 2017. The current status of heavy metal in lake sediments from China: Pollution and ecological risk assessment. *Ecology and Evolution*, 7(**14**), 5454–5466.

Yap, C.K., Tan, S.G. and Omar, H. 2003. Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* under laboratory. *Hydrobiologia*, 498(**1-3**), 151–160.

Yap, C.K., Ismail, A., Tan, S.G. and Rahim, I.A., 2004. Assessment of different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of Pb: field and laboratory Studies. *Water Air Soil Pollution*, 153(**1**), 253-268.

Zhang, J., Wang, Z., Song, Z., Xie, Z., Li, L. and Song, L., 2012. Bioaccumulation of microcystins in two freshwater gastropods from a cyanobacteria-bloom plateau lake, Lake Dianchi. *Environmental Pollution*, 164(**47**), 227-234.

Zheng, J.L., Yuan, S.S., Wu, C.W. and Li, W.Y. 2016. Chronic waterborne zinc and cadmium exposures induced different responses towards oxidative stress in the liver of zebrafish. *Aquatic Toxicology*, 177(**Supplement C**), 261-268.

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