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Study on the accumulation and transformation of lead in liver and muscle of climbing perch (*Anabas testudineus*)

Dang Nguyen Nha Khanh[1,2], Ngo Thi Tuong Vy[2], Tran Ha Phuong[2], Pham Tuan Nhi[3], Vu Quang Huy[3], Nguyen Quoc Thang[4], Do Trung Sy[5], Le Tien Dung[6], Nguyen Duy Khanh[6], Nguyen Thi Kim Phuong[1,3,*]

[1] *Graduate University of Science and Technology, Vietnam Academy of Science and Technology*
[2] *National Institute of Applied Mechanics and Informatics, Vietnam Academy of Science and Technology*
[3] *HoChiMinh City of Institute of Resources Geography, Vietnam Academy of Science and Technology*
[4] *Industrial University of Ho Chi Minh*
[5] *Institute of Chemistry, Vietnam Academy of Science and Technology*
[6] *Institute of Applied Materials Science, Vietnam Academy of Science and Technology*
**Email: nguyenthikimp@yahoo.ca*

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ABSTRACT

Lead (Pb) accumulation in food is being seriously concerned due to its harmful effects on the health in of consumers. In this study, the accumulation and speciation of Pb analysis were carried out on climbing perch (*Anabas testudineus*). The *A. testudineus* was raised in waterborne Pb at concentration of 20, 30, 40 mg/L for Pb in 7 and 28 days. Pb is present in different chemical forms in the food chain, but organic Pb is more toxic than inorganic Pb. In the present study, organic Pb in liver and muscle of *A. testudineus* was extracted with methanol and was measured by inductively coupled plasma-optical emission spectrometry. After 28 days of exposure, total Pb concentration in *A. testudineus* was from 124.23 ± 5.09 to 174.45 ± 5.55 mg/kg dry weight for the liver and from 67.83 ± 5.79 to 153.60 ± 5.78 mg/kg dry weight for the muscle. Organic Pb found in *A. testudineus* was from 8.1 to 9.9 % for the liver and from 5.9 to 12.5 % for the muscle. The accumulation of Pb in *A. testudineus* was quite high however, the conversion from inorganic Pb to organic Pb in *A. testudineus* was relatively low.

Introduction

Recently, world fish consumption has been steadily increasing due to their nutritional and therapeutic benefits. In addition to its important protein source, fish is also rich in essential minerals and unsaturated fatty acids (Omega-3 fatty acids) [1]. The American Heart Association recommends eating fish at least twice a week to achieve a daily intake of Omega-3 fatty acids [2]. The two main ways through which heavy metals get into fish are ingestion (direct consumption of water and food contamination) and non-ingestion (through membranes such as muscles and gills). As a high nutrient level and a major source of protein in the food chain, the human body is therefore largely susceptible to enriched heavy metal concentration in fishes [3]. Heavy metals have been shown to be seriously toxic to all living organisms including human. They compete with nutrient elements in the binding sites of transport

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and storage proteins. The accumulation of heavy metals in fish organs are driven by physiochemical and biological variables such as pH, water temperature, hardness, salinity, alkalinity, exposure duration and feeding habits [4,5].

Lead (Pb) is known to be a highly toxic heavy metal that has adverse effects on human health including nervous system disorders, mental retardation, skeletal hematopoietic function disorder and even death [6]. After entering the cell, inorganic Pb is converted organic Pb compounds. Organic Pb compounds are considered to be more toxic than inorganic Pb. However, both of inorganic and organic Pb compounds may be accumulated in fish and can therefore reach the food chain of human [7].

Anabas testudineus, the climbing perch is an invasive species that can live without water for 6–10 hours [8]. This species is important as a food fish in certain areas of South Asia and in Southeast Asia. *Anabas testudineus* is a favorite Vietnamese dish because meat contains high levels of nutrients, good for health. They often live in all types of fresh water surface: rice fields, ponds, ditches, swamps, rivers...Freshwater fish is a valuable source of food for human consumption. However, in the context of increased environmental pollution and insufficient information on food safety warnings on the aspects of heavy metal pollution in freshwater fish, thus, this study focused on the accumulation of Pb in the gill, liver and muscle tissues of *A. testudineus*. Sub-chronic exposures to Pb were also investigated to determine the speciation of Pb in the liver and muscle tissues of *A. testudineus*.

Experimental

Chemicals

$Pb(CH_3COO)_2$, NaOH, HCl (36%), HNO_3 (65%), H_2O_2 (30%), H_3BO_3, $Na_2B_4O_7$ and glacial acetic acid were analytical grade (Merck, Darmstadt, Germany). Methanol was HPLC grade (Merck, Darmstadt, Germany). The waterborne Pb to expose the fish was made by dissolving $Pb(CH_3COO)_2$ into distilled water.

Fish and conditional experiment

A. testudineus were collected from Tam Sach fish farms, Thu Duc district, Hochiminh city, Vietnam. Similar sized fish, 9 ± 1 cm in length and 12.9 ± 1 g in wet weight, were used for the experiments. Fish were transported in polyethylene bags filled with oxygen. Fish 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 (30 ± 1°C) and illuminated for 10 h. Fish were allowed to acclimate for 12 days before the exposure studies began.

LC_{50} test

A. testudineus were exposed to Pb at concentration of 30, 40, 50, 75, 90, 100, 150, 175 and 200 mg/L under static environment without water change for 96 h. Sixty fish were randomly assigned to each treatment, and each treatment consisted of four tanks with fifteen fish per tank. Fish mortality was recorded after 0, 24, 48, 72, and 96 h of exposed to Pb solution LC_{50} value was calculated by simple graphic method.

Subchronic Pb exposure

Anabas testudineus were exposed to waterborne Pb at 1/6, 1/4, and 1/3 of 96 h LC_{50}. Seventy-five fish were randomly assigned to each treatment; each treatment consisted of three tanks with twenty-five fish per tank (n=3). Water samples for total Pb analysis were collected twice a week and analyzed by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima 2100 DV, Perkin Elmer). Due to water renewal, water samples were collected twice a week for analysis of Pb concentration.

Tissue sampling: On days 7, 14, 21 and 28 of the exposure period, *A. testudineus* were collected randomly from each group and washed with bi-distilled water before gill, liver and muscle tissue sampling. These tissues were freeze-dried for 48h before analysis of Pb concentration species.

Sample preparation

Total Pb determination: Prior to determination, tissues of weight 1.0 g were subjected to preliminary mineralization in presence of 6 mL of a 2:1 v/v mixture of HNO_3 (65%) and H_2O_2 (30%) in the closed vessel. The samples were then heated to 85 ºC for 30 min. The obtained clear liquid was diluted with deionized water to 25 mL and then assayed for concentration of Pb using ICP-OES (Optima 2100 DV, Perkin Elmer). The method had a limit of detection (LOD) of 0 1.20 μg/L for Pb. The assay recovery was 92.2% for Pb.

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Organic Pb compound: The extraction process as described in the previous report [9]: Organic Pb compound in dried liver and muscle tissues were extracted with a microwave-assisted. About 0.5 g of dried tissue and 10 mL of 50% methanol solution (in 70 mmol/L H_3BO_3, 17.5 mmol/L $Na_2B_4O_7$ solution) were added to a 30 mL Teflon tube. The tube was closed and placed in a microwave oven, then heated at 70 °C for 5 minutes under 400 W for 3 times (with a 2 min break between two heating sessions). Then, the solution was cooled to room temperature and filtered through a 0.22 µm membrane filter to obtained the extract. The extract was evaporated to almost dry using nitrogen stream and diluted with 1% HNO_3 before being passed into the 25mL volumetric flask. The final solution was used for the ICP-OES (Optima 2100 DV, Perkin Elmer).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 22.0. Data were expressed by mean ± SD. The significance differences between mean was assessed by LSD ($p < 0.05$).

Results and discussion

LC_{50} value

Figure 1 shows the curve of *A. testudineus* mortality versus Pb concentration.

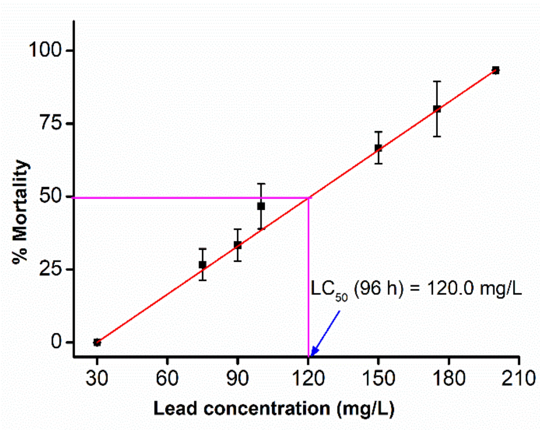

Figure 1: Graphical estimation of LC_{50} 96h for Pb

The equation for Pb concentration mortality in the linear regression was found to be y = 0.549 x - 16.471 with r^2 = 1.00. The 96 h LC_{50} value of Pb in *A. testudineus* was approximately 120 mg/L. No fish death was observed in the control group. Previous study had reported that the 96 h LC_{50} values of Pb for *Oreochromis* sp. was found to be about 3.24 mg/L [10].

A comparison of LC_{50} values indicated that *Oreochromis* sp. was more sensitive to Pb than *A. testudineus*. Different organisms have different levels of sensitivity to metals. These differences are due to natural biological variations between species, reflecting population genetic make-up and individual conditions. This variation is generally small for organisms of the same species, age, and health, whereas the variation is high between species [11].

Accumulation of Pb

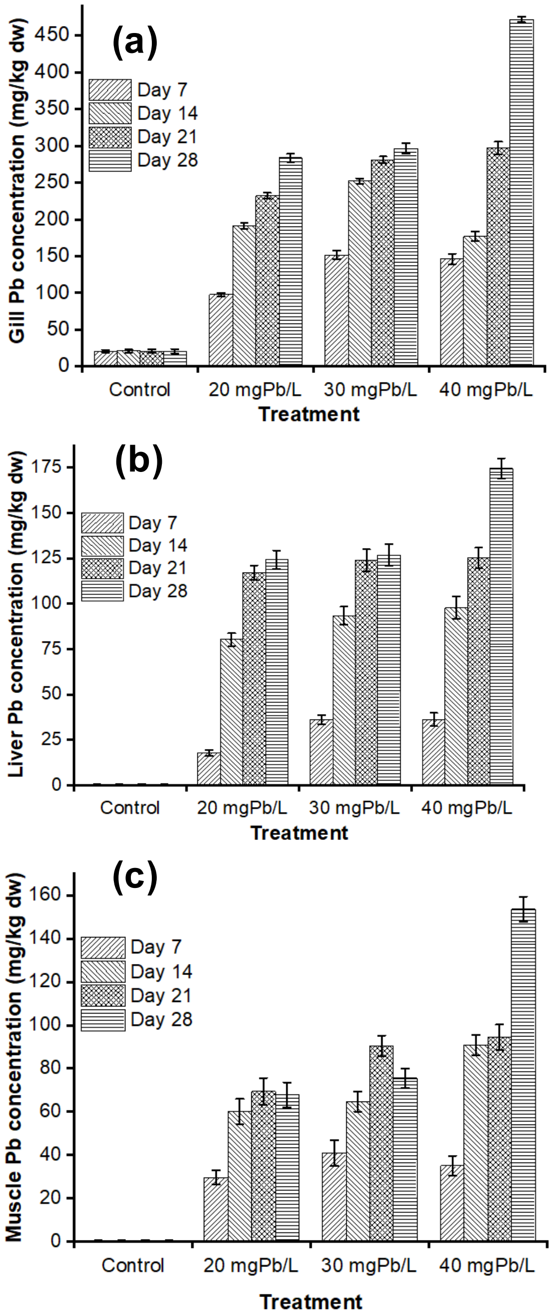

Figure 2: Pb accumulation in *A. testudineus* exposed to Pb (a) Gill (b) Liver and (c) Muscle (mean ± SD, n=3)

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The concentrations of Pb in the gill, liver and muscle of *A. testudineus*. were measured on day 7, 14, 21, and 28 of the exposure phase, and the results are presented in Figure 2. The concentrations of Pb in gill, liver, and muscle increased steadily with increasing Pb concentration in the water and increasing duration of exposure. In this study, the order of Pb accumulation in the *A. testudineus* tissues was gill > liver > muscle.

Previous studies also reported that gills have a high tendency to accumulate heavy metals [12-14]. The gills of fish act as an interface between the environment and the blood. Gills are used for the continuous diffusion of oxygen and the maintenance of pH, and they are also the main sites of heavy metals absorption due to direct contact with the water [15]. In this study, Pb accumulation in the gills of groups exposed to Pb was significantly higher than that of the control group ($p < 0.05$) (Fig. 2a). The concentrations of Pb in the gills were from 97.57 ± 2.33 to 283.71 ± 5.39 mg/kg dry weight, from 151.35 ± 6.01 to 296.53 ± 7.03 mg/kg dry weight and from 146.27 ± 7.13 to 471.84 ± 3.92 mg/kg dry weight at Pb exposures of 20, 30 and 40 mg/L, respectively. These values were about 5- to 23-fold higher than the control group.

The accumulation of Pb in the liver of *A. testudineus* increased linearly at Pb exposures of 20, 30 and 40 mg/L (Fig.2b). After 28 days of exposure at 20, 30 and 40 mg/L, the concentrations of Pb in fish liver reached 124.23 ± 5.09, 126.77 ± 5.97 and 174.45 ± 5.55 mg/kg dry weight, respectively. These values were about 215- to 303-fold higher than those in the control group.

Accumulation of Pb in the muscle of *A. testudineus* significantly increased with increasing Pb concentrations in the water (Fig. 2c). After 28 days of exposure, the Pb accumulation muscle of *A. testudineus* significantly increased in the exposure groups compared to the controls ($p < 0.05$). The accumulation of Pb in fish muscle were from 29.67 ± 3.15 to 67.83 ± 5.79 mg/kg dry weight for the groups exposed to Pb at 20 mg/L, from 41.04 ± 5.90 to 75.61 ± 4.39 mg/kg dry weight for the groups exposed to Pb at 30 mg/L, and from 35.05 ± 4.57 to 153.60 ± 5.78 mg/kg dry weight for the groups exposed to Pb at 40 mg/L. These values were approximately 53- to 256-fold higher than those in the control group.

At day 28 of exposure, accumulation of Pb in the liver was from 1.1 to 1.9- fold higher than that of the muscle. Previous studies have reported that metals accumulate in liver higher than in muscle [1,16,17]; this is probably due to higher metabolic activities in the liver compared to those of muscle [18]. These results are consistent with the hypothesis that accumulation in muscle becomes important only when the liver has reached the maximum storage capacity.

Speciation of Pb

Previous study has shown that organic Pb compounds are more toxic than inorganic Pb [9]. It can be seen in Figure 3, accumulation of total Pb and inorganic Pb in liver of *A. testudineus* highly increased with increasing exposure time and Pb concentration in water whereas, organic Pb in liver slightly increased with increasing exposure time and concentration of Pb in water.

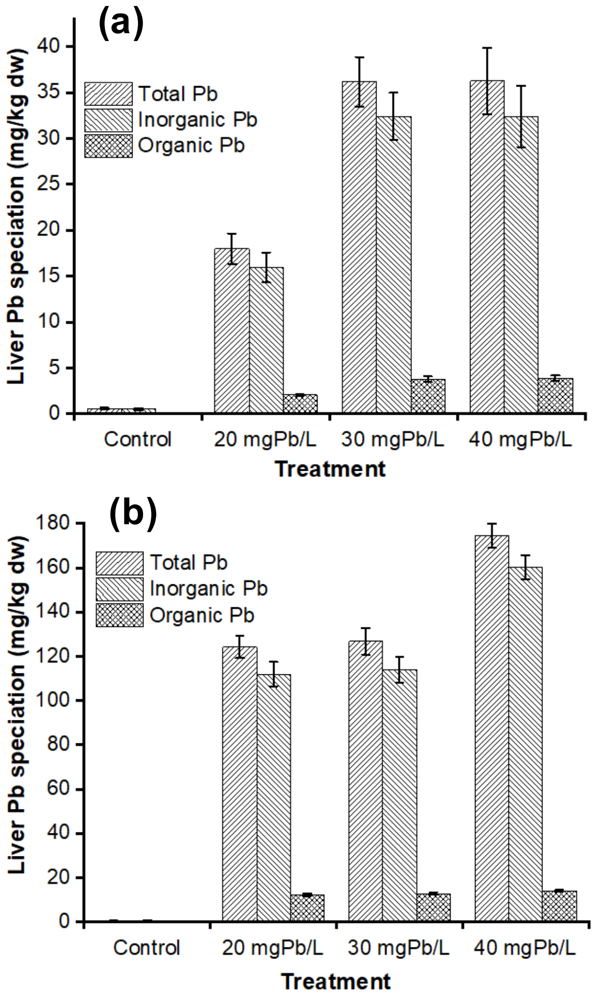

Figure 3: Liver Pb speciation in *A. testudineus* (a) at day 7 and (b) at day 28 (mean ± SD, n=3)

The concentration of organic Pb in liver of *A. testudineus* was found to be from 2.04 ± 0.06 to 3.86 ± 0.31 mg/kg dry weight at day 7 and from 12.28 ± 0.61 to 14.12 ± 0.41 mg/kg dry weight at day 28. The percentage of organic Pb in the liver of *A. testudineus* exposed to Pb decreased with increasing Pb concentration in the water and exposure duration. In

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this study, percentage of organic Pb in the liver of *A. testudineus* exposed to Pb was found to be from 10.6 to 11.3% at day 7 and from 8.1 to 9.9% at day 28. From day 7 to day 28, the total Pb in the liver increased but the percentage of organic Pb in the liver decreased, suggesting that the conversion from inorganic to organic Pb in liver of *A. testudineus* was relatively low. The muscle was able to convert from inorganic to organic Pb compounds rather than liver.

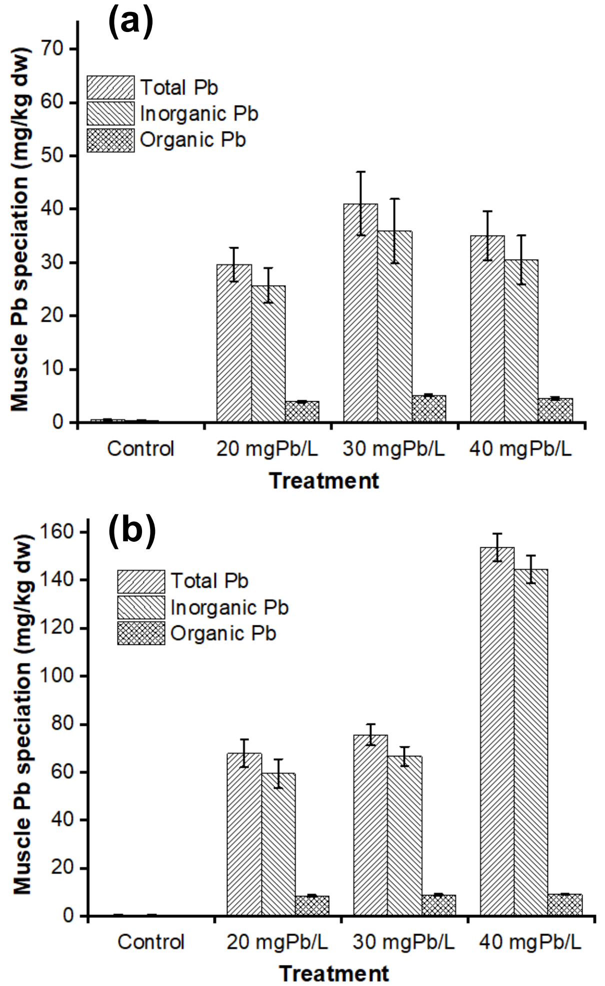

Figure 4: Muscle Pb speciation in *A. testudineus* (a) at day 7 and (b) at day 28 (mean ± SD, n=3)

As seen in Figure 4, the concentration of organic Pb in muscle of *A. testudineus* was found to be from 3.97 ± 0.19 to 5.16 ± 0.20 mg/kg dry weight at day 7 and from 8.46 ± 0.44 to 9.12 ± 0.11 mg/kg dry weight at day 28. The percentage of organic Pb in the muscle of *A. testudineus* exposed to Pb was from 12.6 to 13.4 % at day 7 and from 5.9 to 12.5 % at day 28. Similar to the liver, from day 7 to day 28, the total Pb in the muscle increased but the percentage of organic Pb in the muscle did not increase, suggesting that the conversion from inorganic to organic Pb in muscle of *A. testudineus* was relatively low.

Conclusion

In summary, the work reported here has shown that *A. testudineus* freshwater fish exposed to Pb can accumulate with significant concentration of Pb. The *A. testudineus* has ability to convert from inorganic to organic Pb compounds, however, the conversion from inorganic Pb to organic Pb in the liver and muscle of *A. testudineus* was relatively low.

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