INFLUENCE OF SUB-CHRONIC EXPOSURE TO ARSENIC, CADMIUM, LEAD ON GROWTH AND ACCUMULATION OF ITS IN Oreochromis sp.

Nguyen Quoc Thang¹, Le Van Tan¹,² and Nguyen Thi Kim Phuong²

¹Chemical Engineering Faculty, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao, Go Vap, Ho Chi Minh City, Vietnam
²National Institute of Applied Mechanics and Informatics, Vietnam Academy of Science and Technology

Corresponding Author: levantan@iuh.edu.vn

ABSTRACT

The accumulation and elimination of arsenic (As), cadmium (Cd), lead (Pb) and its effect on Oreochromis sp. growth were studied after exposing fish to 1.0, 1.5 and 3.0 mgAs/L or 0.66, 1.0 and 2.0 mgCd/L or 0.12, 0.18 and 0.33 mgPb/L for 20 days in exposure phase and 10 days in the recovery phase. As Cd and Pb significantly affect on increases in weight of fish. In the exposure phase, the ratio of Metal / Metal H2O in tissues of fish was in the order muscle >> liver > gill in the case As exposure; muscle << gill << liver for Cd exposure, and muscle << liver << gill in the case of Pb exposure. On day 10 of the recovery phase, the elimination of accumulated As, Cd, Pb in the fish organs was approximately from 12% to 29% for As, from 33% to 54% for Cd and from 14% to 39% for Pb.

Keywords: Oreochromis sp., Arsenic, Cadmium, Accumulation, Elimination

INTRODUCTION

Heavy metals are important contaminants in aquatic environments worldwide. Metals pollution has increased with the technological progress of human society. Arsenic (As), lead (Pb), and cadmium (Cd), are popularly known as highly toxic heavy metals. These metals don’t have a function in the physiological processes in the living organisms and cause tissue damage even at very low concentration.¹ Nowadays, with the growing concern of fish nutritional and therapeutic benefits, world consumption of it has increased simultaneously. Fish are not only an important source of protein but also typically have rich contents of essential minerals and unsaturated fatty acids (Omega-3 fatty acids).² However, fish normally occupy higher positions in the aquatic food chain and easily accumulate heavy metals in their organs. Heavy metals in fish cultivate a potential risk, not only for themselves but also to piscivorous birds, mammals and even human beings through food causing chronic or acute diseases.³,⁴ Heavy metals were accumulated in fish as a result of a lot of factors which were water concentrations of metals, exposure times, the temperature of water, oxygen concentration of water, pH, hardness, alkalinity, salinity, and organic carbon level.⁵,⁶ Oreochromis sp. was breed in cages in rivers and lakes. They are not only quite common but also have brought profits for farmers in the Mekong Delta, Vietnam. Farmers are currently suffering from losses due to Oreochromis sp from water pollution. Therefore, the importance of studies of heavy metal bioaccumulation, elimination and its effect on Oreochromis sp. growth, which represent a valuable source of food for Vietnamese, in the context of environmental pollution, seems unquestionable.

EXPERIMENTAL

Fish and Conditional Experiment
Oreochromis sp. in this research were collected from Tu Hai fish farms, ⁸th provincial Highway, Tan An Hoi commune, Cu Chi district, Ho Chi Minh City, Viet Nam. Only similar-sized fish, which were 20 ± 1 cm in length and 300 ± 1 g in weight, were selected for the experiments. This batch of fish was transported in polyethylene bags which were filled with oxygen. Then, the selection of fish was kept in a 500 L tank. The tank was set at the room-controlled laboratory conditions set at 30 ± 1 °C, illumination for 10 h, and 25% daily water renewal. The tank’s water was tested every day in dissolved oxygen = 6.5 ± 0.7 mg/L, pH = 6.3, temperature = 28 ± 4 °C. The fish were allowed to acclimate for 12 days before the start of exposure studies.

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**LC$_{50}$ Test**

*Oreochromis* sp. was exposed to waterborne either NaAsO$_2$ at 0 (control group), 1, 5, 10, 15, 20, 25, 27, 30, 33, 35, and 40 mgAs/L or CdCl$_2$ 0 (control group), 2, 5, 10, 15, 20, 30, 40, and 45 mgCd/L or Pb(CH$_3$OO)$_2$ at 0 (control group), 0.5, 1, 2, 5, 7, 10, 13, and 15 mgPb/L for 96 hrs. In each treatment, forty fish were randomly assigned into consisted of three tanks which were ten fish in every tank. During the acute toxicity test, the fish were not fed; the dead fish were removed immediately from the tank. Fish mortality (%) was recorded after 0, 24, 48, 72, and 96 hrs of exposure to As or Cd or Pb solution. LC$_{50}$ values were calculated by the simple graphic method.

**Subchronic As, Pb, Cd Exposure and Recovery Experiments**

*Oreochromis* sp. was exposed to waterborne As or Pb or Cd at 0 mg/L (control group), 1.00 mg/L, 1.50 mg/L, 3.00 mg/L for As, 0 mg/L (control group), 0.12 mg/L, 0.18 mg/L, 0.33 mg/L for Pb, and 0 mg/L (control group), 0.66 mg/L, 1.00 mg/L, 2.00 mg/L for Cd equal to 0, 1/30, 1/20, and 1/10 of 96 hrs LC$_{50}$. The exposure phase was 20 days and the depuration period was 10 days. In every treatment, seventy-five fish were randomly assigned and distributed of three tanks and every tank contained twenty-five fish (n=3). Twice a week, water samples were collected for analysis to test As or Pb or Cd concentration. After the heavy metal exposure time, the experiment fish were in depuration period of day 10$^{th}$ in control water.

**Sampling**

On day 20$^{th}$ of exposure and day 10$^{th}$ of the depuration period, fish were washed in bi-distilled water and were weighed to compare the effect of As, Pb, Cd concentration in the water on the growth rate to fish. After that, three organs of *Oreochromis* sp. fish (such as muscle, gill and liver) were randomly collected from each group and these organs were dried in an oven at 105 $^\circ$C for 10 hrs before analysis of As or Pb or Cd level.

**As, Pb, and Cd Analysis**

To determine of As, Cd, and Pb concentration, weight 1.000 g of tissues were put into the closed vessel and added 4 mL HNO$_3$ (65%) and 2 mL H$_2$O$_2$ (30%). The closed vessel was then heated to 85 $^\circ$C for 30 min to preliminary mineralization. The obtained clear liquid was diluted to 25 mL with deionized water. The concentration of Pb was assayed for using Inductively Coupled Plasma - Optical Emission Spectrometry (Optima 2100 DV, Perkin Elmer) and concentration of As and Cd were assayed for using Inductively Coupled Plasma – Mass Spectrophotometry (ICP-MS 7700, Agilent, USA). The method had a limit of detection (LOD) of 0.076 $\mu$g/L for As, 1.0 $\mu$g/L for Pb, and 1.0 $\mu$g/L for Cd. The assay recovery was approximately 105.0% for As, 92.2% for Pb, and 94.4% for Cd.$^{7,8}$

**Statistical Analysis**

Using the Design Expert version 11 to evaluate the statistical analysis of the result. Data were expressed by mean ± SD.

**RESULTS AND DISCUSSION**

**The 96 hrs LC$_{50}$ Value of As, Cd, and Pb**

**The Mortality Rate of *Oreochromis* sp. in 96 hrs**

Figure-1 depicts the percentage mortality for different exposure periods at different concentrations of As, Pb, and Cd. *Oreochromis* sp. fish were not died in the first 96 hrs in the control group and the groups exposed to 20 mgAs/L. Meanwhile, 13.3% to 100% of the fish died for 96 hrs in the groups which were exposed to about 20 to 45 mg/L of As concentrations in the waterborne (Fig.-1a).

Fig.-1b shows that there is an increase in the mortality rate of fish with the increased Pb concentrations in the water. None of the fish died in the first 24 hrs for the groups exposed to 0.5 mgPb/L. But in the last 96 hrs of the experiment, approximately 37% of fish died. For the groups exposed to 1.0 mgPb/L, in the first 24 hrs there was about 10% of fish death, there was about 47% of fish died in the last 96 hrs of the experiment. Exposure of *Oreochromis* sp. to 2-13 mgPb/L resulted in about 47 to 76% dead fish. The highest mortality was found in the group exposed to 15 mgPb/L. There was about 60% of fish died in the first 24 hrs, which lasted 72 hrs, about 90% of fish died. None of the controlled fish died during 96 hrs.

In the fig.-1c, fish in all treatment groups died for 96 hrs. Mortality of fish increased with increasing Cd concentration in water. At the group exposed to 2 mgCd/L, no fish died until 72 hrs. But in the last 96 hrs, it was about 7% fish death. About 27% to 100% of the fish died in the groups treatment of 5 mgCd/L to 45 mgCd/L. Meanwhile, control fish did not die for 96 hrs.
**LC50 Value of As, Cd, and Pb**

The 96 hrs LC50 values of fish varied from species to species and from metal to metal. Fig.-2 shows a curve of *Oreochromis* sp. mortality versus As or Cd or Pb concentration. The 96 hrs LC50 value of As, Cd, and Pb for *Oreochromis* sp. were found to be approximately 29.26 mg/L, 19.58 mg/L, and 3.24 mg/L, respectively. In this study, for *Oreochromis* sp., the 96 hrs LC50 value of As was found approximately 29.26 mg/L which was a high level. These results displayed that the highly toxic inorganic arsenic forms were able to be biotransformed to the less toxic organic arsenic forms. Meanwhile, our previous studies showed that inorganic arsenic forms were biotransformed to the less toxic organic arsenic forms which were monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsinebetaine (AsB) in the *Oreochromis* sp. fish in the inorganic arsenic (III) waterbone.9

**Effect of As, Cd, Pb Concentration in the Water on the Growth Rate of Oreochromis sp. (Exposure Time)**

The bodyweight of the controlled fish increased with the increase in exposure time while the bodyweight of fish exposed to As, Pb, Cd are much lower (Table-1). As the toxicity of As, Cd, Pb metals affected fish growth in the case of exposure, these metals were consequently changed to the metabolic demands. The impact of changing metabolic demands on normal growth processes was shown clearly in the group exposed to heavy metal. Fish exposed to higher metal concentrations (1.5-3.0 mg/L for As, 0.18-0.33 mg/L for Pb, 1.0-2.0 mg/L for Cd) grew slower than fish exposed to lower metal concentration (1.0 mgAs/L, 0.66, 0.12 mgPb/L, mgCd/L) and the controlled fish. This demonstrated that the test fish grew to belong to the increase of heavy metal level in the water.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fish Body Weight (gam)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4th</td>
</tr>
<tr>
<td>Control</td>
<td>311 ± 4</td>
</tr>
<tr>
<td>1.0As</td>
<td>306 ± 4</td>
</tr>
<tr>
<td>1.5As</td>
<td>304 ± 2</td>
</tr>
</tbody>
</table>

*Fig.-1: Mortality Rate of Oreochromis sp. exposure to (a) As, (b) Pb, and (c) Cd*

*Fig.-2: Graphical Estimation of LC50 of As, Pb, Cd for Oreochromis sp.*
In the control groups, fish was strong and ate very good but in the treatment groups, fish was exhausted and grew slower than the control fish groups. After 20 days exposure of to heavy metal, in the controlled groups, the fish’s weight increased about 19.3% compared to the starting of the exposure. Meanwhile, in the groups which were exposed to 1-3 mgAs/L, the fish weight only increased by 2.7-5.7% compared to the starting of the exposure.

During the 12th and 20th day of exposure, the weight of the controlled fish increased from 9.7 to 19.3%, while the fish weight of the group which was exposed to 0.66 mgCd/L increased from 4.3 to 6.3% compared to the starting of the exposure. On day 20th after living in the exposure heavy metal, the fish’s weight of the group which was exposed to 1-2 mgCd/L increased by 3.4-6.7% compared to the starting of the exposure. These results were similar to previous studies documented by M Saeed Heydarnejad et al. (2013), the control *Oncorhynchus mykiss* fish and the fish exposed to lower cadmium level in the water grew quicker than fish exposed to the higher cadmium level.\(^9,10\)

The fish weight from the group exposed to 0.13-0.33 mgPb/L increased approximately 2.7-5.3% (on day 12th of the exposure time) and 5.0-8.7% (on day 20th of the exposure time) compared to the beginning of the experiment.

The weight of the control fish was increased about 12.3% after 10th day in the recovery phase, meanwhile, the weight of As treatment fish was not different which was increased less than 1.9%. The weight of Cd and Pb treatment fish increased from 1.9 to 4.7% and from 2.9 to 4.0% compared to the end date of exposure, respectively. These showed that fish exposed to higher As, Cd, Pb concentrations grew slower than fish exposed lower than metal level and the control fish.

**Effect of As, Cd, Pb Concentration in the Water on the Accumulation of its in *Oreochromis* sp (Exposure Time)**

Heavy metal was bound to metallothioneins ligands, metallochaperones ligands, or metal-binding proteins which were intracellular ligands to enter the cells. Besides, metal can enter the cells by efflux of metal through the basolateral membranes. The complexes of heavy metal and hydrophobic ligands can pass the cell membrane of fish by diffusion across the plasma membrane.\(^9,10\) Table-2 demonstrates that the scale Metal\(_{fish\ organisms}\)/Metal\(_{H2O}\) was opposite the level of As, Cd, Pb in treatment water. It could be due to fish being found lacking to realize poison in the case of low exposed metals, cause of metals passes in the cell through membrane protein carry and by diffusion across the plasma membrane.\(^11,12\)

In the case of fish living in the high metals level water, they were realized poison, so that metals pass to the cell mainly by diffusion across the plasma membrane cell. In spite of As, Cd, Pb level in *Oreochromis* sp. fish, which was the group polluted to a high level of As, Cd, Pb in water, were higher than that heavy metal level in fish from the group polluted to low level of As, Cd, Pb in water. Nonetheless, the increases in As, Cd, Pb levels in fish organs were not significant in comparison with the increases of As, Cd, Pb levels in the water. Because of that, the scale of Metal\(_{fish\ organs}\)/Metal\(_{H2O}\) decreased as increasing of As, Cd, Pb levels in the polluted water.

**Table-2: The Ratio of Metal\(_{fish\ organs}\)/Metal\(_{H2O}\) at day 20th of Exposure Time**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Gill</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0As</td>
<td>0.89</td>
<td>1.15</td>
<td>2.27</td>
</tr>
<tr>
<td>1.5As</td>
<td>0.74</td>
<td>0.95</td>
<td>1.75</td>
</tr>
<tr>
<td>3.0As</td>
<td>0.43</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>0.66Cd</td>
<td>1.24</td>
<td>2.79</td>
<td>0.44</td>
</tr>
<tr>
<td>1.00Cd</td>
<td>0.97</td>
<td>2.06</td>
<td>0.32</td>
</tr>
<tr>
<td>2.00Cd</td>
<td>0.72</td>
<td>1.27</td>
<td>0.20</td>
</tr>
<tr>
<td>0.12Pb</td>
<td>71.93</td>
<td>32.30</td>
<td>3.60</td>
</tr>
<tr>
<td>0.18Pb</td>
<td>49.62</td>
<td>30.96</td>
<td>5.00</td>
</tr>
<tr>
<td>0.33Pb</td>
<td>27.37</td>
<td>18.14</td>
<td>2.87</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD (n=3)
The Contaminated Fish Body Weight Changes During Recovery Time

After 20 days of exposure time, the contaminated fish were washed in distilled water and then were transferred to clean water to start recovery time. Looking over the growth of the control fish demonstrated that the fish were healthy, they ate ordinarily while the treatment fish were not healthy and slowly grew in depuration period. In the As, Cd, Pb treatment groups, the weight of fish between day 20th of exposure and day 10th were less different (Table-3). After 10th day lived in freshwater, the weight of As, Cd, Pb treatment fish increased less than 5% while the weight of the control fish increased significantly about 12.3%. The control fish and the lower As, Cd, Pb level treatment fish grew faster than the higher As, Cd, Pb level treatment fish. This shows that the growth of test fish was dose-dependent.

Table-3: Bodyweight Changes between day 20th of Exposure and Day 10th of Recovery Time

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Exposure Phasea</th>
<th>Recovery Phasea</th>
<th>% Fish Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 20th</td>
<td>Day 10th</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>358 ± 5</td>
<td>402 ± 8</td>
<td>12.3</td>
</tr>
<tr>
<td>1.0As</td>
<td>317 ± 4</td>
<td>323 ± 3</td>
<td>1.9</td>
</tr>
<tr>
<td>1.5As</td>
<td>313 ± 3</td>
<td>317 ± 4</td>
<td>1.3</td>
</tr>
<tr>
<td>3.0As</td>
<td>308 ± 4</td>
<td>311 ± 2</td>
<td>1.0</td>
</tr>
<tr>
<td>0.12Pb</td>
<td>326 ± 5</td>
<td>334 ± 5</td>
<td>4.0</td>
</tr>
<tr>
<td>0.18Pb</td>
<td>318 ± 3</td>
<td>322 ± 3</td>
<td>3.1</td>
</tr>
<tr>
<td>0.33Pb</td>
<td>315 ± 3</td>
<td>315 ± 3</td>
<td>2.9</td>
</tr>
<tr>
<td>0.66Cd</td>
<td>319 ± 2</td>
<td>339 ± 6</td>
<td>4.7</td>
</tr>
<tr>
<td>1.00Cd</td>
<td>312 ± 4</td>
<td>328 ± 4</td>
<td>3.2</td>
</tr>
<tr>
<td>2.00Cd</td>
<td>309 ± 4</td>
<td>324 ± 5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

aMean ± SD (n=3)

The Elimination of accumulated As, Cd, Pb in Fish during Recovery Time

Level of As, Cd, Pb in gill, liver and muscle of As, Cd, Pb treatment fish at day 10th of depuration period significantly reduced compared to day 20th of exposure time. The decrease of heavy metal level in gill, liver and muscle of treatment fish was higher than the increase of fish weight. These results demonstrated that Oreochromis sp. could excrete heavy metal in fish tissues. Depuration of As, Cd, Pb was possible due to the subsequent excretion of As, Cd, Pb rich residual bodies. Meanwhile, As, Cd, Pb levels in the control fish were not significant differences in depuration period. The excretion of accumulated As, Cd, Pb from gill, liver and muscle of fish was mainly decided by the function of organs. The elimination rate of accumulated As, Cd, Pb from fish organs was presented in Fig.-3.

Fig.-3: The Elimination of accumulated (a) As, (b) Cd, (c) Pb from Fish Organs at Day 10 of Recovery Time

The ability elimination of accumulated As, Cd, and Pb in fish were liver > gill ≈ muscle, gill > muscle > liver, and gill > liver > muscle, respectively. Oreochromis sp had elimination Cd in its tissues better than As and Pb. The Cd concentration in gill and muscle of treatment fish were quickly decreased from 33.7% to 54.3% and from 27.8% to 48.8%, respectively. Likely expected to a liver role in the excretion of cadmium element from the body, therefore the ability Cd elimination from the liver was slightly slower (26.4 - 34.3%).13-19

CONCLUSION

There was strong evidence for a correlation between As, Cd, Pb concentrations in Oreochromis sp. organs and those in the water. This study demonstrated that As, Cd, Pb in water inhibited Oreochromis sp. growth. The liver and gill are target organ for Cd and Pb accumulation, respectively; in the case of As exposure, a target organ that accumulates As are muscle and liver; those implies that they are also the critical organs for toxic symptoms. A quick decrease of accumulated As, Cd, Pb in fish organs during recovery time was observed.
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