Effect of Hardness on Acute Toxicity of Copper to Tropical Freshwater Biota in Mekong River Water, Laos

Naksayfong Khounnavongsa¹, Chuleemas Boonthai Iwai¹²*, Tham Hoang³

ABSTRACT: Increasing of industrial activities along Mekong River has intensified environmental pollution problems and the deterioration of several ecosystems with the accumulation of many pollutants such as toxic metals. A potentially significant metal contaminant in the Mekong River is copper and its effects to aquatic species. The ecotoxicology of copper on freshwater organisms were studied using field-collected water from two sites of Mekong River in Laos. The study compared the effects of water hardnesses (20±2.8 and 108±0.0 mg/L as CaCO₃). In this study, an US EPA method was used for acute toxicity testing to a juvenile fish Cyprinidae (Labeo rohita) and a Zooplankton Moina macrocopa at different water hardnesses. The acute toxicity of the organism responses to copper at the different water hardnesses of Mekong River water showed that higher water hardness can reduce the toxic effect of copper. Moreover, mortalities were observed at 24, 48, 72 and 96 hr for L. rohita and 24 to 48 hr for M. macrocopa. The results of LC₅₀ with 95% confidence interval for each water hardnesses (20±2.8 and 108±0.0 mg/L as CaCO₃) were 0.038(0.021-0.077) and 0.106 (+) mg/L for L. rohita and 0.004(0.002-0.008) and 0.012(0.009-0.015) mg/L for M. macrocopa, respectively. This study showed that the order of effect of copper toxicity of the Mekong River water with M. macrocopa>L.rohita. It was concluded that the effects of copper to organism was dependent on the water hardness level due to increasing water hardness reduce the toxic effect of copper upon aquatic organisms.

Keywords: Acute toxicity, Copper, Labeo rohita, Moina macrocopa, Lower Mekong Basin

Introduction

The Mekong River is one of the world’s great river, it is the 12th longest river in terms of volume of water and runs for approximately 4,350 km (MRC, 2005, 2011; Shaochuang, Pingli, Donghui and Peidong, 2007), which flows through 6 countries including China, Myanmar/Burma, Laos, Thailand, Cambodia and Vietnam before it flows into the South China Sea. About 60 million people which rely on some extent on the water resources of the Lower Mekong River Basin for food supply and sustainability of their livelihoods due to the fact that resources have the potential to contribute to the economic development of the countries (MRC, 2008, 2010b), the economic development have increased dramatically and have led to dramatic water pollution in the Mekong River.

Heavy metals are a worldwide problem as these metals are indestructible and have toxic effects on living organisms when they exceed a certain concentration limit (Alkarkhi et al., 2009), these metals, which find many useful applications in our life, are very harmful if they are discharged into natural water resources, and may pose finally a serious health hazard. Heavy metals have

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high mobility in aquatic systems and in general may produce high toxicity (Zouboulis et al., 2004). Due to non-biodegradability, metal ions accumulate and their amounts are increased along the food chain. Cu is one of heavy metals which are widely used in various industries and considered seriously as common water pollutant which is often present in industrial wastewaters are hazardous to the aquatic ecosystem, low Cu concentration is essential for aquatic animals. However, at high level of Cu concentration it becomes toxic for animals that live in the environment. All aquatic organisms are directly or indirectly affected by the physical characteristics of their environment, especially the chemical composition of the water (Gillis et al., 2008). Many researchers have been found that water quality parameters may influence on toxicity value. Increasing water hardness reduces the toxic effect of metals in aquatic organisms which have been a subject of many investigations (Kim et al., 2001; Promelas, 2002; Rathore et al., 2003; Martins et al., 2004; Markich et al., 2006) and also the relationship between water hardness and the acute toxicity of Cu in freshwater is well documented. Although many researches have been conducted to toxicity of heavy metals in aquatic organisms, however, the numbers of studies related to the toxic effect of Cu at water hardness on tropical aquatic organisms are limited. Due to the ecotoxicology data in Laos is not available. Therefore, ecotoxicology of copper were investigated on local fresh water organisms at site along Lower Mekong Basin in Laos, toxicity testing for copper will contribute the ecotoxicology data to manage the aquatic environment for revise environmental quality standard in Laos.

### Materials and methods

#### Study Area and Water Sampling

Two different locations were conducted based on different water hardness along the Mekong River parts of Laos (Figure 1), from Vientiane Capital City (17°58’1.18”N/ 102°35’1.66”E) and Pakxan district (Bolikhamxai province) (18°22’23.29”N/ 103°39’43.85”E). Those sites were chosen due to proximity with activities that potentially contribute to pollution of the Mekong River.

Sampling water containers were cleaned by 10% HNO₃ (Suprapur, Merk) and rinsed several times with deionized water prior to use. The container was held in the river at within 100 to 150 m from landing beaches and about 20 to 30 cm below surface water. Water samplings were measured water quality parameters such as pH, EC, TDS and DO by Multiparameter Meter (HANNA HI 9828) and standard EDTA titrant (hardness and alkalinity) were conducted and recorded on the field. Moreover, BOD, DOC, COD by following the US EPA standard method.

#### Experimental Organisms

Tropical freshwater organisms, a juvenile fish Cyprinidae (Labeo rohita) and a Zooplankton Moina macrocopa used in this investigation were obtained from the hatchery maintained by Khon Kaen Department of Fisheries, Thailand.

Juvenile L. rohita was acclimated with tap water by a portable pump for a period of 48 h at 25°C±1°C and are often more sensitive to toxicants than adults and appear healthy, behave normally, and have low mortality in cultures. Therefore, Juvenile L. rohita 1-14 day (s) of age were used for acute toxicity testing.
M. macrocopa was cultured in outdoor circular concrete tanks 100 cm and 30 cm depth. M. macrocopa were chosen second step for acute toxicity testing, due to have a short life cycle.

Experimental Designs

Factorial experiment in Completely Randomized Design (CRD); seven treatments and four replicates, and juvenile organisms often more sensitive to toxicants than adults and appear healthy, behave normally, and low mortality in cultures. Therefore, juvenile L. rohita 1-14 day(s) of age were used for acute toxicity testing. M. macrocopa were chosen the first or second step for acute toxicity testing, due to they have a short life cycle. At the beginning of bioassay, 25°C±1°C at a temperature control room with 16 hr light, 8 hr darkness photoperiod. One organism at a time was randomly selected in aquaria until each of seven treatments and four replicates held 20 fishes at 200 mL and 20 M. macrocopa at 25 mL of test solution volume. The endpoint was the number of dead test organisms at various concentrations was observed at 24 to 48 h for M. macrocopa and at 24, 48, 72 and 96 h for juvenile L. rohita. The death test organisms were determined (EPA, 2002).

Stock Copper Solution

Stock solution of copper (II) sulphate (CuSO$_4$·5H$_2$O) was prepared by dissolve 3.929 g (CuSO$_4$·5H$_2$O) in 1000 mL deionized water. This solution contains 1000 mg/L stock Cu. And then standard Cu solution was diluted 100 mg/L with deionized water for acute toxicity test.

Acute Toxicity Test

Acute toxicity of Cu was conducted on juvenile L. rohita and M. macrocopa at different water hardnesses (20±2.8 and 108±0.0 mg/L as CaCO$_3$), all experiment were performed according to the US EPA procedure for the static no-renewal technique. Therefore, juvenile L. rohita with seven difference Cu concentrations were (0, 0.02, 0.03, 0.05, 0.09 and 0.13 mg/L) at water hardness (20±2.8 mg/L as CaCO$_3$) and (0, 0.02, 0.09, 0.16, 0.23, 0.30 and 0.37 mg/L) at water hardness (108±0.0 mg/L as CaCO$_3$) which were observed at 24, 48, 72 and 96 h. Moreover, M. macrocopa was observed at 24 and 48 hr with seven difference Cu concentrations were (0, 0.001, 0.004, 0.007, 0.01, 0.04 and 0.07 mg/L) at water hardness (20±2.8 mg/L as CaCO$_3$) and (0, 0.007, 0.01, 0.04, 0.07, 0.10 and 0.13 mg/L) at water hardness (108±0.0 mg/L as CaCO$_3$). During experiment, the amount mortality in each container was recorded and water quality purposes DO, pH, EC and temperature were monitored at the beginning and end of the testing.

Statistical Analysis

Mortality data from acute toxicity test on juvenile L. rohita and M. macrocopa at different water hardness of Mekong River were used in determining LC$_{50}$ values and 96% confidence intervals by PROBIT analyzed with SPSS Statistics Version 20 software. In addition, Statistix 8 Analytical software was analyzed factor analysis of variance (ANOVA) was also employed to determine the significant differences of the experiment.

Results

This study was designed to assess the effects of true water hardness (20±2.8 and 108±0.0 mg/L as CaCO$_3$) on the toxicity of Cu to juvenile L. rohita and M. macrocopa. The main chemical
Characteristics and water quality parameters of two field collected samples are given in Table 1. In general, the waters are considered as contaminated by heavy metal concentration (e.g., Mg, Ca, Cr, Pb, Cd, Zn, Mn, Fe and Cu) were detection limit and found that heavy metals are not higher than permissible limits that recommended by National Environmental Standards of (WREA, 2010). Acute toxicity of Cu to Juvenile L. rohita. Cu toxicity decreased with increasing water hardness. At water hardness 20±2.8 mg/L as CaCO₃, the percentage mortality of L. rohita was 100% at 96 hr by 0.13 mg/L Cu (Table 3) had highly significant difference (P<0.001), while percentage mortality was 100% at 96 hr of 108±0.0 mg/L as CaCO₃ at Cu 0.37 mg/L, that has highly significant difference (P<0.001) as well. LC₅₀ values 95% confidence interval, indicating that toxic effect of Cu to by L. rohita, a 3-fold increase in water hardness. At 20±2.8 mg/L as CaCO₃ hardness, the percentage mortality of M. macrocopa was 100% at 48 hr by Cu 0.07 mg/L had highly significant difference (P<0.001), while percentage mortality was 100% at 48 hr of 108±0.0 mg/L as CaCO₃ at Cu 0.13 mg/L, that has highly significant difference (P<0.001) as well. A 3-fold increase in water hardness (from 20±2.8 to 108±0.0 mg/L as CaCO₃) did significantly (P<0.05).

Table 1  Physical and chemical characteristics of water quality of two field collected samples along the Mekong River parts of Laos. n=3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vientiane Capital City</th>
<th>Pakxan district</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.88±0.08</td>
<td>6.79±0.05</td>
</tr>
<tr>
<td>EC (µs/cm)</td>
<td>242±0.00</td>
<td>67±0.00</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>121±0.01</td>
<td>34±0.00</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>7±0.21</td>
<td>7.03±0.34</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>0.98±0.04</td>
<td>0.38±0.08</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>1.20±0.01</td>
<td>0.81±0.34</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>1.89±2.0</td>
<td>3.34±6.9</td>
</tr>
<tr>
<td>Hardness (mg/L as CaCO₃)</td>
<td>108±0.0</td>
<td>20±2.83</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO₃)</td>
<td>100±4.90</td>
<td>34±2.19</td>
</tr>
<tr>
<td>Magnesium (mg/L)</td>
<td>168.64±4.6</td>
<td>10.12±9.82</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td>724.18±2.62</td>
<td>53.35±1.96</td>
</tr>
</tbody>
</table>

Table 2  Acute toxicity endpoints (LC₅₀) calculated for Juvenile L. rohita exposed to Cu (mg/L) at two hardness levels.

<table>
<thead>
<tr>
<th>Hour</th>
<th>LC₅₀ (mg/L) 95% Confidence Limits for conc.</th>
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<tbody>
<tr>
<td></td>
<td>20±2.83 (mg/L as CaCO₃)</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>24</td>
<td>0.083</td>
</tr>
<tr>
<td>48</td>
<td>0.065</td>
</tr>
<tr>
<td>72</td>
<td>0.055</td>
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</table>
Discussion

Influence of hardness on Cu toxicity.

Acute toxicity tests were performed with Juvenile *L. rohita* and *M. macrocopa* to determine the LC$_{50}$ and lethal concentrations of Cu to water hardness. During the study period it was observed that test organisms showed more sensitivity toward Cu at water hardness (20±2.83 mg/L as CaCO$_3$ than 108±0.00 mg/L as CaCO$_3$).

Comparative LC$_{50}$ at water hardnesses 20±2.8 and 108±0.0 mg/L as CaCO$_3$ found a 3-fold increase toxic effect of Cu to juvenile *L. rohita* significantly (P<0.05).

A 3-fold increase in water hardnesses (from 20±2.8 to 108±0.0 mg/L as CaCO$_3$) did significantly (P<0.05) toxic effect of Cu to *M. macrocopa* that overlapping 95% confidence intervals of LC$_{50}$ values.

Conclusion

As the data presented here shown acute toxicity of Cu to juvenile *L. rohita* and *M. macrocopa* at different water hardnesses which are the most sensitive parameters for the evaluation of acute toxicity test effect. The results of LC$_{50}$ with 95% confidence interval for each water hardnesses (20±2.8 and 108±0.0 mg/L as CaCO$_3$) were 0.038 (0.021-0.077) and 0.106 (-) mg/L for *L. rohita* and 0.004 (0.002-0.008) and 0.012(0.009-0.015) mg/L for *M. macrocopa*, respectively. This study showed that the order of effect of copper toxicity of the Mekong River water with *M. macrocopa>*L.rohita*. It was concluded that the effects of copper to organism was dependent on the water hardness level due to increasing water hardness reduce the toxic effect of copper upon aquatic organisms.

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References


