Research article

Ecotoxicology of Copper on Freshwater Fish with Different Water Hardness on the Mekong River, Lao PDR

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Abstract The aquatic resources of the Mekong River are important to support the livelihoods of a large percentage of 60 million or more people living in the Lower Mekong Basin. A study on the impact of heavy metals on tropical freshwater fish in the Mekong River is needed. Ecotoxicology of copper on freshwater fish was studied using field-collected water from two local sites along the Lower Mekong Basin in Lao PDR, which focused on two different water hardness. In this study, US EPA method was used for the acute toxicity test to larvae *Labeo rohita* with 7 different copper concentrations (0, 0.02, 0.09, 0.16, 0.23, 0.3, and 0.37 mg/L) in moderately hardness of water (108±0.00 mg/L as $CaCO_3$) and (0, 0.02, 0.03, 0.04, 0.05, 0.09, and 0.13 mg/L) in soft hardness of water (20±2.83 mg/L as $CaCO_3$). The mortalities of fish were observed at 24, 48, 72, and 96 hr. The results clearly showed that amount of mortalities were increased from low to high copper concentrations. The 96-hr LC_{50} values of copper on larvae *L. rohita* with moderately hardness and soft hardness of water were 0.106 and 0.038 mg/L, respectively.

Keywords acute toxicity, copper, *Labeo rohita*, the lower Mekong basin

INTRODUCTION

The Mekong River is the 12th longest river in the world in terms of volume of water. The river runs for approximately 4,350 km from China through five countries, namely Myanmar, Lao PDR, Thailand, Cambodia, and Viet Nam. The millions of people living in different parts of the Lower Mekong Basin rely on some extent on the water resources of the Mekong River Basin for food supply and sustainability of their livelihoods (MRC, 2010a), due to the fact that the resources have potential to contribute to economic development of the countries. If not properly planned, managed, and monitored, it could also exert tremendous pressure on the basin's ecological health, livelihoods, and water quality.

Copper in the aquatic environment is usually related to anthropogenic sources rather than natural sources (McNeely et al., 1979). Industrial sources of copper include mining, electroplating, petroleum refining, metal works, and foundries. Copper is a micronutrient for both plants and animals at low concentrations and is recognized as all plants and animals (Kapustka et al., 2004). Concentrations of copper have been reported from 0.03 to 0.23 µg/L in surface seawaters and from 0.20 to 30 µg/L in freshwater systems (Bowen, 1985). However, it may become toxic to some forms of aquatic life at elevated concentrations.

Water hardness is one of the water qualities, usually expressed as mg/L $CaCO_3$. It substantially affects metal toxicity but seemingly has little effect on the toxicity of organic chemicals (Sprague, 1985; Mayer, 1988; Inglis, 1972). The relation of water hardness to metal toxicity concerning the lethal tolerance of freshwater fish to copper, is mainly dependent to the hardness of the water; as the increasing hardness reduces the uptake rate of copper by gill tissue. One hypothesis suggested that calcium-magnesium hardness may act intrinsically upon cell-membrane permeability at the gills (Spear and Pierce, 1979). Therefore, the objective of this study was carried out on the tropical fish species Cyprinidae, *Labeo rohita* to evaluate acute toxicity of copper with different water hardness to contribute the ecotoxicology data for the management of aquatic environment towards environmental quality standard revision in Lao PDR.

METERIALS AND METHOD

Location and sampling: The study sites were conducted at two different locations along the Lower Mekong Basin in Lao PDR by focusing on different water hardness. The first study site was Vientiane, the capital city (Lao PDR), located at 17°58′1.18′′N/ 102°35′1.66′′E. The city is around 692,900 inhabitants with a density of 176 people per km^2 (ADB, 2000). Municipal sewage is normally discharged into That Luang wetland and is consequently discharged into the Mekong River downstream. The second study site was Pakxan district, located at 18°22′23.29′′N /103°39′43.85′′E. Sources of pollution of this district included domestic, industrial waste, agricultural runoff, and mining activities. The sites were chosen based on proximity and activities that potentially polluted the Mekong River.

Reagents: All laboratory glassware, polyethylene, and polypropylene were soaked in 10% HNO3 acid for at least 48 hr and rinsed with distilled water more than 3 times prior using. Deionized (DI) water from a Millipore Milli-QTM ultra-pure (<18.2 MΩ/cm) water system was used throughout the study. Water hardness was measured by EDTA titration method. A standard solution of copper 1000 mg/L (Spectrosol grade, Merk) was prepared and diluted 100 mg/L with DI water for acute toxicity test.

Test specimen: Tropical freshwater fish, juvenile Cyprinidae *Labeo rohita* used in this investigation were obtained from the hatchery maintained by Khon Kaen Department of Fisheries, Thailand. The test specimen was conducted at Ecotoxicology Laboratory in Khon Kaen University. Juvenile *L. rohita* were acclimated with tap water by a portable pump for a period of 48 hr at $25^0C\pm1^0C$ and arewere more sensitive to toxicants than adults, appeared healthy, behaved normally, fed well, and had low mortality in cultures12a. Therefore, juvenile *L. rohita* 1-14 day(s) of age were used for acute toxicity test. At the beginning of bioassay, 25ºC±1ºC at a temperature control room with 16-hr light, 8-hr darkness photoperiod. Moreover, copper concentrations for each test chambers were determined based on the factorial experiment in Completely Randomized Design (CRD). One fish was randomly selected at a time from the aquaria to complete the experiment of 7 treatments and 4 replications of 20 fish at 200 mL of test solution volume.

Data analysis: The acute toxicity of copper was determined as the median lethal concentration (LC50) for 96 hr in each exposure time, with 95% confidence intervals. The analysis used the Probit Analysis function of SPSS Version 17, statistical software.

RESULTS AND DISCUSION

Percent mortality of larvae *Labeo rohita* was evaluated at soft hardness and moderately hardness of water (20±2.83 and 108±0.00 mg/L as $CaCO_3$). After being exposed in different periods of time, copper concentrations are resulted in highly significant difference ($P<0.01$), as presented in (Tables 1 and 2). The LC_{50} values of copper to larvae *L. rohita* at 24, 48, 72 and 96-hr for 20±2.83 mg/L as $CaCO_3$ were 0.083 (0.069-0.106), 0.065 (0.06-0.071), 0.055 (0.045-0.069), and 0.038 (0.021-0.077) mg/L; and for 108±0.00 mg/L as $CaCO_3$ were 0.271, 0.157, 0.129, and 0.106 mg/L, respectively. The result of LC_{50} values with 95% confidence limits of copper are summarized in (Table 3) which clearly indicates that at 20±2.83 mg/L as $CaCO_3$ soft hardness of water was more toxic than 108±0.00 mg/L as $CaCO_3$ moderate hardness to larvae *L. rohita*.

Table 1 Percent mortality of larvae *Labeo rohita* at 20±2.83 mg/L as $CaCO_3$ soft hardness at exposure time with different copper concentrations

Exposure concentration, (mg/L Cu)	% mortality on exposure time			
	24hr	48hr	72hr	96hr
0	$0±0^{f}$	$0±0^{e}$	$4±3^{e}$	$10±4^{e}$
0.02	$1±3^{ef}$	$3±3^{e}$	$10±6^{e}$	$18±10^{de}$
0.03	$6±3^{df}$	$14±3^{d}$	$20±4^{d}$	$24±9^{d}$
0.04	$11±5^{cd}$	$21±3^{c}$	$24±3^{d}$	$29±5^{d}$
0.05	$14±8^{c}$	$25±4^{c}$	$39±6^{c}$	$54±10^{c}$
0.09	$46±3^{b}$	$69±5^{b}$	$75±9^{b}$	$80±9^{b}$
0.13	$86±5^{a}$	$91±3^{a}$	$98±3^{a}$	$100±0^{a}$
F-test	***	***	***	***
C.V. %	17.63	9.71	13.7	17.08

Note: Values are mean ± SD (n=4). Difference litters indicate a significant difference (p<0.01) by one-way ANOVA analysis with lsd comparison

Table 2 Percent mortality of larvae *Labeo rohita* at 108±0.00 mg/L as $CaCO_3$ moderately hardness at exposure time with different copper concentrations

Exposure concentration, (mg/L Cu)	% mortality on exposure time			
	24hr	48hr	72hr	96hr
0	$3±3^{d}$	$4±5^{d}$	$6±6^{e}$	$9±9^{e}$
0.02	$4±8^{d}$	$10±11^{cd}$	$15±14^{de}$	$20±17^{de}$
0.09	$5±0^{d}$	$14±3^{c}$	$21±3^{cd}$	$30±0^{cd}$
0.16	$13±3^{d}$	$19±3^{c}$	$26±3^{c}$	$34±6^{c}$
0.23	$40±8^{c}$	$63±9^{b}$	$66±9^{b}$	$68±9^{b}$
0.3	$59±10^{b}$	$95±4^{a}$	$98±3^{a}$	$99±3^{a}$
0.37	$76±9^{a}$	$96±5^{a}$	$99±3^{a}$	$100±0^{a}$
F-test	***	***	***	***
C.V. %	24.38	14.4	14.72	16.11

Note: Values are mean ± SD (n=4). Difference litters indicate a significant difference (p<0.01) by one-way ANOVA analysis with lsd comparison

Acute toxicity of copper in soft water hardness with copper concentration 0.13 mg/L, the mortality at 96 hours of exposure was 100%. On the other hand, 100% mortality at 96 hours in moderately water hardness was observed at copper concentration 0.37 mg/L, due to the toxic effect of heavy metals on freshwater organisms related to hardness (Kim et al., 2001; Plye et al., 2002; Rathor and Khangarot, 2003; Markich et al., 2006).

Comparing the effect of copper at two different water hardness on larvae *L. rohita*, it was observed that copper at the soft water had high toxic to the fish with LC_{50} value of 0.038 (0.021-0.077) mg/L, whereas copper at the moderately hardness of water was less toxic with LC_{50} value of 0.106 mg/L. It could be seen that the tropical freshwater fish was potentially sensitive to toxic elements.

Fish can have a quick response to various copper concentrations in the form of increased surface movements were observed in the first few hours of the experiment, which normalized within 24hr of exposure, thereafter fish settled on the bottom of the chamber. The response of fish mortality with exposure time is shown in Fig. 1. In freshwater, as water hardness increased, heavy metal toxicity decreased due to competition between metal ions and calcium and magnesium ions for the uptake sites of organisms (Javid et al., 2007; Kim et al., 2001; Pyle et al., 2002; Ebrahimpour et al., 2010). According to Penttinen et al., (1998), the uptake of calcium and magnesium ions by the cell membrane caused it to stabilize, and this reduced its permeability to metal ions. Water hardness reduced metal toxicity by saturating gill surface binding sites with Ca^{2+} to the exclusion of metal cations (Pyle et al., 2002).

Table 3 Lethal concentration (LC50 to 95% confidence limits) of copper to larvae *Labeo rohita* at exposure time

	LC50 (Medium Lethal Concentration) mg/L 95% Confidence Limits for conc.			
	24hr	48hr	72hr	96hr
20±2.83 mg/L as $CaCO_3$ soft hardness	0.083 (0.069-0.106)	0.065 (0.06-0.071)	0.055 (0.045-0.069)	0.038 (0.021-0.077)
108±0.00 mg/L as $CaCO_3$ moderately hardness	0.271 -	0.157 -	0.129 -	0.106 -

Note: (-) =95% Confidence limit (lower-upper value exposure time

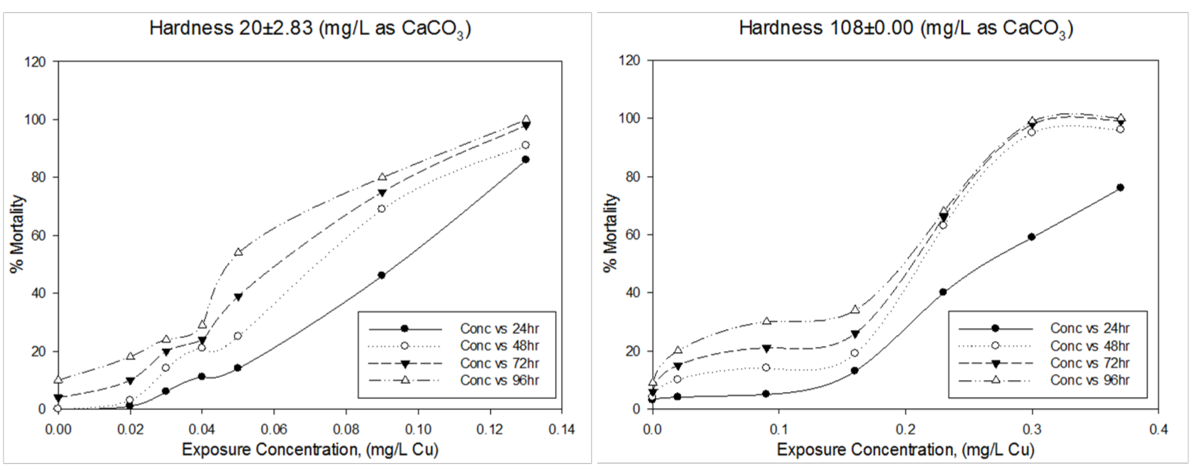

Fig. 1 Relation of percent mortality of larvae *Labeo rohita* to exposure copper concentrations with 2 different water hardness.

CONCLUSION

The toxicity of copper on larvae *Labeo rohita* under low water hardness was higher than in high water hardness. The results of this toxicity tests for copper contribute to ecotoxicology data to the management of aquatic environment towards environmental quality standard revision in Lao PDR.

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