Research article

Influence of Water Hardness on Ecotoxicology of Copper on Aquatic Biota: Implication for the Revision of Water Quality Standardization in Lao PDR.

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Abstract The ecotoxicology of copper on freshwater organisms were studied using field-collected water from two local sites along Lower Mekong Basin in Lao PDR, which focused on different water hardness 20±2.83 and 108±0.00 mg L^{-1} as $CaCO_3$ (water in rainy season), 105±3.35 and 140±4.00 mg L^{-1} as $CaCO_3$ (water in dry season) and distilled water was reconstituted by adding reagent-grade chemicals which reconstituted water hardness 18±3.58 and 86±4.56 mg L^{-1} as $CaCO_3$. In this study, the US EPA method was used for the acute toxicity test to juvenile *Cyprinidae (Labeo rohita)* and *Moina macrocopa* at different water hardness. Mortalities were at 24, 48, 72 and 96 hr for *L. rohita* and 48 hr for *M. macrocopa*. At water hardness of 20±2.83 mg L^{-1} as $CaCO_3$, the percentage mortality of *L. rohita* was 100% at 96 hr by Cu 0.13 mg L^{-1}. The mortality was 100% at 96 hr of water hardness 108±0.00 mg/L as $CaCO_3$ at Cu 0.37 mg L^{-1}. There was a 3-fold increase in water hardness (from 20±2.83 and 108±0.00 mg L^{-1} as $CaCO_3$) and Cu was highly significantly toxic to *L. rohita*. The percentage mortality of *L. rohita* was 100% at 96 hr by Cu 0.37 mg L^{-1}, on 105±3.35 and 140±4.00 mg L^{-1} as $CaCO_3$, and both were highly significant difference ($P < 0.001$). A 8-fold increase in water hardness (from 105±3.35 and 140±4.00 mg/L as $CaCO_3$) and Cu was significantlytoxic to *L. rohita*. At 20±2.83 mg L^{-1} as $CaCO_3$ hardness, the percentage mortality of *M. macrocopa* of 100% was very highly significant at 48 hr by Cu 0.07 mg L^{-1}, while percentage mortality was 100% at 48 hr of water hardness 108±0.00 mg L^{-1} as $CaCO_3$ at Cu 0.13 mg L^{-1}, the difference was highly significant as well. A 3-fold increase in water hardness (from 20±2.83 to 108±0.00 mg L^{-1} as $CaCO_3$) had significant toxic effect of Cu to *M. macrocopa* with overlapping 95% confidence intervals of LC_{50} values. The percentage mortality of *M. macrocopa* of 100% at 48 hr by Cu 0.13 mg L^{-1}, on water hardness of 105±3.35 and 140±4.00 m L^{-1} as $CaCO_3$, were both highly significant. A 2-fold increase in water hardness (from 105±3.35 and 140±4.00 mg L^{-1} as $CaCO_3$) did not significantly ($P > 0.05$) have toxic effect of Cu to *M. macrocopa*. The effects of Cu on organisms depend on water hardness, i.e., increasing water hardness reduce the toxic effect of Cu on aquatic organism.

Keywords Water hardness, aquatic biota, copper

INTRODUCTION

The Mekong Basin supports about 60 million people, most of whom depend directly on the natural resource for their livelihood (MRC, 2010; Snidvongs & Teng, 2006). Moreover, activities that discharge heavy metals directly or indirectly into natural water systems with little or no treatment may cause serious environmental effects. Heavy metals are widely used in industries and are common water pollutants; thus knowledge of their toxicity to aquatic organisms are significance (Rathore & Khangarot, 2003).

Cu is a significance trace element to organisms, its often found in industrial wastewaters which become extremely toxic for aquatic animals as their concentration in water increase. The hardness of water is a major factor which influences the toxic effects of Cu to aquatic organisms because increase in water hardness can decrease Cu toxicity due to competition between metal ions and Ca^{2+} and Mg^{2+} ions for uptake sites of organisms (Gary, 1996; Yim, Kim, & Kim, 2006).

The levels of Cu and its distribution in natural water bodies in Lower Mekong Basin are not well known. Recent investigations suggest that background levels may be higher than expected. However, data on heavy metal toxicity in Laos is limited. Therefore, research on the ecotoxicology of Cu on freshwater biota of Mekong River in Laos is important.

METHODOLOGY

The study sites were 2 locations at 2 different seasons (rainy and dry seasons) along Lower Mekong Basin in Lao PDR with focus on different water hardness; Vientiane (17°58´1.18´´N/ 102°35´1.66´´E), a city of around 692,900 inhabitants with a density of 176 people km^{-1} (Phonvisai, Coowanitwong, Shapkota, Pradhan, & Hossain, 2006). Vientiane discharges its municipal sewage into ThatLuang wetland that discharges into the Mekong River downstream of the city. The other site is Pakxan District (18°22´23.29´´N /103°39´43.85´´E. The sources of pollution in this site include domestic, industrial waste, agricultural runoff and mining activities. Those sites were chosen due to their proximity and are with activities that potentially contribute to pollution of the Mekong River (Table 1).

Table 1 Sampling sites based on different water hardness along the Mekong River parts in Laos (n=3)

Location and Date		Water hardness (mg L^{-1} as $CaCO_3$)	Latitude (N) and Longitude (E)
Rainy season	Pakxan District (13 July,2014)	20±2.83	(17°58'1.18"N/ 102°35'1.66"E)
Rainy season	Vientiane Capital City (12 July,2014)	108±0.00	(18°22'23.29"N/ 103°39'43.85"E).
Dry season	Pakxan District (22 March,2015)	105±3.35	(17°58'1.18"N/ 102°35'1.66"E)
Dry season	Vientiane Capital City (21 March, 2015)	140±4.00	(18°22'23.29"N/ 103°39'43.85"E).

Table 2 Distilled water reconstituted by adding reagent-grade chemicals to make its characteristics similar to that of the Mekong River water (n=3)

Water quality parameters	Reconstituted water hardness	
	Soft water	Moderate hardness
pH	7.06±0.1	7.73±0.1
EC (µs cm^{-1})	36±0.0	347±0.0
TDS (mg L^{-1})	18±0.0	173±0.0
DO (mg L^{-1})	6.97±1.6	8.66±0.9
Hardness (mg L^{-1} as $CaCO_3$)	18±3.58	86±4.56
Alkalinity (mg L^{-1} as $CaCO_3$)	14±2.2	86±3.6

Distilled water was reconstituted to make it similar water hardness to Mekong River water by adding $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCI that following a USEPA procedure to give reconstituted water hardness as $CaCO_3$ for 18±3.58 mg L^{-1} and 86±4.56 mg L^{-1} (Table 2).

Statistical Analysis

Acute toxicity dose-response data, involving quintal response mortality following toxicity of copper on the test species; Cyprinidae *(Labeo rohita)* and *Moina macrocopa* were determined by using of PROBIT Analysis LC_{50} Determination Method (SPSS Statistics Version 20 software). Mortality response of organisms were recorded when the animals sank to bottom of the containers and become motionless. The toxicity response determined at the end of the 96 hr for *L. rohita* and 48 hr for *M. macrocopa*. Significance in 95% confidence interval (95% CI) of detect 48 and 96 hr LC50 value were determined using Chi-Square technique.

RESULTS AND DISCUSSION

Acute Toxicity of Copper on Local Freshwater Organisms; Cypronidae - *Labeo rohita* and *Moina Macrocopa* under Different Water Hardness of Mekong River in Lao PDR during Rainy Season in 2014

Acute toxicity of Cu to juvenile fish Cyprinidae *(Labeo rohita)* during rainy season with water hardness 20±2.8 mg L^{-1} as $CaCO_3$ was similar to that reported by (Perschbacher & Wurts, 1999) in his study on *Channel Catfish* where they reported 100% mortality in treatments containing magnesium-based 20 and 400 mg L^{-1} as $CaCO_3$. These results demonstrate the sensitivity of the organism to Cu concentrations. However, the present study found that at water hardness of 20±2.8 mg L^{-1} as $CaCO_3$, the mortality of *L. rohita* was 100% at 96 hr with Cu concentration of 0.13 mg L^{-1} (CV < 20%), indicating very high significant difference. A similar result was obtained at 108±0.0 mg L^{-1} as $CaCO_3$ at Cu concentration of 0.37 mg L^{-1}. . Comparative LC_{50} at water hardness 20±2.8 and 108±0.0 mg L^{-1} as $CaCO_3$ resulted to a significant 3-fold increase in the toxicity of Cu to *L. rohita* (Table 3).

Table 3 Acute toxicity endpoints (LC_{50}) calculated for juvenile fish Cyprinidae (*Labeo rohita)* exposed to Cu (mg L^{-1}) into rainy season based on different water hardnesses (20±2.8 and 108±0.0 mg/L as $CaCO_3$)

Rainy season				Rainy season			
LC_{50} (mg/L) 95% Confidence Limits for conc.				LC_{50} (mg/L) 95% Confidence Limits for conc.			
Hour	20±2.8 (mg/L as $CaCO_3$)			Hour	108±0.0 (mg/L as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.083	0.069	0.106	24	0.271	−	−
48	0.065	0.06	0.071	48	0.157	−	−
72	0.055	0.045	0.069	72	0.129	−	−
96	0.038*	0.021	0.077	96	0.106*	−	−

*Note: * = significant at 5% level*

Cu effects to different water hardness 20±2.8 mg L^{-1} as $CaCO_3$, the percentage mortality of *Moina macrocopa* was 100% at 48 hr by Cu concentration 0.07 mg L^{-1}, indicating test acceptability highly significant difference, while percentage mortality was 100% at 48 hr of water hardness 108±0.0 mg L^{-1} as $CaCO_3$ at Cu concentration 0.13 mg L^{-1}, that has highly significant difference as well, a 3-fold increase in water hardness (from 20±2.8 to 108±0.0 mg L^{-1} as $CaCO_3$) was significantly different toxicity effect of Cu to *Moina macrocopa* that overlapping 95% confidence intervals of LC_{50} values (Table 4).

Table 4 Acute toxicity endpoints (LC_{50}) calculated for *Moina macrocopa* exposed to Cu (mg/L) into rainy season based on different water hardnesses (20±2.8 and 108±0.0 mg/L as $CaCO_3$)

Rainy season				Rainy season			
LC_{50} (mg/L) 95% Confidence Limits for conc.				LC_{50} (mg/L) 95% Confidence Limits for conc.			
Hour	20±2.8 (mg/L as $CaCO_3$)			Hour	108±0.0 (mg/L as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.011	-	-	24	0.023	0.011	0.044
48	0.004*	0.002	0.008	48	0.012*	0.009	0.015

*Note: * = significant at 5% level*

Acute Toxicity of Copper on Local Freshwater Organisms; Cyprinidae - *Labeo rohita* and *Moina macrocopa* under Different Water Hardness of Mekong River in Lao PDR during Dry Season in 2015

Cu was toxic to Cyprinidae *(Labeo rohita)* during dry season with water hardnesses as $CaCO_3$ 105±3.34 mg L^{-1} and 140±4.0 mg L^{-1}. Cu toxicity decreases with increasing hardness. Pourkhabbaz et al, (2011) found that *Gambusia holbrooki* is sensitive to Cu toxicity in soft, more than hard water. A 12-fold increase in water hardness (25 to 350 mg L^{-1} as $CaCO_3$) substantially reduced the toxicity of Cu (up to 38-fold). In the present study, LC_{50} for juvenile fish Cyprinidae *(Labeo rohita)* was 0.092 (0-0.048) mg L^{-1} at water hardness of 105±3.4 mg L^{-1} as $CaCO_3$, compared to LC_{50} value 0.155 (0.113-0.188) mg L^{-1} at water hardness of 140±4.0 mg L^{-1} as $CaCO_3$. This is a 2-fold significant increase in toxicity on juvenile fish Cyprinidae *(L. rohita)* (Table 5).

Table 5 Acute toxicity endpoints (LC_{50}) for juvenile fish Cyprinidae *(Labeo rohita)* exposed to Cu (mg/L) during the dry season based on different water hardness (105±3.4 and 140±4.0 mg L^{-1} as $CaCO_3$)

Dry season				Dry season			
LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.				LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.			
Hour	105±3.4 (mg L^{-1} as $CaCO_3$)			Hour	140±4.0 (mg L^{-1} as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.145	0.072	0.449	24	0.248	0.226	0.273
48	0.123	0	0.063	48	0.176	0.145	0.204
72	0.107	0	0.049	72	0.161	0.126	0.19
96	0.092*	0	0.048	96	0.155*	0.113	0.188

*Note: * = significant at 5% level*

Table 6 Acute toxicity endpoints (LC_{50}) calculated for *Moina macrocopa* exposed to Cu (mg L^{-1}) into dry season based at different water hardness (105±3.4 and 140±4.0 mg L^{-1} as $CaCO_3$)

Dry season				Dry season			
LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.				LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.			
Hour	105±3.4 (mg L^{-1} as $CaCO_3$)			Hour	140±4.0 (mg L^{-1} as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.055	0.031	0.121	24	0.143	0.063	5.711
48	0.01*	0.005	0.016	48	0.023*	0.003	0.062

*Note: * = significant at 5% level*

The percentage mortality of *Moina macrocopa* was 100% at 48 hr at Cu concentration 0.13 mg L^{-1}, on 105±3.4 and 140±4.0 mg L^{-1} as $CaCO_3$ hardness. A 2-fold increase in water hardnesses as $CaCO_3$ (from 105±3.4 and 140±4.0 mg/L) did not significantly *(P > 0.05*) affect Cu toxicity to *Moina macrocopa* (Table 6).

Acute Toxicity of Copper on Local Freshwater Organisms; Cyprinidae *(Labeo rohita)* and *Moina macrocopa* on Distilled water with Different Water Hardness

The effects of water hardness as $CaCO_3$ at 18±3.6 mg L^{-1} and 86±4.6 mg L^{-1} on acute toxicity of Cu to on juvenile fish Cyprinidae *(Labeo rohita)* at water hardness 18±3.6 mg L^{-1} as $CaCO_3$ was similar to that reported by Olaifa et al, (2004) who worked on juvenile *C*. *Gariepinus* exposed to various Cu concentrations, e.g., 0.6, 0.71 and 0,70 mg L^{-1} at 10.42 mg L^{-1} as $CaCO_3$. Baldwin et al (2003) studied the influence of water hardness on the inhibitory effects of copper on juvenile coho salmon *(Oncorhynchus kisutch)*. However, in the present study found at 18±3.6 mg L^{-1} as $CaCO_3$ hardness, the percentage mortality of *L*. *rohita* was 100%, it was found that at 96 hr with Cu concentration of 0.02 mg L^{-1}, the percentage mortality was 100% at 96 hr of water hardness of 86±4.6 mg L^{-1} as $CaCO_3$ at Cu concentration 0.16 mg L^{-1}. A 7-fold increase in water hardnesses as $CaCO_3$ 18±3.58 and 86±4.56 mg L^{-1} did not significantly affect Cu toxicity on *L*. *rohita)* (Table 7).

Table 7 Acute toxicity endpoints (LC_{50}) of juvenile fish Cyprinidae *(Labeo rohita)* exposed to Cu (mg L^{-1}) into distilled water hardness (18±3.6 and 86±4.6 mg L^{-1} as $CaCO_3$)

Soft hardness				Moderately water hardness			
LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.				LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.			
Hour	18±3.6 (mg L^{-1} as $CaCO_3$)			Hour	86±4.6 (mg L^{-1} as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.013	0.008	0.087	24	0.105	0.042	0.835
48	0.008	0.006	0.013	48	0.067	0.033	0.121
72	0.006	0.004	0.008	72	0.052	0.028	0.087
96	0.006*	0.004	0.007	96	0.042*	0.017	0.083

*Note: * = non-significant at 5% level*

Table 8 Acute toxicity endpoints (LC_{50}) for *Moina macrocopa* exposed to Cu (mg L^{-1}) at different water hardness (18±3.6 and 86±4.6 mg L^{-1} as $CaCO_3$)

Soft hardness				Moderately water hardness			
LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.				LC_{50} (mg L^{-1}) 95% Confidence Limits for conc.			
Hour	18±3.6 (mg L^{-1} as $CaCO_3$)			Hour	86±4.6 (mg L^{-1} as $CaCO_3$)		
	Estimate	Lower	Upper		Estimate	Lower	Upper
24	0.000797	0.000521	0.00083	24	0.024895	0.004465	0.06329
48	0.000493*	0.000087	0.000965	48	0.013725*	0.004918	0.024119

*Note: * = non-significant at 5% level*

The percentage mortality of *Moina macrocopa* at was 100% at 48 hr by Cu concentration 0.04 mg L^{-1} had highly significant difference *(P< 0.001)*, while percentage mortality was 100% at 48 hr of 86±4.6 mg L^{-1} as $CaCO_3$ at Cu concentration 0.13 mg/L, that has highly significant difference *(P < 0.001)*, a 28-fold increase in water hardness (18±3.6 and 86±4.6 mg L^{-1} as $CaCO_3$) did not significantly *(P > 0.05)* toxicity effects of Cu to *(Moina macrocopa)* that overlapping 95% confidence intervals of LC_{50} values (Table 8).

CONCLUSION

High water hardness could reduce toxic effect of copper on (indicate species tested. As a result of LC_{50} with 95% confidence interval in water hardnesses as $CaCO_3$ (20±2.8 mg L^{-1}, 108±0.0 mg L^{-1}, 105±3.35 mg L^{-1}, and 140±4.00 mg L^{-1} were 0.038 (0.021-0.077) mg L^{-1}, 0.106 mg L^{-1}, 0.092 (0-0.048) mg L^{-1} and 0.155 (0.113-0.188) mg L^{-1} for *L. rohita*. Moreover, *M. macrocopa* were 0.004 (0.002-0.008) mg L^{-1}, 0.012 (0.009-0.015) mg L^{-1}, 0.01 (0.005-0.016) mg L^{-1} and 0.023 (0.003-0.062) mg L^{-1}, respectively.

Distilled water with soft hardness and moderately water hardness were reconstituted water similar as true water of Mekong River (18±3.6 and 86±4.6 mg L^{-1} as $CaCO_3$), the LC_{50} with 95% confidence interval of *L. rohita* were 0.006 (0.004-0.007) mg L^{-1} and 0.042 (0.017-0.083) mg/L. and *M. macrocopa* were 0.000493 (0.000087-0.000965) mg L^{-1} and 0.013 (0.0049-0.0241) mg L^{-1}.

The results of this toxicity tests for copper will contribute to ecotoxicology data to manage the aquatic environment for revise environmental quality standard in Lao PDR.

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