Research article

Influence of Dissolved Organic Carbon on the Ecotoxicology of Copper on Aquatic Biota: Implication for the Revision of Water Quality Standardization in Cambodia

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Abstract The Mekong River is one of the world's greatest river systems and sustains human life and ecosystems. The livelihoods of 60 million people who live along the Lower Mekong Basin (LMB) rely on both the economic resource and the ecological health of the river. In this study, US EPA method was used for the acute toxicity with different water dissolved organic carbon (DOC) of Mekong River in Cambodia on *Chironomus javanus* and fish Nile tilapia (*O. niloticus*) to modify the effecting of DOC on copper toxicity. Both *C. javanus* and Nile tilapia were significantly less sensitive to copper in water high DOC (5.74 mg/L DOC), compared to water low DOC (1.12 mg/L DOC) exposures. The effect of DOC, as humic acid source on the acute toxicity of copper (Cu) to *C. javanus* and Nile tilapia also was investigated. The mortalities for both species increase with increasing copper concentration, but LC_{50} value decreased as more toxic on Nile tilapia and *C. javanus*. This gave an order of toxicity of copper in water with low DOC > water with high DOC at the end point of LC_{50}. DOC might provide protection against Cu toxicity in the freshwater in term of completive between copper form and DOC. The result of the LC_{50} with 95% confidence limit obtained at 48 hr in tap water on *Moina macrocopa*, *C. javanus*, Grass Carp (*Ctenopharyngodon idella*) and Nile tilapia were 12 µg/L, 16399 µg/L, 118 µg/L and 1383 µg/L, respectively. This gave an order of toxicity of copper in tap water with *M. macrocopa*> Grass Carp > Nile tilapia> *C. javanus*. Also, it could be noted that Moina was the most sensitive followed by Grass Carp, Nile tilapia, and *C. javanus* to copper. Present study indicated that water chemistry parameters can influence on copper toxicity to tropical freshwaters biota. Exposures in this series of laboratory experiment will provides a worst-case scenario and useful for determine the risk assessment of copper on Mekong tropical freshwater animals.

Keywords copper, water quality, aquatic biota

INTRODUCTION

The livelihoods of 60 million people who live along the Lower Mekong Basin (LMB) rely on both the economic resource and the ecological health of the river MRC (2015). However, the development activities during the past decade and currently, including mining, industries, agriculture, deforestation and household wastes, have caused of extensive soil erosion and contributed increasingly to transfer of environmental levels of heavy metals especially copper (Cu) into the Mekong River (Ti and Facon, 2004; Coates et al., 2006).

Copper (Cu) is known as the important that all living organisms require its small amounts (5-20 μg/g) to survive Solomon (2009). However, too much Cu concentration more than (20 μg/g) will become toxic Wright and Welbourn (2002) and Bradl (2005)**.** Cu has been documented as one of the most toxic metals to aquatic organism and ecosystem (Bradl, 2005; Carreau and Pyle, 2005; Scudder et al., 1988). Impacts of Cu on an aquatic atmosphere are complex and depend on the physicochemical characteristics of water as mentioned by Kamunde and MacPhail (2011) and Nadella et al. (2009). Therefore, the acute toxicity of Cu to fish, invertebrates and other aquatic organisms are influenced by water quality parameters such as hardness, alkalinity, pH and dissolved organic carbon (DOC) (Linbo et al. 2009; Santore et al., 2001; U.S.EPA, 2002). And the most effective parameter for reducing of Cu toxicity to fish is DOC (Linbo et al., 2009). According to Liu and Sheu (2003) and U.S.EPA (2002) DOC is a vital water quality parameter and it is also a primary food source in the aquatic food web which supports growth of microorganisms and Complex to the metal form.

Many research papers were designed and conducted on ecotoxicology of Cu worldwide, but most of them focused on temperate aquatic species. The information on the impact of toxicity effects of soluble copper on the tropical aquatic biota is limited. So the ecotoxicology of Cu on aquatic biota with Mekong River Cambodia compared with dosed distilled and tap water will be a good representative for tropical aquatic species. In the present study, the ecotoxicology of copper on aquatic biota under different water DOC were investigated in order to help and protect the Mekong River in the future and for implication for the revision of water quality standardization in Cambodia.

OBJECTIVE

The objective of the study is focus on ecotoxicology of copper on aquatic biota under different water dissolved organic carbon.

METHODOLOGY

Water sampling: In this study, water samples were collected at two sites in Cambodia's Mekong River, which focus on different water dissolved organic carbon. The site 1 is located in Stung Treng at 13°30'52.50"N/105°55'54.00"E, next to the Lao PDR border. The site 2 is located in the Kampong Cham at 11°59'18.77"N/105°28'10.26"E, next to the Vietnam border.

Test organisms: The native organisms used in the present study were Moina (*Moina macrocopa*), *Chironomus javanus*, Grass Carp (*Ctenopharyngodon idella*) and Nile tilapia (*Oreochromis niloticus*). These species were provided by the Department of Fisheries, Khon Kaen, Thailand and have been cultured in at ecotoxicology laboratory of Khon Kaen University, Thailand.

Chemical and test procedure: The standard stock solution (100 mg/L) for studied metals was freshly prepared by dissolving of copper sulfate $CuSO_4$ $5H_2O$. The test organisms were subjected to different concentrations of the stock copper solution in each container. The control was kept in experimental water without adding copper.

Preparation of the standard DOC: The concentration DOC was augmented using a commercially available humic acid (Aldrich Humic acid (AHA), Sigma-Aldrich, st, Louis, MO, USA). DOC test water were then diluted to the final concentrations (1 and 5 mg/L DOC) to which the animal were exposed**.**

DOC analysis: The dissolved organic carbon concentration in each sample of the filtered (0.45 μm) exposure water was determined by using a Shimadzu total organic carbon analyzer (model 5050A; Mandel Scientific, Guelph, ON, Canada). The total organic carbon in each sample was calculated automatically by subtracting inorganic carbon from total carbon.

Toxicity test: Acute copper toxicity experiments were performed for a 4-d period (96h) using small fishes at 5 days old, the second instar larva of *Chironomus javanus* and for a 2-day period for Moina

(*Moina macrocopa*) at < 1 day old. The number of dead organisms were counted every 24 hours and removed from aquarium as soon as possible. During the toxicity test, organisms were not fed. The experimental were performed at room temperature of 25±1C°, with a Photoperiod of 16h light: 18h darkness. All control result in lower mortality, less than 10% which revealed the acceptability of the test (U.S.EPA, 2002).

Statistical analysis: Toxicological dose-response data involving quintal response (mortality) following toxicity of copper on the test species were determined by used of Probit Analysis LC50 Determination Method (SPSS, version 19 software). The rate response determined at the end of the 96-h for Grass Carp (*Ctenopharyngodon idella*) and Nile tilapia (*Oreochromis niloticus*), and 48-h for Moina (*Moina macrocopa*) and Chironomids (*Chironomidus javanus*). Significance in 95% confidence interval (95% CI) of detect 48 and 96 hour LC50 value were determined using the Chi-Square technique (Ezeonyejiaku et al., 2011).

RESULTS AND DISCUSSION

Water quality: The water quality parameters measured during the test at site 1 and site 2 were pH 7.77 ± 0.02 and 7.83±0.00, Conductivity 191± 1.53 and 192.33±1.03 µS/cm, TDS 45±005 and 50±0.89 mg/L, dissolve oxygen 10.46±0.05 and 8.23±0.04 mg/L, and total hardness (mg^{2+} and Ca^{2+}) 88±4 and 112±4 mg/L as CaCO3, respectively. The mean value of other water quality parameters such as DOC, BOD and alkalinity were 5.74±0.08 and 1.12±0.26 mg/L, 541.86±7.39 and 542.86±7.39 mg/L, 1.33±0.20 and 0.4±0.17 mg/L and 118.66±4.61and 113.33±2.30 mg/L, respectively. And a summary of measured heavy metal data for all experiments (e.g., Cu, Zn, Mn, Fe, Pb, Cd, Cr, Mg and Ca) were shown in (Table 1).

Table 1 Physical- chemical composition of Mekong River in Cambodia

Physical-chemical variable (units)	Site1	Site2
pH	7.83±0.00	7.77±0.01
Temperature (C°)	27.4±0.26	27.38±0.25
DO (mg/L)	8.23±0.04	10.46±0.05
EC (µS/cm)	192.33±1.03	191.66±1.12
TDS (mg/L)	50±0.89	45.66±0.81
Alkalinity (mg/L as $CaCO_3$)	113.33±2.30	118.66±4.61
Hardness (mg/L as $CaCO_3$)	112±4	98.66±8.32
BOD (mg/L)	0.4±0.17	1.33±0.20
DOC (mg/L)	1.12±0.26	5.74±0.08
Cu (µg/L)	0.005±4.98	0.005±3.38
Zn (µg/L)	0.003±4.72	0.002±0.14
Mn (µg/L)	0.004±0.13	0.007±0.28
Fe (µg/L)	0.033±0.14	0.022±0.38
Pb (µg/L)	0.005±5.60	0.002±2.85
Cd (µg/L)	0.0002±2.24	0.0009±0.11
Cr (µg/L)	0.019±1.40	0.017±0.74
Mg (µg/L)	>5*	>5*
Ca (µg/L)	>5*	>5*

**: The limitation of analytic is not determined we need to make more dilution, Mean (±SE), n=3*

The Effluence of DOC of Mekong River on Copper Sensitivity

A strong relationship between DOC concentration and copper toxicity showed in (Table 2). Both Nile tilapia and Chironomid were significantly less sensitive to copper at water high (5.74 mg/L DOC), compared to water low (1.12 mg/L DOC) water exposures. The protective effect of increasing water dissolved organic carbon against metal toxicity of copper has been reported in a wide range of aquatic life and thus many water quality regulation are adjusted for water DOC USEPA (1996, 2002). DOC were found to provide significant protection from acute copper exposure as the LC_{50} at water (5.74 mg/L DOC) was more than two-fold higher than (1.12 mg/L DOC) of fish Nile tilapia; and more than one-fold at water (5.74 mg/L DOC) was higher than (1.12 mg/L DOC) of Chironomid. Although there has been limited study into the effect of exposure water DOC on copper toxicity in Nile tilapia and Chironomid, Gillis et al. (2008) studied the acute toxicity test of copper to glochidia (larvae) of freshwater mussel under different water hardness and dissolved organic carbon reported that the addition of DOC (as Aldrich Humic Acid) 1.6 mg/L with the soft water was the result of decrease in Cu sensitive as ten- fold increase in EC_{50} of E. triquetra. In addition, it is interesting to note that the copper toxicity was affected by the DOC. The outcomes of the present study come in agreement with the out finding of Mclntyre et al. (2008) who reported that DOC levels of 1-6 mg/L were found to partially restore olfactory capacity in salmon that were exposed to copper.

Table 2 LC_{50} with 95 percent confidence interval of copper on Chironomid (*Chironomus javanus*) and Nile tilapia (*Oreochromis niloticus*) of two different DOC of Mekong River

Species	DOC (mg/L)	LC_{50} with 95% Cl (µg/L)			
		24h	48h	72h	96h
Nile tilapia	5.74	1228 (1138-1340)	1052 (890-1296)	939 (771-1185)	742 (562-981)
	1.12	1236 (1128-1371)	806 (494-1334)	561 (129-1177)	397 (123-761)
Chironomid	5.74	8237 (5471-32105)	5033 (3035-88359)	2206 (-)	853 (-)
	1.12	2864 (-)	2443 (-)	983 (-)	707 (-)

CL= Confidence limit, LC_{50}= Median lethal concentrations, (-) = 95% Confidence limit (lower-upper value) exposure at 96 hours

The Effluence of DOC from Humic Acid Source on Copper Sensitivity

DOC provided by the addition of Humic Acid alone to test waters significantly decrease copper toxicity to Both Chironomid and Nile tilapia. The mortalities for both species increased with increasing copper concentration, and the LC_{50} values decreased indicating more toxicity on Nile tilapia and Chironomid. This gave an order of toxicity of copper in water with low DOC > water with high DOC at the 96hr LC_{50}. DOC appears to provide protection against Cu toxicity in the freshwater by completing between free copper and DOC.

Acute Toxicity with Tap Water

The LC_{50} 48 hrs values for copper were 12 µg/L in Moina, 16399 µg/L in Chironomid, 294 µg/L in Grass Carp and 1869 µg/L in Nile tilapia, respectively (table 3). This gave an order of toxicity of copper in tap water with Moina > Grass Carp > Nile tilapia > Chironomid. The outcomes of the present study came in agreement with those of Mastin and Rodgers Jr (2000) who reported that *D. magna* was the most sensitive of nontarget animals tested for all the tree copper herbicides. In similar toxicity experiments that exposed a heterogeneous assemblage of test organisms to copper, cladocerans (e.g., Ceriodaphnia and Daphnia) were the most sensitive species Schubauer-Berigan et al. (1993) and Dobbs et al. (1994). According to Pokethitiyook et al. (1987) reported that the mean value 24, and 48 hr LC_{50} for copper in *Moina macrocopa* with water pH 29 were 19 µg/L and 17 µg/L, respectively. However, these reports were higher than the present study, suggesting the different in methodologies. The mean value 24, and 48 hr LC_{50} for $CuSO_4$ $5H_2O$ in *C. javanus* were 24127 and 16399 µg/L, respectively. Hence, the acute test of 48 and 24 hr showed an opposite relationship between LC_{50} and exposure time, increase in the concentration reduces the time to kill 50% of *C. javanus*. Previous studies, the 48 hr LC_{50} for $CuSO_4$ was 1073 µg/L in *C. ramousus* third instar larvae Majumdar and Gupta (2012). Other outcomes Mastin and Rodgers Jr (2000) reported that 48 hr LC_{50} of midge larvae *C. tentans* exposed to Clearigate and Cutrine-Plus were 373.5 and 460.9 µg/L, These values were considerably lower than responding value in *C. javanus*, indicating higher vulnerability to Cu.

Table 3 LC_{50} with 95 % confidence interval of copper to Moina (*Moina macrocopa*) ,Chironomid (*Chironomus javanus*), Grass Carp (*Ctenopharyngodon idella*) and Nile tilapia (*Oreochromis niloticus*) of tap water

Species	Test time	LC_{50} with 95% Cl (µg/L)
Moina *(Moina macrocopa)*	24hr	46 (33-68)
	48hr	12 (-)
Chironomid *(Chironomus javanus)*	24hr	24127 (13264-36397)
	48hr	16399 (-)
Grass Carp *(Ctenopharyngodon ldella)*	24hr	330 (293-373)
	48hr	294 (238-353)
	72hr	232 (180-293)
	96hr	188 (126-275)
Nile tilapia (*Orecochromis nileticus*)	24hr	2388 (2261-2592)
	48hr	1869 (1157-5235)
	72hr	1740 (-)
	96hr	1383 (859-2718)

CL= Confidence limit, $LC_{50=}$ Median lethal concentrations, (-) = 95% Confidence limit (lower-upper value) at exposure time

CONCLUSION

DOC appears to provide protection against Cu toxicity in the freshwater by complexing between free Cu and DOC. The present study indicated that water chemistry parameters can influence Cu toxicity to tropical freshwater biota. Exposure of the test species to this series of laboratory experiments has provided useful data for determine the risk of Cu in Mekong river water compared with dosed distilled and tap water, and for implication for the revision of water quality standardization in Cambodia.

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