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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Received 14 January 2016 Accepted 11 July 2016 (*Corresponding Author)

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