Effect of Dissolved Organic Carbon (DOC) on Acute Toxicity of Copper to Tropical Freshwater Biota in Mekong River, Cambodia

Lyly Soeung[1], Chuleemas Boonthai Iwai[1,2*] and Tham Hoang[3]

ABSTRACT: The aquatic resources of the Mekong River are importance to supporting the livelihoods of a large percentage of 60 million who live along the Lower Mekong Basin. Ecotoxicology of copper on tropical freshwater biota was studied using field-collected water from 2 local sites along Lower Mekong Basin in Cambodia, which had two different water dissolved organic carbon (5.74 mg/L DOC and 1.12 mg/L DOC). In this study, US EPA method was used for the acute toxicity with two different water DOC on Chironomid *(Chironomus javanus)* and fish Nile tilapia *(Oreochromis niloticus*) to modify the effecting of dissolved organic carbon (DOC) on copper toxicity. The result showed that the LC_{50} with 95% confidence limit obtained were 742 μg/L in Chironomid and 853μg/L in Nile tilapia with high (5.74 mg/L DOC), respectively. In term of low (1.12 mg/L DOC) test water, the LC_{50} with 95% confidence limit obtained were 707μg/L in Chironomid and 397μg/L in Nile tilapia, respectively. 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 complexing between free copper and DOC. The present study indicated that water chemistry parameters can influence copper 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 copper in Mekong river water.
Keywords*:* Ecotoxicology, Copper, Dissolve organic carbon, Lower Mekong Basin

Introduction

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 MRC (2013a, 2013b and 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) and Coates et al. (2006). Although the water quality of the Mekong River is considered to be remarkably good, some areas of the Mekong Delta, which are adversely inflicted by high population density, irregular mining activities, and increasing agricultural activities, have experienced worsening water quality MRC (2015).

[1] Department of Plant Sciences and Agricultural Resources, Land Resources and Environment Section, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

[2] Integrated Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, 40002 Thailand

[3] Ecotoxicology and Risk Assessment Laboratory, Department of Environmental Sciences, Institute of Environmental Sustainability, BVM Building 314, Loyola University Chicago, 1032 W. Sheridan Rd, Chicago, IL 60660

* Corresponding author: chulee_b@kku.ac.th, chuleemas1@gmail.com

Copper is known as the important that all living organisms require its small amounts (5-20 µg/g) to survive. However, too much copper concentration more than (20µg/g) will become toxic Wright and Welbourn (2002) and Bradl (2005). Copper has been documented as one of the most toxic metals to aquatic organism and ecosystem Bradl (2005), Carreau and Pyle (2005) and Scudder et al. (1988). The report Solomon (2009) and Taub Frieda (2004) showed that exposure to copper concentration can make fish lose their sense of smell, thus reducing their appetite, food intake and population.

Impacts of copper on an aquatic atmosphere are complex and depend on the physicochemical characteristics of water Kamunde and MacPhail (2011) and Nadella et al. (2009). Therefore, the acute toxicity of copper to fish, invertebrates and other aquatic organisms are influenced by water quality parameters such as hardness, alkalinity, pH and dissolved organic carbon result in increased copper LC50 values in freshwater organism Linbo et al. (2009), Santore et al. (2001) and U.S.EPA (2002). And the most effective parameter for reducing of copper toxicity to fish is DOC Linbo et al. (2009). 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 Liu and Sheu (2003) and U.S.EPA (2002).

Many research papers were designed and conducted on ecotoxicology of copper worldwide, McIntyre et al. (2008), Linbo et al. (2009), Shuhaimi-Othman et al. (2013), Majumdar and Gupta (2012), Duarte et al. (2009), Lagrana et al. (2011), Nebeker et al. (1988) and Mastin et al. (2000), 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 copper on local species with Mekong River Cambodia will be a good representative for tropical aquatic species. In the present study, the ecological of copper on tropical test species larvae Nile tilapia *(Oreochromis niloticus*) and the second instar larvae of Chironomid *(Chironomus javunus)* under different water DOC were investigated in order to help and protect the Mekong River in the future and to set a standard for heavy metal digestion in the Mekong River in Cambodia, as well as the Mekong River Basin.

Methodology and Method

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 that joins by the combined flow of three major tributaries, the Sesan, Sekong and Srepok. 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.

Organisms

Chironomid *(Chironomids javanus)* Midge larvae were cultured at ecotoxicology laboratory of Khon Kaen University, Thailand. Fish Nile tilapia *(Orecochromis nileticus)* larvae were provided by Department of Fisheries, Khon Kaen, Thailand. The tested fish larva was immediately collected after hatching in oxygenate bags to the laboratory and handle properly to minimize injury in order to reduce the number of dead organisms. Average weight of Nile tilapia was 9.717$\pm$0.040 mg which used for acute toxicity testing U.S.EPA (2002). Young organisms are often more sensitive

than adults Dung et al. (2005). For this reason, the early life stages of fish and invertebrate are required for all tests. In a given test, all organisms should be taken from the same source in order to minimize the diversity of response to experimental materials U.S.EPA (2002). The test was conducted at Ecotoxicology laboratory in Khon Kaen University.

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 (0.00, 450, 500, 600, 1000, 1500 µg/L) for the fish and (0.00, 500, 800, 1000, 3000, 5000 µg/L) for Chironomid of the stock copper solution in each container combined with high (5.74 mg/L DOC) and low (1.12 mg/L DOC). The control was kept in experimental water without adding copper. Water quality parameters (temperature, DO, alkalinity, hardness and pH) used in containers were periodically determined before toxicity test. In addition, the experimental medium was aerated in order to keep the amount of DO not less than 6 mg/L U.S.EPA (2002).

Acute toxicity test

In this study, US EPA method was used for the acute toxicity with two different water DOC on Chironomid *(Chironomus javanus)* and fish Nile tilapia *(Oreochromis niloticus)* to modify the effecting of dissolved organic carbon (DOC) on copper toxicity. Acute copper toxicity experiments were performed for a 4-d period (96h) using small fishes at 5 days old and the second instar larva of Chironomid. 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± $1C^0$, with a Photoperiod of 16h light: 8h darkness. All control results in lower mortality, less than 10% which revealed the acceptability of the test. In addition, the acute toxicity was assessed for each of the two species in standardized US.EPA (2002).

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.

Statistical analysis

The acute toxicity effects of studies heavy copper on the test species, Nile tilapia and Chironomid were determined by the use of Probit Analysis LC_{50} Determination Method (SPSS, version 19 software). The rate response determined at the end of the 96th hour. Significance in 95% confidence interval (95%CI) of detected 96 hour LC_{50} value was determined using the Chi-Square technique Ezeonyejiaku et al. (2011).

Result and discussion

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±0.89mg/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 pa-

rameters 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±0.17mg/L and 118.66±4.61and 113.33±2.30mg/L, respectively. And a summary of measured heavy metal data for all experiments (e.g., Cu, Zn, Mn, Fe, Pb, Cd, Mg, Cr and Ca) were shown in (**Table 1**).

Mortality rate and exposure times

The data from acute toxicity test of copper for Nile tilapia *(Oreochromis niloticus)* and Chironomid *(Chironomus javanus)* revealed that the mortality of organisms increased with increasing copper concentration and exposure time. However, the mortality rate of fish Nile tilapia and Chironomid with (1.12 mg/L DOC)> (5.74 mg/L DOC) (**Figure 1** and **Figure 2**). The present study indicated that Copper was found become toxic to Nile tilapia responding higher than Chironomid from the difference concentration-response obtained with the difference water dissolved organic carbon.

The effluence of DOC 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. 74mg/L DOC), compared to water low (1.12mg/L DOC) water exposures.

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 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). Dissolved organic carbon 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 (1.12mg/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 DOC 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 McIntyre 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. Therefore, Brooks et al. (2008) demonstrated that the addition of DOC into the exposure media could provide the germlings of the macroalgae with protection against copper toxicity by increased the EC_{50} value of 117.3 µg/L at a corrected DOC as (DOC from humic Acid only) in the seawater. These authors have also mentioned that the concentration 1.6 mg/L DOC was not found to protected copper toxicity. However, the concentration about 2 mg/L DOC was found to protected copper toxicity, with increased in the EC50 value. Overall, our results suggest that even though many of the lakes and rivers in which endangered are found in the tropical have been polluted by many sources such as agriculture-runoff, industrial discharge and household wastes MRC (2013), that the increased organic carbon in these water bodies may provide protection from acute copper exposure.

Acute toxicity

The result showed that the LC_{50} with 95% confidence limit obtained were 742 µg/L in Chironomid *(Chironomus javanus)* and 853µg/L in Nile tilapia *(Oreochromis niloticus)* with high (5.74 mg/L DOC), respectively. In low (1.12 mg/L DOC) test water, the LC_{50} with 95% confidence limit obtained were 707µg/L in Chironomid and 397µg/L in 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 complexing between free copper and DOC. Similarly, (Linbo et al. 2009, Santore et al. 2001, U.S.EPA 2002) reported that Copper toxicity of fish, invertebrates and other aquatic organisms are influenced by water quality parameters such as hardness, alkalinity, pH and DOC; and the most effective parameter for reducing of copper toxicity to fish is DOC Linbo et al. (2009). Previous studies, Taweel et al. (2013) showed the LC_{50} 96 hr value for copper was 1093 µg/L on the fingerline Tilapia fish *(Orecochromis nileticus)*. However, this result is

higher than present study. But, the results of previous studies did not mention about DOC concentrations, the 96 hr LC_{50} in *C. tentans* and *C. ramousus* were 170 and 183µg/L, respectively Shuhaimi-Othman et al. (2013) and Majumdar and Gupta (2012). These values are considerably lower than responding value in *C. Javunus* in the present study with all water DOC.

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

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

Conclusion

Present study indicated that water chemistry parameters can influence on copper toxicity to tropical freshwaters biota. Dissolved organic carbon (DOC) appears to provide protection against Cu toxicity in the freshwater by complexing between free copper and DOC. Both Chironomid and Nile tilapia were significantly less sensitive to copper in water high (5.74 mg/L DOC), compared to water low (1.12 mg/L DOC) exposures. Overall, our results suggest that even Mekong River water in which endangered is found in the some areas have been polluted by many sources such as agriculture-runoff, industrial discharge and household wastes that the increased organic carbon in the water bodies may provide protection from acute copper exposure. The present study indicated that water chemistry parameters can influence copper 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 copper in Mekong river water. Other acute toxicity studies of copper under the different water quality parameters with more tropical organisms species should be encouraged.

Acknowledgements

The authors would like to thank to Integrated Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University and the research collaboration

project between Loyola University Chicago and Khon Kean University supported by International Copper Association, Ltd. (ICA).

References

Bradl, H. 2005. Heavy Metals in the Environment: Origin. Interaction and Remediation, Elsevier/Academic Press, London.

Brooks, S. J., T. Bolam, L. Tolhurst, J. Bassett, J. La Roche, M. Waldock, and K. V. Thomas. 2008. Dissolved organic carbon reduces the toxicity of copper to germlings of the macroalgae, Fucus vesiculosus. Ecotoxicology and Environmental Safety. 70(1): 88-98.

Carreau, N. D., and G. G. Pyle. 2005. Effect of copper exposure during embryonic development on chemosensory function of juvenile fathead minnows (< i> Pimephales promelas</i>). Ecotoxicology and Environmental Safety. 61(1): 1-6.

Coates, D., O. Poeu, U. Suntornratnana, T. Nguyen, and S. Viravong. 2006. Biodiversity and fisheries in the Mekong River Basin. Mekong development series.

DePalma, S.G,S., W. R. Arnold, J. C. McGeer, D. G. Dixon, and D. S. Smith. 2011. Effects of dissolved organic matter and reduced sulphur on copper bioavailability in coastal marine environments. Ecotoxicology and environmental safety. 74(3): 230-237.

Duarte, R. M., A. C. Menezes, L. da Silveira Rodrigues, V. M. de Almeida-Val, and A. L. Val. 2009. Copper sensitivity of wild ornamental fish of the Amazon. Ecotoxicol Environ Saf. 72(3): 693-698.

Dung, L.Q., N.M. Cuong, N.T. Huyen, and N.D Cu. 2005. Acute toxicity test to determine the effects of copper zinc and cyanide on cobia (Rachycentron canadum) resources in North Vietnam. Australia Journal Ecotoxicology. 11(3): 163-166.

Gillis, P. L., R. J. Mitchell, A. N. Schwalb, K. A. McNichols, G. L. Mackie, C. M. Wood, and J. D. Ackerman, 2008. Sensitivity of the glochidia (larvae) of freshwater mussels to copper: Assessing the effect of water hardness and dissolved organic carbon on the sensitivity of endangered species. Aquatic Toxicology. 88(2): 137-145.

Kamunde, C., and R. MacPhail. 2011. Effect of humic acid during concurrent chronic waterborne exposure of rainbow trout (Oncorhynchus mykiss) to copper, cadmium and zinc. Ecotoxicology and Environmental Safety. 74(3): 259-269.

Majumdar, T., and A. Gupta. 2012b. Acute and chronic toxicity of copper on aquatic insect Chironomus ramosus from Assam, India. Journal of environmental biology/Academy of Environmental Biology, India. 33(1): 139-142.

Mastin, B., and J. Rodgers Jr. 2000. Toxicity and bioavailability of copper herbicides (Clearigate, Cutrine-Plus, and copper sulfate) to freshwater animals. Archives of Environmental Contamination and Toxicology, 39(4): 445-451.

MRC. 2013a. 2011. Annual Water Quality Data Assessment Report . pp. 59. Vientiane, Lao PDR.

Nebeker, A. V., M. A. Cairns, and C. M. Wise. 1984. Relative sensitivity of Chironomus tentans life stages to copper. Environmental Toxicology and Chemistry. 3(1): 151-158.

Santore, R. C., D. M. Di Toro, P. R. Paquin, H. E. Allen, and J. S. Meyer. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. Environmental Toxicology and Chemistry. 20(10): 2397-2402.

Taweel, A., M. Shuhaimi-Othrnan, and A. Ahmad, 2013. Tilapia Fish (Oreochromis niloticus). Journal of Biological Sciences. 13(5): 365-371.

U.S. EPA. 1996. Ecological effects test guidelines, OPPTS 850.1085, Fish acute toxicity mitigated by humic acid. EPA 712-C-96-117, Washington, DC.

U.S.EPA. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency.