Research article

Ecotoxicology of Copper on Local Freshwater Organisms in Mekong River Cambodia

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Abstract The protection of aquatic habitat from damage and understanding of both sensitivity of aquatic organisms to contaminant and ecological effects. Mekong River quality criteria of aquatic life for metals are largely driven by the extremely sensitive small organisms toxicity which are the Mekong native species. In this study we assessed the toxicity of Copper in the Mekong river water with Chironomids Species (*Chironomus javanus*) and Nile tilapia (*Oreochromis niloticus*). Acute toxicity effect of copper concentration to freshwater animals occur after the exposure at tested with Mekong water was studied by observing mortality and LC_{50} over a 24 hours test period. The LC_{50} with 95% confidence limit of the 96-hours toxicity was performed to contrast responses of Chironomids Species *(C. javanus)* and Nile tilapia (*O. niloticus)*. The result showed that the LC_{50} with 95% confidence limit obtained were 742 µg/L in Chironomids Species *(C. javanus)* and 853µg/L in Nile tilapia *(O. niloticus)*. Copper (Cu) is a big concern for environment, human and aquatic organisms because it can accumulate in to plant and animals via food web. The out coming of this series of laboratory experiment will provides a worst-case scenario and useful for determine the risk assessment of copper on local freshwater organisms in Mekong River Cambodia as well as Mekong River Basin.

Keywords ecotoxicology, acute toxicity, copper, local freshwater animals, Mekong River

INTRODUCTION

The resources of the Mekong Basin, such as fishes, other aquatic resources and plants are play main role in food security, income and livelihood for many people across the Lower Mekong Basin. Around 60 million people live in the lower Mekong Basin and most of people in the basin are closely linked to the Mekong and resources of it to support their livelihood (MRC, 2007; Ferguson et al., 2011). The development activities during the past decade and up to now, including mining, agriculture, deforestation, grazing and urbanization have caused of extensive soil erosion and contribute increasingly to environmental levels of heavy metals especially copper (Cu) into water body (Ti and Facon, 2004; Coates et al., 2006)

Copper mining was known as the important sector for economics. Therefore, it represents an essential in all living organisms that is required in small amounts (Heath, 1995). Humans, other animals, fish and shellfish require 5-20 micrograms per gram (µg/g) for carbohydrate metabolism and the function of more than 30 enzymes. However, too much of copper concentration which exceed 20 microgram per gram (µg/g) will be toxic, was explained by Wright and Welbourn (2002) and Bradl (2005). Copper has been documented as one of the most toxic metals to aquatic organism and ecosystem (Scudder et al., 1998; Bradl, 2005; Carreau and Plye, 2005). Heavy metal

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contamination of copper (Cu) is a big concern for environment, human and aquatic organisms. Copper (Cu) does not break down in the environment because of that it can accumulate in to plant and animals.

The United States Environmental Protection Agency (US.EPA) has issued a guideline for conducting early-life-stage toxicity test suitable for acute and chronic toxicity tests used for measuring the aggregate toxic chemicals in an effluent or receiving water to freshwater and marine organisms (US. EPA, 2002). In Cambodia, the information on the impact of toxicity effects of soluble copper on the tropical aquatic biota is limited. Therefore, many research papers were designed and conducted on ecotoxicology on copper everywhere in the world, but most them were the different species and different from local species in the Mekong River. The out coming of toxicity tests for copper will contribute data to aquatic environments and ecotoxicological freshwater system for environmental quality standard in order to help and protect the Mekong River in Cambodia as well as Mekong River Basin.

OBJECTIVES

The objective of studying is focus on ecotoxicology of copper on freshwater biota of Nile tilapia *(Oreochromis niloticus)* and Chironomids species *(Chironomus javanus)* with Mekong River in Cambodia.

METHODOLOGY

Sampling

This study was conducted in the Lower Mekong Basin of Cambodia which located in the Kampong Cham Province (Fig. 1).

Fig. 1 Map showing the sampling location

Organisms

Native species of Mekong River fish Nile tilapia *(O. nileticus)* larvae were obtained from the 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 and stress physiological in order to reduce the number of dead organisms. The test was conducted at Ecotoxicology laboratory in Khon Kaen University. Average weight of Nile tilapia was

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9.717±0.040 mg which used for acute toxicity testing. Young organisms are often more sensitive than adults. For this reason, the use of early life stages such as fish fingerling is required for all tests. In a given test, all organisms should be taken from the same source in order to minimize the organisms diversity of response to experimental materials (US.EPA, 2002).

Chironomids Species *(C. javanus)* midge larvae were cultured at ecotoxicology laboratory of Khon Kaen University, Thailand. Organisms were cultured in a glass container, covered with a net to trap emerging adults. Since the aquarium already contains male and female species, mating and production of eggs is possible. To produce eggs of similar age, each egg mass collected was placed on the beaker containing 25 mL of tap water that was aged overnight. After two days, when all eggs were hatched, larvae were transferred on a 14 inches x 10 inches x 6 inches aquarium and given fish flakes for food. This was used as substrate. Overlying water was being replaced every three days. The second instar organisms were placed individually in micro test tubes file with test solutions. Tube was added, just enough for the organisms to create their own tubes.

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 (450, 500,600 1000, 1500 µg/L) for the fish and (500, 800, 1000, 3000, 5000 µg/L) for *C. javanus* of the stock copper solution in each container. 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 (table 2). In addition, the experimental medium was aerated in order to keep the amount of DO not less than 6 mg/L (Ezeonyejiaku et al., 2011).

Acute Toxicity Test

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 *(C. javanus)*. 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± 1 °C, with a Photoperiod of 12h light: 12 darkness. All control result in lower mortality, less than 10% which revealed the acceptability of the test (US. EPA, 2002).

Water Quality

The water quality parameters measured during the test were pH 7.77 ± 0.02, Conductivity 191± 1.53 µS/cm, TDS 45±005mg/L, dissolve oxygen 10.46±0.05mg/L, and total hardness (mg^{2+} and Ca^{2+}) 88±4 mg/L as $CaCO_3$. The mean value of other water quality parameters such as DOC, BOD and alkalinity were 5.74 ±0 mg/L, 1.33±0.20 mg/L and 104±0 mg/L, respectively (Table 1).

Table 1 Water quality parameter of Mekong River for acute toxicity of copper on Nile tilapia and *C. javanus*

Parameters	Result Water quality of Mekong River	US. EPA, 2002
pH	7.77±0.02	6.8-8.4
Temperature (°C)	28±0.03	25±1
DO (mg/L)	10.46±0.05	Above 80%
EC (µS/cm)	191±1.53	-
TDS (mg/L)	45±0	-
Alkalinity (mg/L)	104±0	-
Hardness (mg/L)	88±4	-
BOD (mg/L)	1.33±0.20	-
DOC (mg/L)	5.74± 0	-

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Statistical Analysis

All the results were carried out by analysis of variance (ANOVA) with Duncan's Multiple Rang Test (DMRT) by Statistic 8 software (version 8, USA) on the Factorail experiment in Completely Randomized Design (CRD). The significance was reported at $p<0.05$ levels. Toxicological dose-response data involving quintal response (mortality) following toxicity of copper on the test species, Nile tilapia and *C. javanus* larvae were determined by 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 hours LC_{50} value was determined using the Chi-Square technique (Ezeonyejiaku et al., 2011).

RESULTS AND DISCUSSION

The data from acute toxicity test of copper for Nile tilapia *(O. niloticus)* and *C. javanus* larvae released that the mortality of organisms increased with increasing copper concentration and exposure time (Table 2). Copper was found toxic to Nile tilapia responding higher than *C. javanus*. The Mean value 24, 48, 72 and 96 hr LC_{50} for $CuSO_4$ $5H_2O$ in the Nile tilapia were 1228, 1052, 939 and 742 µg/L. Hence, the acute test of 96, 72, 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 the tilapia fish. Previous studies (Taweel et al., 2013) showed the LC_{50} 96 hr value for copper was 1093 µg/L on the fingerline Tilapia fish *(O. niloticus)*. However, this result is higher than present study. Various authors in different parts of the world including tropical (Shuhaimi-Othman et al., 2013; Mastin and Rodgers, 2000, Taylor et al., 1991) have observed same and recorded differential toxicity of heavy metal compounds against different test animals. Cusimano et al., (1986) and Solbe (1984) also reported that the acting metal (copper) might be due to the physicochemical characteristics of the test medium, species and ages of fishes used and their susceptibility rates to the test chemical.

Mean 24, 48, 72 and 96 hr LC_{50} values for Cu as $CuSO_4$ $5H_2O$ in the *C. javanus* are presented in the table 2. The 24 hr LC_{50} for $CuSO_4$ was 8237 µg/L which sharply declined to 5033, 2206 and 853 µg/L at 48, 72 and 96 hr, respectively. Also, it could be noted that Copper effect to the *C. javanus* in the long time exposure. The 48 hr LC_{50} for $CuSO_4$ was 1073 µg/L in *C. ramousus* third instar larvae (Majumdar and Gupta, 2012) and the 96 hr LC_{50} in *C. tentans* and *C. ramousus* 170 and 183µg/L, respectively (Shuhaimi-Othman et al., 2013; Majumdar and Gupta, 2012). These values are considerably lower than responding value in *C. javanus*, indicating higher vulnerability to Cu in the former two species. Mastin and Rodgers (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, respectively. On the other hand, Copper sulfate was an order of magnitude less toxic to *C. tentans* than Clearigate and Cutrine-Plus with a mean 48 hr LC_{50} 1,136.5 µg/L (Mastin and Rodgers, 2000).

Percentage mortality of Nile tilapia (*O. niloticus*) and *C. javanus* larvae to copper solution with Mekong River water for 96 hours are presented in Fig. 2. From the concentration- response obtained, it could be seen that *C. javanus* was more sensitive than Nile tilapia. Lower threshold responses of *C. javanus* and Nile tilapia were 538 and 462.5 µg/L, respectively. And upper thresholds of response of $CuSO_4$ with 100% mortality were observed for *C. javanus* and Nile tilapia at 5095 and 1595 µg/L, respectively.

Table 2 LC_{50} Value of $CuSO_4$ $5H_2O$, for Nile tilapia and *C. javanus*

Species	LC_{50} with 95% CL (µg/L)			
	24h	48h	72h	96h
Nile tilapia	1228 (1138-1340)	1052 (890-1296)	939 (771-1185)	742 (562-981)
C. javanus	8237 (5471-32105)	5033 (3035-88359)	2206 (-)	853 (-)

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

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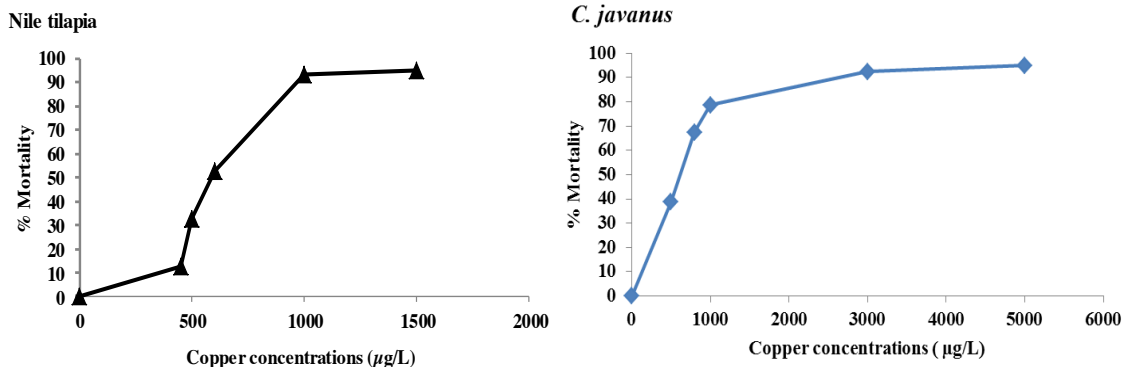

Fig. 2 Percentage mortality of Nile tilapia and *C. javanus* larvae to copper solution with Mekong River water for 96 hours

CONCLUSION

Present study indicated that mortalities for both species increase with increasing copper but LC_{50} value decreased as more toxic on Nile tilapia and *C. javanus*. The 96 hr LC_{50} for $CuSO_4$ $5H_2O$ in the Nile tilapia and *C. javanus* were 742 and 853 µg/L, respectively. *C. javanus* was found more sensitive than Nile tilapia to copper.

Copper sulphate is still commonly used in the study area and in many developing countries to control the water pollution. It is also used as fungicide in agricultural fields study area where from it could be transported into the freshwater ecosystems via surface run off. Therefore, the high toxicity of $CuSO_4$ to non- target organisms like aquatic organisms needs to be taken into careful consideration before applying it indiscriminately in freshwater system. Due to the lack of the data on ecotoxicology of copper on the local species of Mekong River, The further studies on the concentration of heavy metals in other tropical freshwater should be studied.

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