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Copper Toxicity to Tropical Water Flea *Daphnia carinata* and Freshwater Micro-algae *Scenedesmus*

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Abstract

Sai Gon river is one most the most important river of South-eastern region of Vietnam by providing water resources and others vital natural services for millions of people in this area. In recent years, proportion with the sharply increasing of human activities in industry and agriculture, the river is continuously loaded with xenobiotics released by anthropogenic activities. Among pollutants, heavy metals are considered as the most toxic elements to aquatic living organisms and human health. The aim of this study is to assess the sensibility of freshwater microalgae *Scenedesmus* and water flea *Daphnia carinata*, two fresh water species from Vietnam to copper (Cu). After physical and chemical characterization, field water samples from upstream Sai Gon River was used as dilution water in toxicity tests. With water flea *D. carinata*, the EC50 value of 48h immobilization experiment was 1.90 µg/L. Growth inhibition of the *Scenedesmus* algae cells was determined following exposure for 72 h, and EC50 values was 78.2 µg/L. The results showed that Cu is highly toxic to both species, and water flea *D. carinata* was more sensitive than freshwater algae *Scenedesmus*. Based on the observed high sensitivity with Cu, both *D. carinata* and algae *Scenedesmus* are potential tools for the assessment of copper pollution in fresh water of Sai Gon river.

Keywords: Acute toxicity, Daphnia, Algae, Freshwater, Copper, Sai Gon river

1. Introduction

In Vietnam, fresh surface water pollution and deterioration has been an important problem because of its impact on human health and aquatic ecosystem. However, monitoring of fresh water quality are limited due to the shortage of human and financial resources. In recent years, the quickly induction of urbanization and industrialization become main reasons of increasing

water demands, and water pollution caused by anthropogenic activity. Therefore, it called for a better procedure to monitor the quality of water in Sai Gon river system, which are important water resources for Ho Chi Minh, the biggest city in Southern of Vietnam. Among variety of contaminants which can pollute the fresh water and toxic to human health, heavy metals are great concern due to its toxicity and persistence once introduced into the aquatic environment (Beiramzadeh et al., 2019; Saberi et al., 2018). The objective of this study was to investigate the possibility of using tropical fresh water living creatures to detect the contaminated Cu in the water due to its toxicity. This study is going to contribute to provide a simple and cost effective method to quickly assess the toxicity of fresh water quality, to protect the water resource and health of water users.

Metals are among the most intensively studied pollutants in fresh water environments. Many of metals are important for living processes at very low concentrations, but at higher doses they become toxic (Warnau et al., 1995). Metals can be introduced into environment from many anthropogenic activities such as industrial, agricultural, and mining processes, then they become tolerant pollutants and pose significant risks on living creatures in the ecosystem including (Lanctôt et al., 2016; Schwarzenbach et al., 2010; Tomasiks & Warren, 1996). While some metals play vital roles in living processes of organisms, some others do not. On another hand, it is worth to note all metals become toxicants while reach a concentration threshold (Wetzel, 2001). The previous studies have pointed out that metals are indestructible and can be accumulated in body of organisms (Lau et al., 1998; Waykar & Shinde, 2011), then transferred to higher trophic levels of the food chain (Ikemoto et al., 2008). The toxic effects of metals to living organisms have been well defined and considered as a major threat to aquatic biodiversity (Millennium Ecosystem Assessment, 2005; Dinh Van et al., 2013; Lanctôt et al., 2016; Moldovan et al., 2013). Also, it has been found that toxicity of dissolved metals in water is regulated by variety of water physical and chemical characteristics such as pH, alkalinity, dissolved organic carbon (DOC) and hardness (De Schamphelaere & Janssen, 2004; Hoang et al., 2004; Jo et al., 2010; Linbo et al., 2009; Ryan et al., 2009). Therefore, it is essential that the living organisms used in toxicity detection test must be sensitive to the suspected metal – copper in context of Sai Gon river's water characteristics. In this study, the water sample was collected from the upstream of Sai Gon river, and used as dilution water in the toxicity test to define the sensitivity level of tropical *D. carinata* and algae *Scenedesmus*.

The experimental sensitivity to Cu of the two tropical freshwater organisms obtained through this study can serve as starting point for developing a cost-effective pollution monitoring procedure. The two living organisms were chosen due to high sensitivity of micro-crustacean *Daphnia* to dissolved heavy metals in water, while planktonic algae are easy to culture, and require only small laboratory space and simple equipment. Algae are primary producers of which population growth inhibition can be used as criterion of response in toxicity test. Moreover, the inhibition of algae's population in aquatic environment can imply the chain reaction on ecological food chains in water environment.

The purpose of this study is to develop and optimize a procedure using a battery of organisms for use in routine monitoring of freshwater of Sai Gon river. One of the first criteria for toxicity test is sensitivity of organism to contaminant of interest. Therefore, we aim to

develop a practical process which enable to detect Cu pollution in fresh water using a battery of organisms. The test battery consists of two species representatives of two consecutive trophic levels: micro algae *Scenedesmus* sp. (primary producer), and *D. carinata* (primary consumer).

2. Materials and Methods

2.1 Water Samples Collection

Surface water was collected from the upstream of Sai Gon river (Dau Tieng freshwater reservoir). The water sample was transferred to the Environmental Toxicology Laboratory, Institute for Environment and Resources in Hochiminh City, filtered through 0.45 μm syringe filter (Sartorius, Germany) and stored at 4 ℃ prior to the tests.

2.1.1 Water Samples Characteristics

The filtered waters from Dau Tieng reservoir was analyzed for water quality parameters that may affect the bioavailability of dissolved metals and the survival and growth of the two organism of test battery, alkalinity and hardness, pH, trace metals and pesticides. Total hardness was determined based on concentrations of Ca^{2+} and Mg^{2+}, metals were analysis by ICP/MS.

2.1.2 Test Organisms

Organisms used in the present study were *D. carinata* and freshwater algae *Scenedesmus* sp. These species were collected from the field in Vietnam and have been cultured in the Ecotoxicology Laboratory, Institute for Environment and Resources, Vietnam National University – Hochiminh City for over a year. *D. carinata* were cultured in 1.2 L beakers with 1.0 L of COMBO medium (Kilham et al., 1998). The light intensity was approximately 1000 lux. The crustaceans were fed with a mixture of green alga (*Chlorella* sp.) and YCT (yeast, cerrophyl and trout chow digestion), prepared according to the U.S. Environmental Protection Agency Method (US EPA, 2002) with a modification to the algal culture medium, which was the COMBO medium. Algae *Scenedesmus* sp. were culture in COMBO medium.

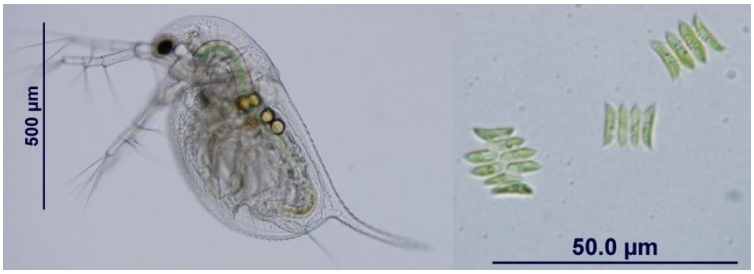

Figure 1. Morphology of *D. carinata* (neonate) and algae *Scenedesmus* sp. used in this study

2.1.3 Acute Toxicity Tests

The 48-h static nonrenewal acute toxicity tests were conducted following the guidelines of the US EPA methods (US EPA, 2002) with two adjustments of: i) light regime (a photoperiod

of 12 h:12 h light: dark at a light intensity of ca. 1000 Lux) and ii) temperature (27 ± 1 ℃) for tropical species. Neonates of *D. carinata* (age ≤ 24 h) were used for testing. Each treatment had four replicates and each replicate consists of 10 neonates in 40 mL of exposure solution in a 50-mL polypropylene cup. The neonates were fed during the pre-exposure duration but starved during the tests (US EPA, 2002). Copper treatments were prepared by spiking Cu in constituted medium prepared with field-collected water. $CuSO_4.5H_20$ was used as Cu salt. Five concentrations of Cu were prepared for each metal exposure. Controls were prepared by transferring the neonates into the constituted medium without metal addition.

We checked daily for immobilized organisms and removed them from the cups. Immobilization data were used to determine median lethal concentrations (48 h-EC50). At the end of the test, test solution in one of the four replicates was randomly taken (in each metal concentration) for the metal analysis by ICP/MS.

2.1.5 Algal Inhibition Test

Bioassays were performed using the green algae *Scenedesmus* sp. To analyze the toxic effect of Cu on the algal growth, serial concentrations of $CuSO_4.5H_20$ were tested using the COMBO media prepared without EDTA (Kilham et al., 1998). The initial inoculum cell density was $2 \pm 0.2\ 10^4$ cells/mL, and the assays were performed in triplicate using 125 mL flasks containing 25mL of medium. Cultures were incubated at 24 ℃ in constant light (4000 Lux), and the algal growth was estimated by absorbance readings at 750nm after 96h incubation. The effective concentrations of metal inducing 50% effect (EC50) were calculated by plotting the values for the percent inhibition in average specific growth rate against the logarithmic value of the test substance concentration. Using the regression equation, etc., determine the 50% inhibition concentration (EC50).

3. Results & Discussion

The aim of this study is to develop a practical and cost effective procedure to detect copper contamination in freshwater of Sai Gon river. The two organisms were selected due to they originated from tropical freshwater environment and therefore easy to use in Vietnam environmental condition.

In order to evaluate the applicable of the procedure in detection the introducing of Cu into freshwater of Sai Gon river, field water sample was obtained, and spiked with Cu at different concentration, then put in our toxicity test procedure with a battery of two organisms to detect Cu contamination. The chemical analyzes result of freshwater from upstream of Sai Gon river showed good quality, without metals or herbicides contamination (table 1).

Table 1. Dissolved metal concentrations (µg/L) and physical characteristics of filtered field water from Saigon River used for the test. BDL, below detection limits of the ICP/MS. N/A, not available

Nr.	Parameter	Value	Nr.	Parameter	Value
1	TSS (mg/L)	5	17	Cd (mg/L)	BDL (LOD = 0.00004)
2	Hardness (mg $CaCO_3$/L)	14	18	Pb (mg/L)	0.0032
3	COD (mgO_2/L)	7	19	Cr (mg/L)	0.006
4	N-$NH4^+$ (mg/L)	BDL (LOD = 0.03)	20	Cu (mg/L)	0.09
5	Cl^- (mg/L)	4.1	21	Ni (mg/L)	BDL (LOD = 0.004)
6	N-$NO3^-$ (mg/L)	0.24	22	Mn (mg/L)	0.01
7	P-$PO4^{3-}$ (mg/L)	BDL (LOD = 0.01)	23	Hg (mg/L)	BDL (LOD = 0.0003)
8	Total N (mg/L)	BDL (LOD = 1)	24	Se (mg/L)	BDL (LOD = 0.006)
9	Total P (mg/L)	0.02	25	Ag (mg/L)	BDL (LOD = 0.003)
10	SO_4^{2-} (mg/L)	2.17	26	Lindan (µg/L)	BDL (LOD = 0.006)
11	Al (mg/L)	3.34	27	Aldrin (µg/L)	BDL (LOD = 0.01)
12	Ca (mg/L)	9.91	28	Dieldrine (µg/L)	BDL (LOD = 0.01)
13	Mg (mg/L)	1.73	29	Endosulfan (µg/L)	BDL (LOD = 0.01)
14	Na (mg/L)	2.42	30	4,4'-DDT (µg/L)	BDL (LOD = 0.01)
15	K (mg/L)	2.35	31	4,4'-DDE (µg/L)	BDL (LOD = 0.01)
16	As (mg/L)	BDL (LOD = 0.0005)	32	4,4'-DDD (µg/L)	BDL (LOD = 0.01)

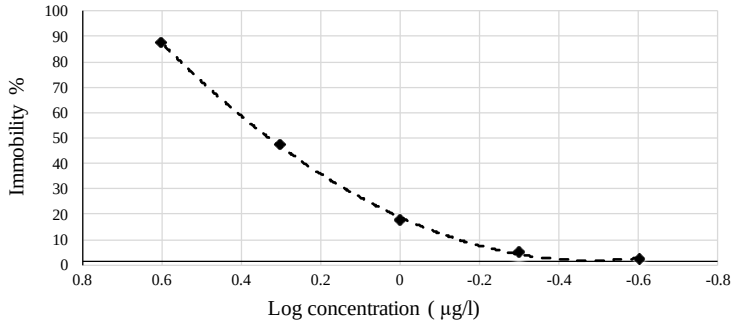

Figure 2. *Daphnia* concentration-immobilization rate curve. Log concentration of copper is presented in x axis, and immobility percentage of *Daphnia* at 48 hours is showed in y axis

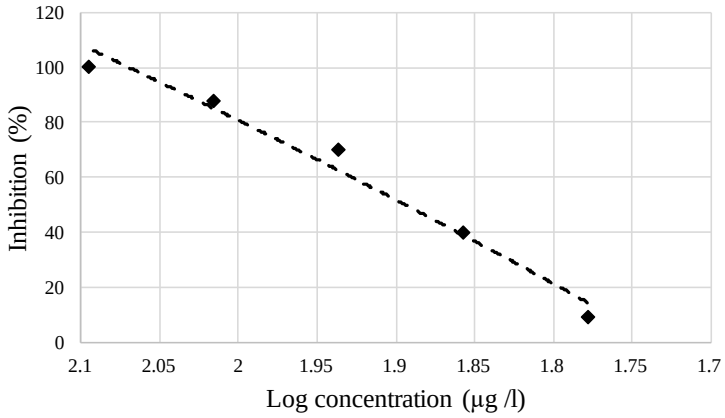

Figure 3. Algal concentration-inhibition (growth rate) curve. Log concentration of copper is presented in x axis, and growth rate inhibition percentage of *Scenedesmus* algae at 72 hours is showed in y axis

Copper is an essential micro-nutrient for living organisms by involving in varieties of protein functions, and cell respiratory. Food are the main resource for aquatic living (Bervoets & Blust, 2003; Campbell et al., 2008). However, at high level Cu can be toxic by disrupting ion homeostatis, inhibiting the anhydrase enzyme in cell cytoplasma, and group of Na+, K + -ATPases which helps to maintain osmotic equilibrium and membrane potential in cells (Giacomin et al., 2014).

Copper can be introduced into environment through electrical product wastes (Lopez et al., 2011). Due to increasing amount of this type of waste in recent year, it is the risk of Cu contamination of the freshwater reservoir and therefore it is required better supervision to detect Cu pollution in this freshwater body. For *D. carinata*, with the modified ISO test medium with pH 7.0, EC 168.8 (µS/Cm), DO = 6.8 (mg/L) and temperature 29^{o}C, the EC50 value of 48h immobilization experiment was 1.90 µg/L (95% CI = 1.58 – 2.33 µg/L). The result showed that *D. carinata* in this study was more sensitive to copper than *Daphnia magna* with EC50 was 13 µg/L (Okamoto et al., 2015).

The results demonstrated that the proposed toxicity testing procedure with *D. carinata* is sensitive and can be considered as a potential tool for Cd pollution monitoring control in freshwater of Sai Gon river. The EC50 value was 1.90 µg/L for Cu make this organism a good tool for detection Cu pollution. According to the Vietnam's national standards (QCVN 08), the best surface water quality can have up to 100 µg/L of Cu, this level was known to have no effect on human health. The study of Winner and Farrel has confirmed that members of the family Daphnidae represent some of the most acutely sensitive aquatic species to copper. In more details, they observed that all four species including *D. magna*, *D. pulex*, *D. parvula*, and *D. ambigua* exhibited reductions in survival at Cu concentrations > 40 µg/liter (Winner & Farrell, 2011). Therefore, with EC50 value 1.90 µg/L the proposed testing procedure using *D. carinata* is very sensitivity and easy to apply to detect copper contamination.

Beside of *D. carinata*, freshwater microalgae *Scenedesmus* sp. was also use to dectect copper pollution. The combination use of different organisms could increase the reliability of heavy metal pollution detection. Using COMBO medium without EDTA prepared with water collected from the upstream of Sai Gon river. The test medium has pH = 7.6, EC = 278 (µS/cm), DO = 6.9 (mg/L) and temperature 26^{o}C. The result pointed out the growth inhibition effect of Cu on the fresh water algae. The IC50 value was 78.2 µg/L (95% CI = 63.4 – 93.3 µg/L) was lower than *Scenedesmus subspicatus* (180 µg/L) which has been reported previosly (Tisler & Zagorc-Koncan, 2003). In Vietnam's national standards the allowance value of Cu level in A1 surface water which can be used for domestic and other purposes is 0.1 mg/L (QCVN 08). Therefore, the EC50 value of *S. cenedesmus* in this study was lower than the legal threshold of water quality. And therefore, it is a potential tool to detect Cu contamination water samples.

The result of this study, in consistant with previous others, confirm the high toxicity of Cu on fresh water algae. Cu contamination, lead to eliminate the population of the primary producer – algae, and then severely impact the aquatic organisms at high levels of the food chains such as microcrustacean, insect, bivalve, and fish (Wright et al., 2002).

In agreement with trigger value for copper of 1.4 µg/L was derived using the statistical distribution method with 95% protection provided by of the ANZECC & ARMCANZ (2000) guidelines (ANZECC & ARMCANZ, 2002) the result of this study emphasized the necessary and provide a way to detect Cu pollution at very low level to protect the aquatic ecological environment.

4. Conclusion

The result showed that both two organisms are potential bioindicators for the assessment of copper pollution in Sai Gon river. However, water flea *D. carinata* was more sensitive than freshwater algae *Scenedesmus*. Beside of high sensitivity, the toxicity test procedure using two tropical aquatic organisms is also cost effective, and easy to apply. The proposed procedure in this study can be used to daily test water quality to detect the introduction of toxic pollutants such as heavy metal Cu. In combining with the following detail chemical analysis can be combined later to precisely identify the inspect toxic pollutants, the proposed toxicity test in this study can contribute to monitoring water quality and protect human health form risks related to consuming of heavy metals contaminated waters.

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