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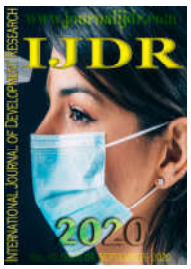

RESEARCH ARTICLE OPEN ACCESS

SENSITIVITY OF WATER FLEA *DAPHNIA CARINATA* AND FRESHWATER MICROALGAE *SCENEDESMUS* FROM VIET NAM TO CADMIUM

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ABSTRACT

In South-eastern region of Viet Nam, Sai Gon river not only provides drinking water for million people, but also supports biodiversity of local freshwater ecosystem. However, due to development of 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 Viet Nam to cadmium (Cd). After physical and chemical characterization, field water samples from upstream of Sai Gon River was used as dilution water in toxicity tests. With water flea *D.carinata*, the EC50 value of 48h immobilization experiment was 7.62 µg/Lfor Cd. Growth inhibition of the algae cells was determined following exposure for 72 h, and EC50 values of Cd was 328.5 µg/L. The results showed that Cd is highly toxic to both species, and water flea *D.carinata* was much more sensitive than freshwater algae *Scenedesmus*. Based on the observed high sensitivity with Cd, *D.carinata* is a potential bioindicator for the assessment of Cd pollution in fresh water ofSai Gon river. While Cd-tolerance algae *Scenedesmus* calls for further investigation on metal uptake capacity and utilization in Cd contaminated water treatment.

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INTRODUCTION

In Viet Nam, fresh surface water pollution and deterioration has been an important problem because of its impact on human health and aquatic ecosystem. In practical, detail and extensive 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 Viet Nam. 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 tolerance once introduced into the aquatic environment.

The objective of this study was to investigate the possibility of using tropical fresh water living creatures to detect the contaminated Cd 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 human(Lanctôt *et al*., 2016; Schwarzenbach *et al*., 2010; Tomasiks and 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 and 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).Recently, 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 and Janssen, 2004; Hoang *et al*., 2004; Jo *et al*., 2010; Linbo *et al*., 2009; Ryan *et al*., 2009). Therefore, it is necessary to take the water samples of interested area, and use it to prepare the toxicity test using tropical organism to define the sensitivity level. In this study, two tropical freshwater microcrustacean and phytoplankton species, including *D. carinata*. and Scenedesmus sp., were screened in terms of sensitivity to Cd for cost effective pollution monitoring. The two living organisms were chosen due to high sensitivity of microcrustacean 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 quality. Sensitivity isone of the most important criteria for toxicity test to detect the contaminant of interest. Therefore, we aim to develop a practical process which enable to detect Cd 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 *Daphnia carinata* (primary consumer).

MATERIALS AND METHODS

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°C prior to the tests.

Water samples characteristics: The filtered waters from Dau Tieng reservoirwas 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 con- centrations of Ca^{2+} and Mg^{2+}, metals were analysis by ICP/MS.

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 Ecotoxi- cology 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 Pro- tection 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 *Daphnia carinata* (adult, female) used in this study

Figure 2. Morphology of colonies of *Scenedesmus* sp. under a microscope. Scale bar: 20 μm

Acute toxicity tests: The 48h 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 °C) 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). Cadmium treatments were prepared by spiking Cd in constituted medium prepared with field-collected water. $CdSO_4.8/3H_20$ was used as Cd salt. Five concentrations of Cd 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.

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	Total 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	$N\text{-}NH4^+$ (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	$N\text{-}NO3^-$ (mg/l)	0,24	22	Mn (mg/l)	0,01
7	$P\text{-}PO4^{3-}$ (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)

Immobilization data were used to determine median lethal concentrations (48 h-LC50). 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.

Algal inhibition test: Bioassays were performed using the green algae *Scenedesmus* sp. To analyze the toxic effect of Cd on the algal growth, serial concentrations of $CdSO_4.8/3H_20$ were tested using the COMBO media prepared without EDTA (Kilham *et al*., 1998).The initial inoculum cell density was 2±0.2 x 10^4 cells/mL, and the assays were performed in triplicate using 125mLflasks containing 25mL of medium. Cultures were incubated at 24°C 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).

RESULTS AND DISCUSSION

The aim of this study is to develop a practical and cost effective procedure to detect cadmium contamination in the freshwater of Sai Gon river. The two organisms were selected due to they originated from tropical freshwater environment and therefore easy to use in Viet Nam environmental condition. In order to evaluate the applicable of the procedure in detection Cd contamination in freshwater of Sai Gon river, field water sample was obtained, and spiked with Cd at different concentration, then put in our toxicity test procedure with a battery of two organisms to detect Cd contamination. The chemical analyzes result of freshwater from upstream of Sai Gon river showed good quality, without metals or herbicides contamination (Table 1). Cadmium was known for its toxicity on human neuron system, and long-term exposure can increase risk of kidney diseases (Kido *et al*., 2003). Cd 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 Cd contamination of the freshwater reservoir and therefore it is required better supervision to detect Cd 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°C, the experimental EC50 was7.62 µg/L.

In consistent with previous study, that Cd has high toxicity with *Daphnia carinata* with EC50 was 3.6 µg/L. The results clearly demonstrated that the proposed toxicity testing procedurewith *D. carinata* is sensitive and can be considered as auseful tool for Cd pollution monitoring control in freshwater of Sai Gon river.

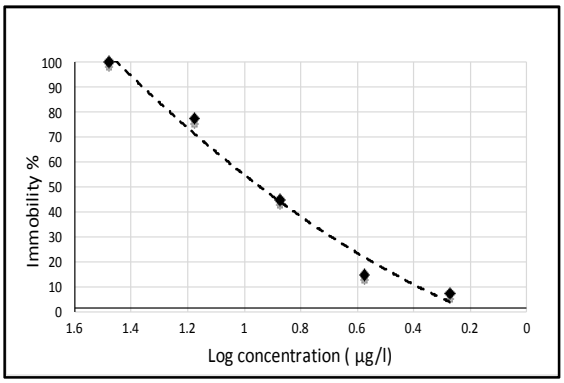

Figure 3: *Daphnia* concentration-immobilization rate curve. Log concentration of cadmium is presented in x axis, and immobility percentage of *Daphnia* at 48 hours is showed in y axis.

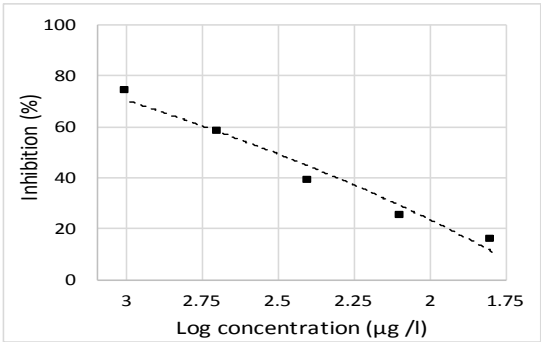

Figure 4. Algal concentration-inhibition (growth rate) curve. Log concentration of cadmium is presented in x axis, and growth rate inhibition percentage of *Scenedesmus* algae at 72 hours is showed in y axis

According the environmental law of Viet Nam, the best surface water quality can have up to 50 µg/l of Cd, this level was known to have no effect on human health. The EC50 value was 7.62 µg/l for Cd make this organism a promise tool for detection Cd pollution. Beside of *D. carinata*, freshwater microalgae *Scenedesmus* sp. was also use to dectect Cadmium 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°C. The result revealed the growth inhibition effect of Cd on the fresh water algae.The IC50 value was 328.5 µg/Lwas higher than *S. obiquus* (58 µg/L)(Monteiro *et al*., 2011). Beside of that, Cadmium is strictly control at very low level in surface water due to its high toxicity to human health. In Viet Nam, the highest allowance value of Cd in surface water is 10µg/L. Therefore, the EC50 value of *Scenedesmus* in this study was more than 30 times higher than the legal threshold of water quality. And therefore, it is only useful to detect heavily Cd contamination water samples. However, the tolerance of the algae to the toxic heavy metal Cd give a hint for new strategy of using it in bioremediation of Cd contamination waste water by simply adding required nutrients to support algae growth and they may extract Cd from water into its biomass. Of course, this potential strategy need further study to investigate the ability to absord and accumulate Cd in cells of the fresh water algae.

Conclusion

The result showed that water flea *D. carinata* was much more sensitive than freshwater algae *Scenedesmus* to Cd contamination in water, and therefore is potential bioindicator for the assessment of Cd pollution in Sai Gon river. Beside of high sensitivity, the toxicity test procedure using two tropical aquatic organisms is also cost effective, and easy to apply. This procedure can be used to daily test water quality to detectthe introduction of toxic pollutants such as heavy metal Cd. Moreover, the tropical *D. carinata* due to its sensitivity to heavy metals contaminants should be further investigated to use as first diagnosis test to detect toxicity in freshwater of Sai Gon River. 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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