

THE TOXICITY OF LEAD TO THE FRESHWATER MICROALGAE *Scenedesmus* AND THE WATER FLEA *Daphnia carinata*

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SUMMARY

Sai Gon river is an important source for water supply in Ho Chi Minh City. However, its water quality is degrading gradually due to rapid population growth, increasing urbanization, and industrialization. The river is continuously loaded with xenobiotics released by anthropogenic activities. Among pollutants, heavy metals are considered the most toxic elements to aquatic living organisms and human health. The aim of this study is to assess the sensibility of freshwater microalga *Scenedesmus* sp. and water flea *Daphnia carinata*, two freshwater species from Viet Nam to lead (Pb). After physical and chemical characterization, field water samples from the upstream of Sai Gon River were used as dilution water in toxicity tests. With water flea *D. carinata*, the EC50 value of 48 h immobilization experiment was 121.64 µg/L for Pb. Growth inhibition of the algae cells was determined following exposure for 96 h, and EC50 values of Pb was 14.76 mg/L. The results showed that Pb was highly toxic to *D. carinata* and harmful to freshwater algae *Scenedesmus* sp. Based on the observed high sensitivity with Pb, *D. carinata* is a potential bioindicator for the assessment of Pb pollution in the Sai Gon River. While lead-tolerance alga *Scenedesmus* sp. calls for further investigation on metal uptake capacity and utilization in the Pb contaminated water treatment.

Key words: acute toxicity, *Daphnia*, algae, freshwater, Lead (Pb), Sai Gon River, *Scenedesmus* sp.

INTRODUCTION

In Viet Nam, fresh surface water pollution and deterioration is an important problem because it has a significant impact on human health and aquatic ecosystem. However, practically monitoring of freshwater quality is still inadequate and facing with many challenges such as human resources, equipment, and financial issues. In recent years, the quick induction of urbanization and industrialization have become the main reasons for increasing water demands, and water pollution caused by anthropogenic activity. Therefore, it urgently called for a better procedure to monitor the quality of water in the Sai Gon River system, which are important water resources for Ho Chi

Minh, the biggest city in South of Viet Nam. Among the variety of contaminants that can pollute the fresh water and toxic to human health, heavy metals are of great concern due to their toxicity and tolerance once introduced into the aquatic environment. The objective of this study was to investigate the possibility of using tropical freshwater living creatures to detect the contaminated Pb in the water through the acute toxicity test. The results of this study are useful for developing a practical and cost-effective procedure to early detect lead pollution in surface water.

Metals are among the most intensively studied pollutants in freshwater environments. Many 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 the environment from many anthropogenic activities such as industrial, agricultural, and mining processes, then they become tolerant pollutants and pose significant risks to living creatures in the ecosystem including humans (Lanctôt *et al.*, 2016; Schwarzenbach *et al.*, 2010; Tomasiks, Warren, 1996). While some metals play vital roles in the living processes of organisms, some others do not. On another hand, it is worth of noting that all metals become toxicants while reaching a concentration threshold (Wetzel, 2001). The previous studies have pointed out that metals are indestructible and can be accumulated in the 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 on living organisms have been well defined and considered as a major threat to aquatic biodiversity (Lanctôt *et al.*, 2016; Millennium Ecosystem Assessment (Program), 2005; Moldovan *et al.*, 2013; Van *et al.*, 2013). Recently, it has been found that toxicity of dissolved metals in water regulated by the variety of water physical and chemical characteristics such as pH, alkalinity, dissolved organic carbon (DOC) and hardness (De Schampelaere, Janssen, 2004; Hoang *et al.*, 2004; Jo *et al.*, 2010; Linbo *et al.*, 2009; Ryan *et al.*, 2009).

In this study, two tropical freshwater microcrustacean and phytoplankton species, including *D. carinata* and *Scenedesmus* sp. were screened in terms of the sensitivity to Pb for cost-effective pollution monitoring. The two living organisms were chosen due to the 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 a criterion of response in toxicity tests. Moreover, the inhibition of algae's population in the aquatic environment can imply the chain

reaction on ecological food chains in the water environment (Kumar *et al.*, 2014).

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 the sensitivity of an organism to the contaminant of interest. Therefore, we aim to develop a practical process that enables us to detect of Pb pollution in freshwater using a battery of organisms. The test battery consists of two species representatives of two consecutive trophic levels: microalga *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) on April 4, 2019. The water sample was transferred to the Environmental Toxicology Laboratory, Institute for Environment and Resources in Ho Chi Minh 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 reservoir was analyzed for water quality parameters that may affect the bioavailability of dissolved metals and the survival and growth of the two organisms of test battery, alkalinity and hardness, pH, trace metals and pesticides (EPA 3510C and EPA 8270D). Total hardness was determined based on concentrations of Ca²⁺ and Mg²⁺ which analysed with ICP-OES (Perkin Emler), metals were analyzed using ICP-MS 7700x (Agilent).

Test organisms

Organisms used in the present study were *D. carinata* and freshwater alga *Scenedesmus* spp. 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 – Ho Chi Minh 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, cerrophyll 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* spp. 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 μ m.

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 °C) for tropical species. Neonates of *D. carinata* (age \leq

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). Lead treatments were prepared by spiking Pb in a constituted medium prepared with field-collected water. $\text{Pb}(\text{NO}_3)_2$ was used as Pb salt. Five two-fold serial dilution concentrations starting from 200 μg Pb/L to 12.5 μg Pb/L 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-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 Pb on the algal growth, a two-fold serial dilutions starting from 20 mg Pb/L to 1.25 mg Pb/L serial concentrations of $\text{Pb}(\text{NO}_3)_2$ were tested using the COMBO media prepared without EDTA (Kilham *et al.*, 1998). The initial inoculum cell density was $2 \pm 0.2 \cdot 10^4$ cells/mL, and the assays were performed in triplicate using 125mLflasks containing 25 mL of medium. Cultures were incubated at 24°C in constant light (4,000 Lux), and the algal growth was estimated by absorbance readings at 750nm after 96 h 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

In order to evaluate the application of the procedure in detection of Pb once introducing

into freshwater of Sai Gon river, a field water sample was obtained, and spiked with Pb at different concentration, then put in our toxicity test procedure with a battery of two organisms to

detect Pb contamination. The chemically analyzed 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.

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-NH ₄ ⁺ (mg/l)	BDL (LOD = 0.03)	20	Cu (mg/l)	0.090
5	Cl ⁻ (mg/l)	4.10	21	Ni (mg/l)	BDL (LOD = 0.004)
6	N-NO ₃ ⁻ (mg/l)	0.24	22	Mn (mg/l)	0.010
7	P-PO ₄ ³⁻ (mg/l)	BDL (LOD = 0.01)	23	Hg (mg/l)	BDL (LOD = 0.0003)
8	TotalN (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)

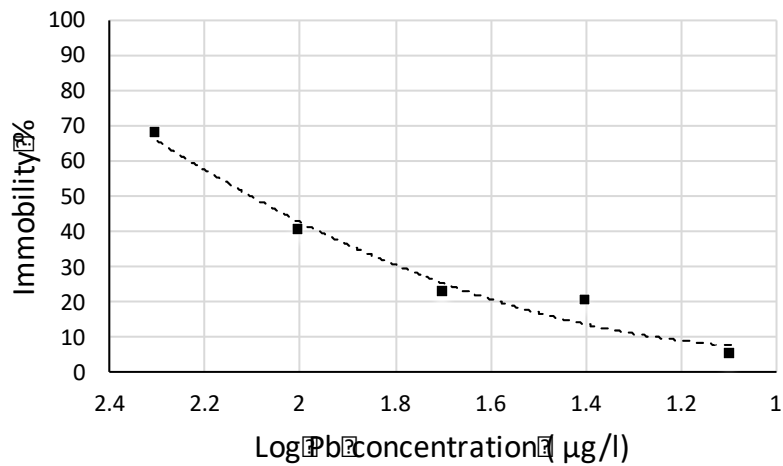


Figure 3. *Daphnia* concentration-immobilization rate curve. Log concentration of lead is presented in x axis, and immobility percentage of *Daphnia* at 48 hours is showed in y axis.

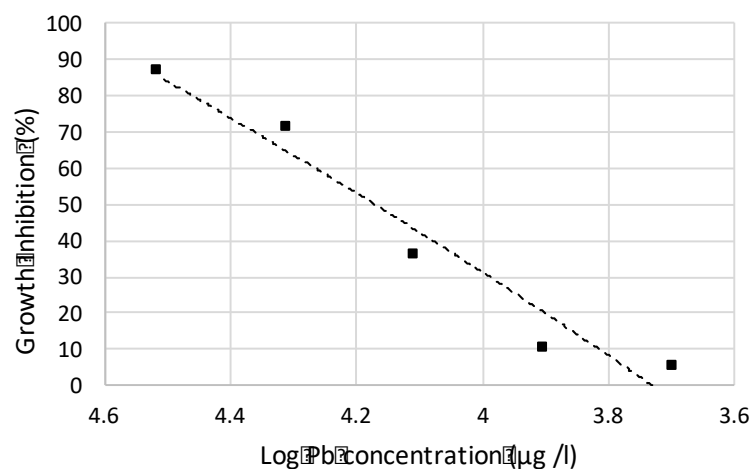


Figure 4. Algal concentration-inhibition (growth rate) curve. Log₁₀ concentration of lead is presented in x axis, and growth rate inhibition percentage of *Scenedesmus* alga at 72 hours is showed in y axis.

Lead is a popularly used metal in a variety of industrial products such as batteries, cable sheaths, machinery manufacturing, shipbuilding, light industry, lead oxide, radiation protection, and other industries.

Lead can accumulate in the skin, muscle, kidney, and liver of fish. At a level of 100 ppb, lead can restrain the functions of fish gills. Therefore, people who consume lead contaminated fish can be exposed to risk of lead-contamination at high level (Wright, Welbourn, 2002). When lead level reaches 500 ppb in an algae cell biomass, the inhibition of photosynthesis enzymes can happen. The reduction of photosynthesis, in turn, significantly declines the algae's population, which causes successive consequence of food supply shortage for other aquatic organisms. The toxicity of lead is higher in the water environment with low pH and hardness.

Due to the increasing extent of industrialization in recent years, it is a risk for Pb contamination in the freshwater reservoir, and therefore it is required better supervision to detect Pb 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 EC₅₀ value of 48 h immobilization experiment was 121.61 µg/L. The

result showed that the sensitivity of *D. carinata* to Pb in this study was higher than *Daphnia magna* with EC₅₀ of 290 µg/L (Okamoto *et al.*, 2015), 694.6 µg/L (Kim *et al.*, 2017). Besides that, the toxicity testing procedure showed that *D. carinata* was sensitive to Pb in the spiked Dau Tieng water. According to the environmental law of Viet Nam, the highest Pb levels in surface water can be up to 50 µg/l, this level was known to have no effect on human health. Therefore, with EC₅₀ value 121.61

µg/l the proposed testing procedure using *D. carinata* is enough sensitivity to detect lead contamination surface water.

Beside *D. carinata*, freshwater microalgae *Scenedesmus* sp. was also used to detect lead 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 Dau Tieng Reservoir, the test medium has pH = 7.6, EC = 278 (µS/cm), DO = 6.9 (mg/L) and temperature 26°C. The result pointed out the growth inhibition effect of Pb on the fresh-water algae. The IC₅₀ value was 14.76 mg/L was higher than *Scenedesmus regularis* (7.2 mg/L) which has been reported previously (Wan Omar *et al.*, 2017). Wan Omar *et al.* (2017) found that at level 7.2 mg/L, Pb caused a significant reduction of total

protein amount in algae cells. However, the observed growth inhibition effect of Pb on the fresh-water algae *Scenedesmus* sp. only happens at high concentration, and lead tolerant ability of the freshwater algae.

Lead is strictly controlled at a low level in surface water due to its high toxicity to human health. In Viet Nam, the highest allowed value of Pb in the surface water is 50 µg/L. The EC50 value of *Scenedesmus* in this study was almost 30 times higher than the legal threshold of the water quality, and seemed not to be suitable for detecting Pb contamination in water. However, the *Scenedesmus* sp. in this study proved to have a high tolerance to heavy metal Pb and could be a potential candidate for bioremediation of Pb contamination wastewater.

CONCLUSION

The result in this study showed that the water flea *D. carinata* was much more sensitive than the freshwater alga *Scenedesmus* to Pb contamination in water, and therefore this species is a potential bioindicator for the assessment of Pb pollution in Sai Gon river. This toxicity test using the water flea *D. carinata* can be used to detect the introduction of heavy metal Pb into water of the Sai Gon River as the first protection barrier. The toxic water samples then can be subjected to further analysis to identify the contaminated toxic pollutants. Beside of that, the results revealed that *Scenedesmus* sp. is a promising organism for the bioremediation of Pb contaminated water due to their apparent tolerance to Pb.

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ĐỘC TÍNH CỦA KIM LOẠI CHÌ TRONG NƯỚC ĐỐI VỚI VI TẢO *Scenedesmus* VÀ BỌ NƯỚC *Daphnia carinata*

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TÓM TẮT

Sông Sài Gòn là nguồn nước sinh hoạt quan trọng của thành phố Hồ Chí Minh. Tuy nhiên, hiện nay chất lượng nước sông đang dần bị suy giảm do sự gia tăng nhanh chóng của dân số, cùng với sự gia tăng quá trình đô thị hóa và công nghiệp hóa. Sông Sài Gòn phải liên tục tiếp nhận các nguồn nước thải từ nhiều hoạt động khác nhau của con người và cùng với đó là các chất ô nhiễm. Trong số các chất thải thì kim loại nặng được đánh giá là một trong những nhóm chất có độc tố cao nhất đối với các thủy sinh vật và con người. Mục tiêu của nghiên cứu này nhằm đánh giá độ nhạy của vi tảo nước ngọt *Scenedesmus* và bọ nước *Daphnia carinata* đối với kim loại chì (Pb). Sau khi thu nhận, phân tích các chỉ tiêu hóa lý, các mẫu nước thu từ thượng nguồn sông Sài Gòn được sử dụng làm dung dịch pha loãng trong thử nghiệm độc học. Đối với bọ nước *Daphnia*, nồng độ gây bất động (immobilized concentration) 50% EC50 tại thời điểm 48 giờ là 121,64 $\mu\text{g/L}$. Thử nghiệm ức chế sự tăng trưởng tế bào tảo được theo dõi trong 72 giờ, và giá trị ức chế tăng trưởng 50% EC50 là 14,76 mg/L . Kết quả nghiên cứu cho thấy Pb có độc tính cao với loài bọ nước *D. carinata*, và có hại đối với vi tảo *Scenedesmus*. Dựa trên đặc điểm nhạy cảm với kim loại chì, bọ nước *D. carinata* có thể được dùng như một chỉ thị sinh học tiềm năng trong việc đánh giá ô nhiễm chì trong nước sông Sài Gòn. Trong khi đó, loài tảo *Scenedesmus*, với khả năng chống chịu mạnh với kim loại chì, có thể cần quan tâm nghiên cứu nhằm ứng dụng trong chiến lược xử lý sinh học các vùng nước ngọt ô nhiễm kim loại chì.

Từ khóa: *độc tính cấp, Daphnia, tảo, nước ngọt, chì, sông Sài Gòn, Scenedesmus sp.*