Growth and Metal Removal Efficiency of The Green Algae *Schroederia setigera* and *Selenastrum bibraianum* Exposed to Nickel, Zinc, and Cadmium

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INTRODUCTION

Nowadays, more attention has been paid to heavy metal pollution as the major consequence of the rapid development of industrialization and urbanization. Among trace metals, Ni and Zn are essential components while Cd are non-essential for living things [1]. However, when exceeding a certain concentration, these metals could be toxic to organisms. Thus, the occurrence of these metals at high levels could be one of the biggest concerns for the environment, ecosystem, and human health.

Microalgae play an essential role in aquatic ecosystems such as producing oxygen and being the food source for other organisms in higher trophic levels. Therefore, the adverse effects of metals on microalgae could strongly change the structure of aquatic ecosystems. There have been numerous studies on the detrimental impacts of Zn, Ni, and Cd on the growth, photosynthesis and morphological abnormalities of microalgae [2]. On the other hand, due to the rapid growth and bio-absorption capacity, many microalgae have been known as potential organisms for metal removal with high efficiency and a friendly mean to the environment. Various investigations on the growth of green algae (e.g. *Scenedesmus*, *Chlorella*) and their metal removal capacity were reported [3]. However, the responses of many green algae (e.g. *Schroederia*, *Selenastrum*) under the exposure to heavy metals have not been fully understood. Therefore, this study investigated the growth and heavy metal removal efficiency of the two green algae *Schroederia setigera* and *Selenastrum bibraianum* isolated from Vietnam under the exposures to Zn, Ni, and Cd.

MATERIALS AND METHODS

- **Materials and Methods**

- The metal solutions ($Ni(NO_3)2$, $Zn(NO_3)2$, and $Cd(NO_3)2$) at 1000 mg/L (ICP/MS standard analysis) were purchased from Merck (Germany) and used as mother solutions for the experiments.

- The algal samples were collected from the Nhieu Loc-Thi Nghe Canal in Hochiminh City. The green algae *S. setigera* Lemmermann 1898 and *S. bibraianum* Reinsch 1866 (Fig. 1) were isolated by the pipetting and washing method [4].

- The algae were cultured in the Z8 medium [5] under the laboratory conditions (at 27 ± 1 °C, light intensity around 2500 Lux and light: dark cycle of 12h:12h) [6].

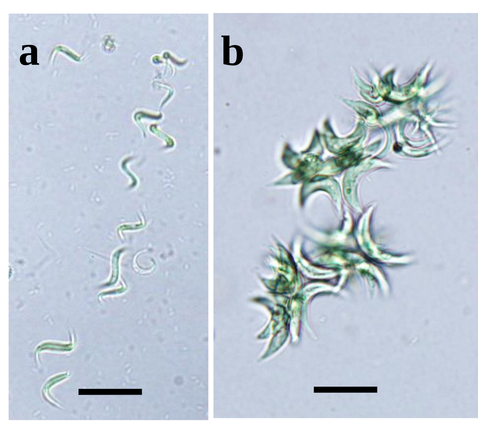
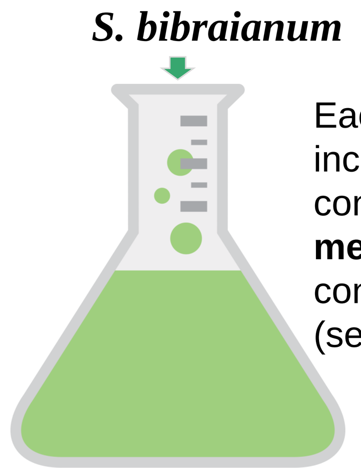


Figure 1 - Schroederia setigera (a) and *Selenastrum bibraianum* (b). Scale bars = 20 µm.

- **Studying the development of the algae upon exposures to three trace metals**



S. setigera or **S. bibraianum**

Each algal species was incubated in 250 mL flasks containing **200 mL of Z8 medium** at the test concentrations of Cd, Ni or Zn (see Table 1)

N= 4

There were **four replicates** with **a similar initial density**

Table 1. Summary of the test concentrations of Cd, Zn, and Ni.

No.	Abbreviations of the exposures	Cd (µg/L)	Zn (µg/L)	Ni (µg/L)
1	**Control**	**0**	**0**	**0**
2	**Cd5**	**5**	-	-
3	**Cd200**	**200**	-	-
4	**Zn100**	-	**100**	-
5	**Zn200**	-	**200**	-
6	**Ni100**	-	-	**100**
7	**Ni200**	-	-	**200**

- The tests **lasted 18 days**.

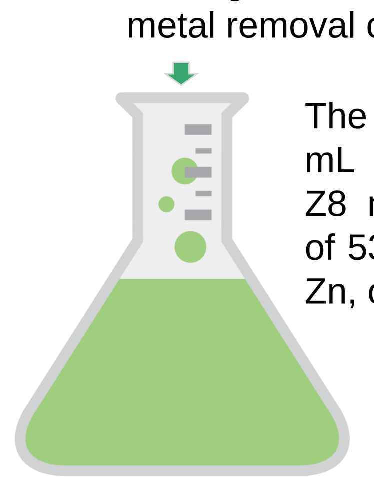
- The algal density in each culturing flask was determined **at the start** and **every two days** of the exposures.

- The algae were counted with a Sedgewick Rafter counting chamber$_{\text{(Graticules Optics, England)}}$ [6].

- **Studying the metal removal of the algae upon exposures to three trace metals**

- This experiment lasted for 16 days.
- The metal concentrations were determined at the day 0 (starting day), day 8th, and day 16th (end of the experiment).
- The Electrothermal Atomic Absorption Spectrometric Method (PinAACle 900Z, Perkin Elmer, USA) was used for metal analysis [6].

S. setigera was selected for testing the metal removal capacity



The alage was incubated in 300 mL flasks containing 250 mL of Z8 medium at the concentration of 537 µg/L for Ni, or 734 µg/L for Zn, or 858 µg/L for Cd.

N= 3

Three replicates were conducted for each test concentration

- **Data treatment**

The growth rate (R) of microalgae was calculated by the equation of R = (lnX1-lnX2)/(t1-t2); where X1 and X2 are algal density at time t1 and t2 [7].

The formula of (E%) = 100x(M1-M2)/M1 was applied to determine the metal removal efficiency by algae; where M1 and M2 are metal concentration at the beginning and the end of the test.

RESULTS AND DISCUSSION

- **Development of the *algae* under exposure to trace metals**

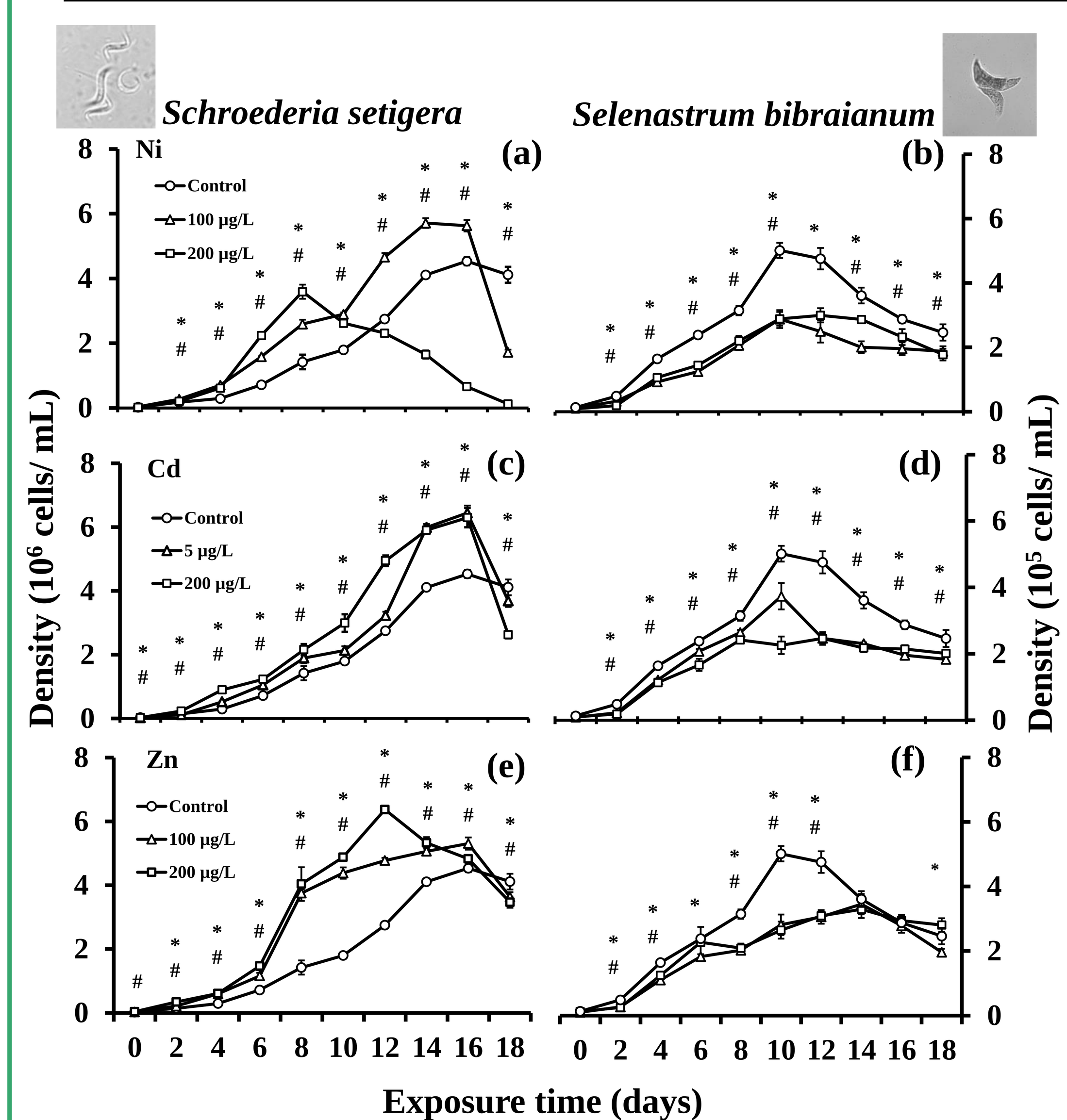


Figure 2 – The density of *Schroederia setigera* and *Selenastrum bibraianum* under exposure to Ni (a, b), Cd (c, d), and Zn (e, f) during 18 days

- The results showed that exposures to three trace metals caused inhibitory on the growth of *S. bibraianum*.

- Seriously, the cell size of *S. bibraianum* in the 200 µg Zn/L treatment was around two times smaller than the control (9.2 ± 2.5 µm compared to 18.9 ± 2.5 µm; Fig. 3).

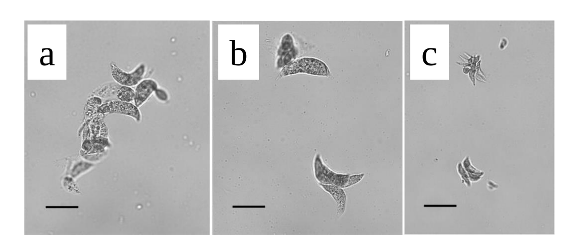


Figure 3 – *Selenastrum bibraianum* in control (a, b) and Zn200 treatment (c). Scale bars = 20 µm.

- In contrast, Zn and Cd at the concentration up to 200 µg/L did not inhibit the growth of *S. setigera* over 18 days of exposure.

- Similarly, the *S. setigera* also grew well during the first 8 days exposed to Ni at 200 µg/L before inhibited after that.

- Previous studies indicated that $Ni_{(100\ \mu g/L)}$ could impair the chlorophyll content and carbon assimilation, while both Zn and Cd strongly affect the photosynthesis and growth of microalgae [8] that help to explain the inhibitory of on the growth of *S. bibraianum* in our study.

- In contrast, Le et al $_{(2019)}$ found a faster development of the green alga *Scenedesmus protuberans* treated with higher than 100 µg Cd/L [9]. The alga *Tetraselmis* sp. could have a Zn tolerance up to 250 µg/L [10]. Besides, the toxic effects of heavy metals on the algae were dependent on the concentrations, exposure time, and the sensitivity of species. These results could be great support to explain the tolerance of *S. setigera* to the metals (Cd, Ni, Zn) in our study.

- **Metal removal by the alga *Schroederia setigera***

Table 2 – Metal removal efficiency of ***Schroederia setigera***

Metals	Metal concentrations (µg/L)			Uptake ratio (%)	
	Starting day	After 8 days	After 16 days	After 8 days	After 16 days
Ni	537	485	472	10	12
Zn	734	292	252	**60**	**66**
Cd	858	750	712	14	18

- The Zn removal efficiency in our study is in line with the study of Kutlu & Mutlu$_{(2017)}$, in which the green alga *Dunaliella* sp. had high efficiency in the Zn removal (up to 85%), but could only reduce less than 10% of Cd out of the test solution [11].

- Besides, Vo et al.$_{(2020)}$ indicated that the diatom *Cyclotella* sp. isolated from Vietnam had a high capacity for both Cr and Cd removal, reaching 99-100% [3]. Wang & Wood$_{(1984)}$ showed the alga *Scenedesmus* sp. could remove 65% of Ni after 24 days [12].

- The difference in Cd and Ni removal efficiency in our study compared to the previous studies may be due to different algae species were used [11]. Moreover, the metal removal capacity of the algae could be affected by the characteristics of the algal culture (e.g. the chemical/ physical characteristic of the medium, the cultural conditions, exposure times, etc.) [12].

CONCLUSIONS

Our study showed the different responses of two green alga *S. setigera* and *S. bibraianum* isolated from Vietnam under exposure to Ni, Cd, and Zn at the concentrations between 5 and 200 µg/L. Although these metals did not cause inhibitory on the growth of *S. setigera*, the growth of *S. bibraianum* was inhibited during 18 days of the exposure.

Our results indicated that Ni and Zn at the concentrations within the permissible limit according to Vietnam Technical Regulation for surface water quality (QCVN 08-MT:2015/BTNMT) could cause negative effects on the growth of the *S. bibraianum*. Therefore, further investigations on the safe concentrations of these metals should be suggested to protect the ecosystem balance.

On the other hand, the alga *S. setigera* had high efficiency in Zn removal, thus using *S. setigera* as an organism for biotechnology to treat Zn in wastewater is recommended.

References:

1. Walker C.H., Hopkin S.P., Sibly R.M., Peakall D.B. - Principles of ecotoxicology, 2nd edition, Taylor & Francis, London, 1996.
2. Ouyang H., Kong X.Z., HE W., Qin N., He Q., Wang Y., Wang R., Xu F. - Effects of five heavy metals at sub-lethal concentrations on the growth and photosynthesis of Chlorella vulgaris, Environmental Chemistry, 25 (2012) 3363-3370.
3. Vo M.T., Nguyen V.T, Vo T.M.C., Bui T.N.P., Dao T.S. - Responses of green algae and diatom upon exposure to chromium and cadmium, Vietnam Journal of Science, Technology and Engineering, 62(1) (2020) 69-73.
4. Belcher H. and Swale E. - Culturing algae – a guide for schools and colleges, The Ferry House, UK, 1988.
5. Kotai J. - Instructions for preparation of modified nutrient solution Z8 for algae, Norwegian Institute for Water Research, Oslo B-11(69), 1972, 1-5.
6. APHA - Standard methods for the examination of water and wastewater, 22nd Edition, American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC. USA, 2012.
7. Lobban C.S., Chapman D.J., Kremer B.P. - Experimental phycology – a laboratory manual, Cambridge University Press, 1988.
8. Azeez P.A. and Banerjee D.K. - Nickel uptake and toxicity in cyanobacteria, Toxicological & Environmental Chemistry, 30:1-2 (1991) 43-50.
9. 11Le V.P., Vo M.T., Le N.H.S., Nguyen N.H., Hoang P.T., Vo T.M.C., Dao T.S. - Development of freshwater microalgae under exposure to atrazine and cadmium, Journal of Science Technology Development – Natural Sciences, 3(4) (2019) 299-306.
10. Kumar D., Santhanam P., Ananth S., Deve A.S., Nandakumar R., Prasath B.B., Jeyanthi S., Jayalakshmi T., Ananthi P. - Effects of different dosages of zinc on the growth and biomass in five marine microalgae, International Journal of Fisheries and Aquaculture 6(1) (2014) 1-8.
11. Kutlu B. and Mutlu E. - Growth and bioaccumulation of cadmium, zinc, lead, copper in Dunaliella sp. isolated from Homa lagoon, eastern Aegean Sea, Indian Journal of Geo Marine Science, 46(06) (2017) 1162-1169.
12. Wang H.K. and Wood J.M. - Bioaccumulation of nickel by algae, Environmental Science & Technology, 18 (1984) 106-109.

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