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GROWTH AND METAL REMOVAL EFFICIENCY OF THE GREEN ALGAE *SCHROEDERIA SETIGERA* AND *SELENASTRUM BIBRAIANUM* EXPOSED TO NICKEL, ZINC, AND CADMIUM

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Abstract. Heavy metal contamination is among the globally environmental and ecological concerns. In this study we assessed the development of the two green algae *Schroederia setigera* and *Selenastrum bibraianum* under exposures to 5 - 200 µg/L of Ni, Zn, and Cd in the laboratory conditions. Heavy metal removal efficiency of *S. setigera* was also tested in 537 µg Ni/L, 734 µg Zn/L, and 858 µg Cd/L. We found that the exposures with these heavy metals caused inhibitory on the growth of *S. bibraianum*. The *S. bibraianum* cell size in the 200 µg Zn/L treatment was around two times smaller than the control. However, Zn and Cd at the concentration of 200 µg/L did not inhibit the growth of *S. setigera* over 18 days of exposure. The *S. setigera* also grew well during 8 days exposed to Ni at the same concentration. Besides, the alga *S. setigera* could remove 66 % of Zn, 18 % of Cd and 12 % of Ni out of the test medium after 16 days of incubation. The Vietnam Technical Regulation related to metals should be considered for ecological protection. We recommend to test the metal removal by the alga *S. setigera* at pilot scale prior to apply it *in situ*.

Keywords: green algae, heavy metals, toxicity, water treatment technology.

Classification numbers: 3.4.2, 3.6.1.

1. INTRODUCTION

Nowadays, more attention has been paid to heavy metal pollution in the environment due to its negative effects on the environmental quality and ecological health. The rapid development of industrialization and urbanization in lasted decades have led to the metal pollution in aquatic environment [1]. Like many other metals, Ni and Zn are mainly derived from industrial waste and agricultural runoff [2], whereas the major emission of Cd is from mining and smelting

activities [3]. In water environment, Cd, Zn, and Ni concentrations could be higher than 190, 3700, and 800 µg/L, respectively [4, 5].

Being natural elements, some trace metals (e.g. Zn, Ni) have been known as the essential components for the normal physiological and biochemical activities of living things [6]. On the other hand, other metals (e.g. Cd, Hg) are not only non-essential elements for living organisms but also toxic to organisms even at low concentrations [7]. Among trace metals, Ni is one of the essential elements for enzyme functions hence plays an important role in cellular physiology, while Zn is a basic component for numerous enzymes related to photosynthesis and metabolisms of plants [8, 9, 10]. However, when exceeding a certain concentration, these metals could cause negative effects on organisms, especially Ni at high concentration could act as a potential carcinogen [8, 10]. Similarly, the toxicity of Cd has been reported on numerous organisms such as detrimental effects on plant physiology or altering enzymatic activities [8]. 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 [11, 12]. 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 in laboratory conditions [3, 9, 10]. In contrast, due to rapid growth and bio-absorption capacity, many microalgae have been known as potential organisms for metal removal with a high efficiency and a friendly mean to the environment [3, 13, 14]. Various investigations on the growth of green algae (e.g. *Scenedesmus*, *Chlorella*) and their metal removal capacity were reported in the world [15, 16, 17, 18, 19]. However, such studies in Viet Nam were scared (see Vo *et al*. [3], Dao *et al*. [20]). Responses of many other green algae (e.g. *Schroederia*, *Selenastrum*) under the exposure with heavy metals have not been fully understood. This study investigated the growth and heavy metal removal efficiency of the two green algae *Schroederia setigera* and *Selenastrum bibraianum* from Viet Nam under the exposures to heavy metals of Zn, Ni, and Cd.

2. MATERIALS AND METHODS

2.1. Organisms and chemicals for the experiments

The algae samples were collected from the Nhieu Loc-Thi Nghe Canal in Hochiminh City by the phytoplankton net with a mesh size of 20 µm [21]. The green algae *S. setigera* Lemmermann 1898 and *S. bibraianum* Reinsch 1866 (Fig. 1) were morphologically identified following the systematic taxonomy of Prescott [22] and isolated under microscope by the pipetting and washing method [23]. After isolation, the algae were cultured in the Z8 medium [24] under the laboratory conditions at the temperature of 27 ± 1 ^{o}C, a light intensity of around 2500 Lux and light: dark cycle of 12h:12h [25].

The metal solutions ($Ni(NO_3)_2$, $Zn(NO_3)_2$, and $Cd(NO_3)_2$) at the concentration of 1000 mg/L (ICP/MS standard analysis) were purchased from Merck (Germany) and used as mother solutions for the experiments. All the test medium including Z8 [21], and Z8 containing metals were filtered through 0.22 µm sterilized cup (Millipore Corporation) before starting the experiments.

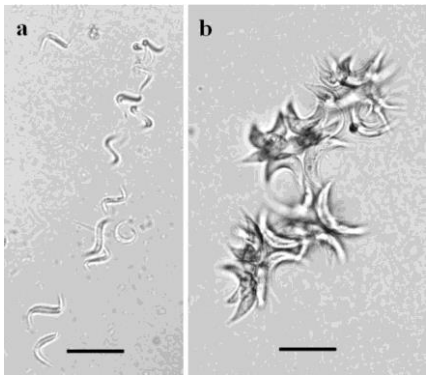

Figure 1. The isolated algal species of *Schroederia setigera* (a) and *Selenastrum bibraianum* (b) used for experiment. Scale bars = 20 µm.

2.2. Experimental setup

We conducted two experiments to: (1) study the development, and (2) study the metal removal of the algae upon exposures to the three trace metals. In the first experiment, the test was conducted according to Muhaemin [17] with minor modifications. Briefly, each algal species was incubated in 250 mL flasks containing 200 mL of the medium at the two different concentrations including 5 and 200 µg/L of Cd, or 100 and 200 µg/L of Ni or Zn. The test concentrations were chosen basing on the Vietnam Technical Regulation for surface water safety (QCVN 08-MT:2015/BTNMT) and the metal concentrations found in nature [4, 5]. Besides, the control was prepared in parallel with the metal exposures by culturing the algae in the medium without metal addition. There were four replicates (n = 4) with a similar initial density of algae in each test concentration. The pH values in each treatment including the control were measured (Metrohm 744) at the beginning and end of the test and did not alter significantly, ranged from 7.3 - 7.6. The experiment on the growth of the algae exposed to metals lasted 18 days in the laboratory conditions as mentioned above. At the starting and every two days of the experiment, sub-samples (2 mL) from each culturing flasks were collected, fixed with Lugol solution, and the algae were counted with a Sedgewick Rafter counting chamber (Graticules Optics, England) under the microscope (Optika B150, Italy) [21]. Every time of algal enumeration, at least 400 cells of *S. setigera* and 400 colonies of *S. bibraianum* were counted to get a reliable algal density as guided by Sournia [21]. At the start of experiment, the mean densities of *S. setigera* in the flasks of the control, 100 µg Ni/L, 200 µg Ni/L, 5 µg Cd/L, 200 µg Cd/L, 100 µg Zn/L, and 200 µg Zn/L were 24850, 35825, 25950, 23575, 28425, 28800, and 35448 cells/mL. Similarly, the densities *S. bibraianum*, at the start of experiment, in the control, 100 µg Ni/L, 200 µg Ni/L, 5 µg Cd/L, 200 µg Cd/L, 100 µg Zn/L, and 200 µg Zn/L were 13025, 11150, 8500, 9200, 10200, 9900, and 12150 cells/mL.

Based on the results of the first experiment, *S. setigera* was selected for the second experiment, testing the metal removal capacity. In the second experiment, the *S. setigera* was incubated in the 300 mL flask containing 250 mL of the medium at the concentration of 537 µg/L for Ni, or 734 µg/L for Zn, or 858 µg/L for Cd. These metal concentrations were determined by the chemical analysis with the Electrothermal Atomic Absorption Spectrometric Method (PinAACle 900Z, Perkin Elmer, USA) [25]. All the test solutions used for the experiment were filtered through the 0.2 µm filter as mentioned above. For each test concentration, three replicates were conducted (n = 3). The pH and electrical conductivity of the

test solutions were measured with a multi-detector (Metrohm 744) whereas the hardness and alkalinity were determined by titration [25]. Previously investigations found that some physical and chemical parameters such as pH, hardness and alkalinity could regulate the bioavailablity and toxicity of metals. For example, when the alkalinity, hardness and pH decrease the concentrations of free ionic metals increase consequently bioavailability and toxicity enhancement of the metals [26, 27]. This experiment lasted for 16 days. At the day 0 (starting day), day 8th, and day 16th (end of the experiment), sub-samples were taken from each treatment by filtering 50 mL algae solution through the 0.45 µm filter (Sartorius, Germany), and acidified with HNO_3 (Merck, Germany). The sub-samples were used to determine metal concentrations (PinAACle 900Z, Perkin Elmer, USA) for evaluating the metal removal efficiency of the alga, *S. setigera* [25].

2.3. 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 [28]. The Kruskal-Wallis test (Sigma plot 12.0) was applied to calculate the statistically significant difference of the density and the growth rate between the control and metal exposures. Additionally, the metal removal efficiency by algae was determined following the formula of

$$(E\%) = 100 \times (M1-M2)/M1,$$

where M1 and M2 are metal concentration at the beginning and the end of the test.

3. RESULTS AND DISCUSSION

3.1. Development of the *S. setigera* and *S. bibraianum* under exposure to trace metals

Over 18 days of the test with *S. setigera*, the algal density in the control and the exposure to Zn (100 and 200 µg/L), Cd (5 and 200 µg/L), and Ni (100µg/L) constantly increased during the first 16 days of the experiment and began to decline at the end of the test (Fig. 2a, c, e). Compared to the control, the growth of *S. setigera* in these metal exposures was not inhibited but even significantly higher ($p < 0.05$; Kruskal-Wallis test) during the 16 days of the experiment, the log and stable phases of the algal development. However, the density of *S. setigera* treated with 200 µg Ni/L decreased after 8 days of incubation (Fig. 2a). The density of *S. bibraianum* in the control and the metal exposures steadily increased during the first 10 days of the test, then decreased until the end of the experiment. However, the density of *S. bibraianum* in all metals exposures was lower than the control during incubation (Figs. 2b, d, f). Seriously, we found a much smaller cell size of *S. bibraianum* treated with 200 µg Zn/L of which the cell length in Zn treatment (9.2 ± 2.5 µm) was around two times smaller than that in the control (18.9 ± 2.5 µm) (Fig. 3). The cell size was not among the planned endpoints of the microalgae of our study, hence this record seemed to be qualitative rather than quantitative.

During the first 8 days of experiment, the mean growth rate of *S. setigera* in the control was 0.50 fold/day. However, that in the three metal exposures was from 0.54 - 0.61 fold/day, significantly higher than the control (Table 1). Over the 16 days of experiment, the period to get a stable developmental phase of *S. setigera*, the growth rate of *S. setigera* in the control was 0.32 fold/day which was similar to the growth rate in 100 µg Zn/L and 200 µg Cd/L, but lower than the growth rate in the 5 µg Cd/L and higher than that growth rate in other treatments (100 µg Ni/L, 200 µg Ni/L, and 200 µg Zn/L; Table 1).

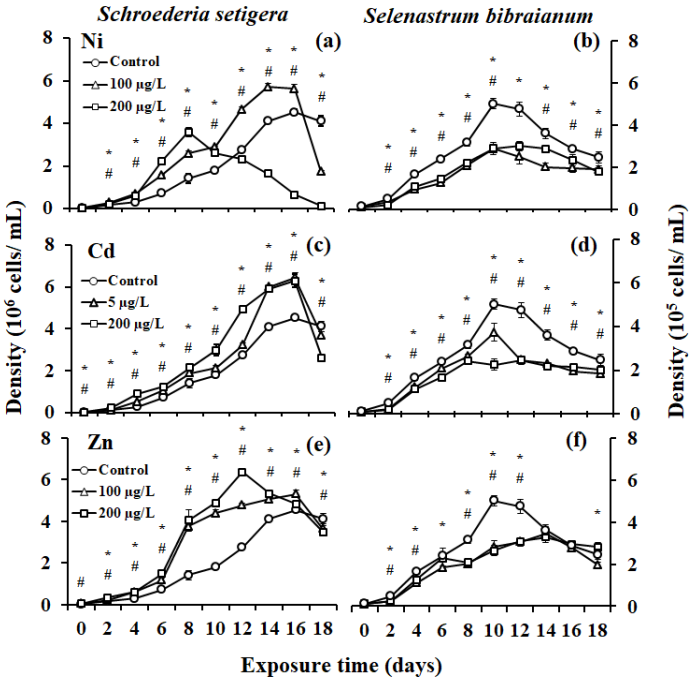

Figure 2. The density of *Schroederia setigera* and *Selenastrum bibraianum* under exposure of Ni (a, b), Cd (c, d), and Zn (e, f) during 18 days of the experiment. The asterisks (*) indicated the significant difference of the algal density between the control and the lower metal concentrations (5 µg/L of Cd; 100 µg/L of Zn or Ni) by the Kruskal-Wallis test ($p < 0.05$). The symbol (#) indicated the significant difference of the algal density between the control and the higher metal concentration (200 µg/L of Cd, Zn or Ni) by the Kruskal-Wallis test ($p < 0.05$).

Table 1. Growth rate of *S. setigera* and *S. bibraianum* exposed to Ni, Zn and Cd. The asterisk "*" indicated the statistic difference between the control and exposures by Kruskal-Wallis test.

Exposures	**Growth rate (fold/day)**			
	Schroederia setigera		*Selenastrum bibraianum*	
	During 8 days	During 16 days	During 8 days	During 16 days
Control	0.50 ± 0.012	0.32 ± 0.005	0.39 ± 0.003	0.19 ± 0.003
Ni 100 µg/L	0.53* ± 0.004	0.31* ± 0.001	0.36* ± 0.012	0.18* ± 0.005
Ni 200 µg/L	0.61* ± 0.010	0.20* ± 0.004	0.41* ± 0.006	0.21* ± 0.006
Zn 100 µg/L	0.61* ± 0.004	0.33 ± 0.002	0.38* ± 0.004	0.21* ± 0.006
Zn 200 µg/L	0.59* ± 0.014	0.31* ± 0.003	0.36* ± 0.007	0.20 ± 0.006
Cd 5 µg/L	0.55* ± 0.013	0.35* ± 0.009	0.42* ± 0.005	0.19 ± 0.004
Cd 200 µg/L	0.54* ± 0.025	0.34 ± 0.007	0.39 ± 0.007	0.19 ± 0.003

In the experiment with *S. bibraianum*, the growth rate of the alga in the control was higher than that of 100 µg Ni/L, 100 and 200 µg Zn/L, similar to that of 200 µg Cd/L, but lower than

that of the remaining metal treatments. The growth rate of *S. bibraianum* over 16 testing days in the control was higher than that of the 100 µg Ni/L, similar to that of 200 µg Zn/L and two Cd treatments, and lower than that of 200 µg Ni/L and 100 µg Zn/L (Table 1).

The growth of *S. bibraianum* in our results was in line with several previous studies which showed an inhibitory on the algal growth in metal treatments. Fezy *et al*. [29] noted that the growth rate of the diatom *Navicula pelliculosa* exposed to 100 µg Ni/L reduced around 50 % after 14 days. Moreover, Ni (100 µg/L) also had impaired the chlorophyll content and carbon assimilation of the cyanobacterium, *Spirulina platensis*, and significantly reduced in the growth rate of another cyanobacterium, *Anacystis nidulans* [30]. Similarly, some other studies indicated Cd at high concentrations had adverse influences on the metabolism of the cells and could cause an inhibitory on the algal growth. The presence of Cd at the concentration up to 500 µg/L caused a 50 % reduction in the cell density of the green alga *Dunaliella salina* due to the reduction in the content of essential elements (e.g. Mg, Ca) for the metabolisms in the cells of the alga [31]. Besides, the inhibitory of Cd at the high concentration of around 500 µg/L on the growth of two algal species *Scenedesmus acuminatus* and *Scenedesmus protuberans* isolated from Viet Nam was also reported [3]. Moreover, Ouyang *et al*. [9] indicated that both Zn and Cd at the concentration of 325 µg/L and 560 µg/L, respectively, impaired the photosynthesis and growth of the green alga *Chlorella vulgaris*. On the other hand, the toxic effects of heavy metals on the algae were dependent on the concentrations, exposure time, and the sensitivity of species [9, 32]. Hence, previous studies help to explain the different responses of two algae under exposure to Ni, Cd, and Zn in the current experiment.

In this study, we did not measure the biochemical characteristics of *S. setigera* and *S. bibraianum* exposed to metals. However, it is found that at high concentrations, Zn out competed other metals in binding on the active sites in biochemical metabolisms in cells, consequently productivity inhibition [33]. More specific, Zn induced a significant changes of reactive oxygen species in cells of green alga *Raphidocelis subcapitata* [34] hence energy cost for the cells. Besides, Zn could replace Mg in chlorophyll molecules, and affect the water-splitting of photosystem II hence impacting photosynthesis and reducing in chlorophyll a content in cells of microalgae [34]. Therefore, in the current study, the exposure to Zn could disorder some processes and caused energy cost in *S. bibraianum* consequently cell length reduction of this species. We suggest that there should be studies on the biochemical responses of the *S. bibraianum* exposed to metals to clarify.

Our results also revealed the alga *S. bibraianum* is more sensitive than *S. setigera* in the exposure to the metals Ni, Cd and Zn. The concentrations of Cd, Ni and Zn in Vietnam Technical Regulation (QCVN) which are allowed in surface water are 5, 100, and 500 µg/L, respectively. However, in our study, *S. bibraianum* was negatively impacted by the tested metals at the concentrations within the QCVN (08-MT:2015/BTNMT). Therefore, there should be studies to adjust the levels of Cd, Zn and Ni in the QCVN for ecological health protection.

The tolerance of *S. setigera* to the metals (Cd, Ni, Zn) in our study is in line with several previous investigations. Le *et al.* [35] found a faster development of the green alga *S. protuberans* treated with higher than 100 µg Cd/L. The alga *Tetraselmis* sp. could have a Zn tolerance up to 250 µg/L [36]. The diatom *Cyclotella* sp. could have similar growth rates in control and Cd treatment [3]. Dao *et al*. [20] reported the well growth of the cyanobacterium *Pseudanabaena mucicola* exposed to more than 1000 µg Cr/L.

Besides, the metals Zn and Ni are among the essential elements for many biochemical processes in the algae [8, 9, 10] so the presence of these metals in the test medium may enhance the development and vegetative reproduction of *S. setigera* consequently density increase.

Apparently, Cd is not an essential element, but could cause toxic effects on organisms. The alga *S. setigera* could be tolerant to Cd as mentioned above and confirmed below (subsection 3.2). However, it is unknown why the metal Cd induced the algal density increase. Further investigations on the biochemical responses of microalgae to Cd are suggested to clarify. Because of the metal tolerance of *S. setigera*, this species was used in the second test on the metal removal capacity and the metal tolerance of this species is confirmed as below.

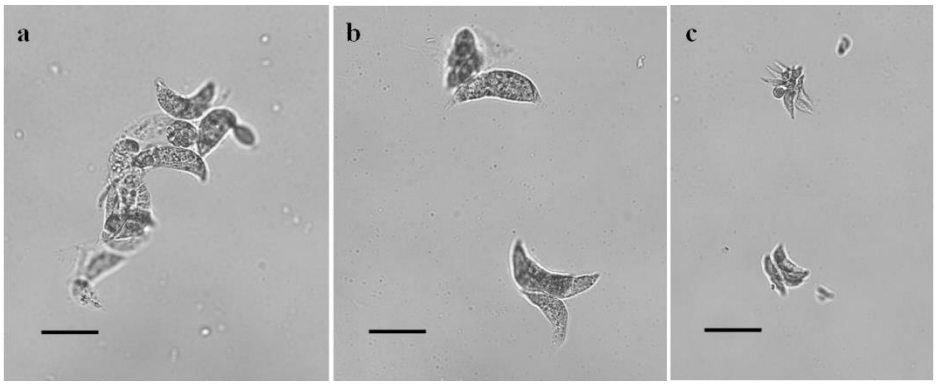

Figure 3. The green alga *Selenastrum bibraianum* in control (a, b) and Zn200 (c) indicating the much smaller cell size in the treatment with 200 µg Zn/L. Scale bars = 20 µm.

3.2. 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 pH of the alga solutions was from 7.1 - 7.2, quite similar among the treatments with three metals (Ni, Zn, Cd) which are favorable for algal development [25]. Similarly, the hardness, the alkalinity, and the electrical conductivity in each treatment ranged from 39 - 42 (mg $CaCO_3$/L), 34 - 51 (mg $CaCO_3$/L), and 810 - 860 (µS/cm), respectively. The hardness and alkalinity revealed the soft water characteristics which enhance the metal mobility in water. During the time of incubation, the alga *S. setigera* grew well in all metal treatments and the growth rate of the alga after 8 and 16 days of the exposure ranged from 0.27 - 0.3 folds/day, and 0.17 - 0.2 folds/day, respectively. The mean concentrations of Ni, Cd, and Zn were 485, 292, and 750 µg/L after 8 days of incubation and those at the end of the test (16 days) were 472, 252, and 712 µg/L, respectively (Table 2). We found that *S. setigera* could remove around 60 % of Zn out of the solutions after 8 days and up to 66 % after 16 days of the incubation. The alga

removed 10 and 12 % of Ni after 8 and 16 days of incubation, respectively. For Cd treatment, the alga was able to remove the metal 14 and 18 %, respectively, after 8 and 16 days of the experiment.

Microalgae isolated from Viet Nam were known to have a high efficiency in metal removal in the laboratory conditions. The cyanobacterium *P. mucicola* isolated from Viet Nam could reduce 71 % of Cr in the test medium after 7 days of incubation [20]. The green algae *S. acuminatus* and *S. protuberans* could remove up to 96 % of the metal Cr out of the solutions after 14 days, while the diatom *Cyclotella* sp. had a high capacity for both Cr and Cd removal, reaching 99 - 100 % [3]. Therefore, the Cd removal capacity by *S. setigera* in the current study is much lower than by some other microalgae from Viet Nam. However, the Zn removal efficiency in our study is in line with the study of Kutlu & Mutlu [37], in which the authors found that 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. In contrast, Geisweid & Urbach [38] reported that the green alga *Eremosphaera viridis* had high efficiency in Cd removal reaching 85 %. Wang & Wood [39] showed the alga *Scenedesmus* sp. could remove 65 % of Ni after 24 days of exposure which is much higher than the Ni removal by *S. setigera* in our study. 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. Vo *et al*. [3] indicated that the diatom *Cyclotella* sp. had higher efficiency in Cd removal (up to 99 %) compared to two green algae *S. acuminatus* and *S. protuberans* (only 6 - 13 %). Similarly, previous studies have showed differences in Cd removal efficiency among the algae species such as *C. vulgaris*, *Ankistrodesmus braunii*, *E. viridis*, *Dunaliella* sp., and *Spirulina platensis* [37, 38]. Moreover, the metal removal capacity of the algae could be affected by the characteristics of the algal culture (e.g. the chemical/ physical characteristics of the medium, the cultural conditions, exposure times, etc.). Wang and Wood [39] showed the difference of the Ni removal capacity of the algae at different pH conditions, in which the alga *Scenedesmus* sp. was cultured in the medium at the pH of 9 could remove up to 65 % of Ni that was higher compared to the culture at the pH of 4 (23 %). Besides, Kutlu and Mutlu [37] indicated that the metal removal efficiency of the alga *Dunaliella* sp. could be higher when exposed to metals at lower concentrations. Similarly, the time of the exposures and light conditions (e.g. light: dark cycle) in the incubation could also affect the metal removal capacity of the alga *Scenedesmus* sp. which was reported elsewhere [39]. These results could be great support to explain the differences in the metal removal capacity of the alga *S. setigera* in our study compared to previous studies. To our knowledge, it is the first investigation on the metal removal capacity of the green alga *S. setigera*. Our results revealed that the alga *S. setigera* had a high efficiency in the Zn removal (up to 66 %), thus *S. setigera* is recommended as an organism for Zn contaminated wastewater treatment biotechnology.

4. CONCLUSIONS

This study showed the different responses of two green alga *S. setigera* and *S. bibraianum* isolated from Viet Nam 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. The exposure to 200 µg Zn/L caused the significant reduction of cell length of *S. bibraianum.* 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, we also

found that 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. We also suggest to study Zn metal removal by the alga *S. setigera* at pilot scale within a period of around one week. In our knowledge, this is the first report on the growth and metal removal efficiency of *S. setigera* exposed to Ni, Zn and Cd. Further investigations on the toxicity of metals on microalgae as well as the metal removal by microalgae are highly suggested.

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