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Responses of green algae and diatom upon exposure to chromium and cadmium

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Abstract:

In this study, the detrimental effects of trace metals on the growth of phytoplankton and the phytoremediation potential of microalgae from Vietnam are elucidated. Two green algae *Scenedesmus accuminatus var biseratus* and *Scenedesmus protuberans,* along with the diatom species *Cyclotella* sp*.,* were exposed to chromium (Cr) and cadmium (Cd) at three distinct concentrations ranging from 5-761 µgl^{-1} and 18-667 µgl^{-1}, respectively, over a period of 14 days. The results indicated that *S. acuminatus var biseratus* and *Cyclotella* sp. were relatively tolerant to Cr, even at the highest test concentration, while the growth rate of S. *protuberans* was significantly inhibited when exposed to 660 µgl^{-1} of Cr. Only *Cyclotella* sp. showed a high Cd tolerance, whereas Cd at concentrations of 493 and 607 µgl^{-1} prohibited the growth rate of *S. acuminatus* and *S. protuberans*, respectively. Moreover, at the concentrations tested, all three algal species could remove 90-100% of the Cr out of the test medium. The diatom *Cyclotella* sp. could reduce up to 99% of Cd whereas the two green algae could only do not remove more than 13% of Cd from the test medium. We strongly recommend the *Cyclotella* sp. as a candidate for phytoremediation in metal-contaminated water. Our results contribute vital information toward solutions that environmental experts and managers are searching for to resolve pollution caused by trace metal contaminants.

Keywords: ***Cyclotella*** **sp.,** ***Scenedesmus accuminatus var biseratus*****,** ***Scenedesmus protuberans,*** **trace metals.**

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Introduction

Recently, an increase in the concentration of trace metals (e.g. chromium, zinc, copper) in bodies of water such as rivers, lakes, and reservoirs caused by anthropogenic activities has been a concern. Although trace metals (e.g. Cu, Ni, Zn) at low concentrations are essential to the life and growth of organisms, at critical concentrations these metals have been demonstrated to cause harmful effects on the ecosystem and human health [1, 2].

Among the trace metals, Cd and Cr are usually found in industrial wastewater. While Cd is chiefly sourced from mining activities, ceramics, and other industrial activities [3], Cr is derived from tanneries, industrial electroplating, and wood preservation [4]. Cr mainly exists in the environment as two types, hexavalent chromium and trivalent chromium. According to a previous study, chromium (VI) can cause mutation, DNA destruction, genetic modification, and cancer. In contrast, Cr (III) is essential for protein, fat, and carbohydrate metabolism and is an encouraged supplement to the daily diet [4]. On the other hand, the toxicity of Cd is very disturbing to organisms due to its unique properties such as being highly toxic even at low concentrations and its long digestion time [5].

In aquatic ecosystems, microalgae, including green algae and diatom, are primary producers and play an critical role in the food web [6]. Moreover, microalgae are very sensitive to small environmental changes [7]. Therefore, many studies on microalgae exposed to trace metals (e.g. Cd, Cr) at high concentrations have been conducted to evaluate the toxicity of these contaminants. Previous investigations indicated that both Cd and Cr are essential for algal development, however, at a particular concentration, these elements can interfere with biochemical and cellular processes that cause reduced growth or even death in microalgae [8-10].

In Vietnam, more and more attention has been focused on solutions to environmental challenges, especially trace metal pollution. Many studies demonstrated that there has been a

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sharp rise in trace metal concentration in the water bodies of Vietnam, mainly due to wastewater discharge [11-13]. Moreover, Vietnam is located in a tropical area that has a high diversity of species, including phytoplankton. However, to our knowledge, there are few studies on the negative effects of trace metals on microalgae strains originating from Vietnam [14, 15]. Therefore, this study aimed to investigate the development and the absorption capacity of two green algal strains, *Scenedemus acuminatus var biseratus* and *Scenedemus protuberans*, and the diatom species, *Cyclotella* sp., isolated from Vietnam after their exposure to two common trace metals, Cd and Cr, in laboratory conditions.

Materials and methods

The colonial freshwater green algae *Scenedesmus accuminatus var biseratus*, *Scenedesmus protuberans,* and the unicellular brackish water diatom species *Cyclotella* sp. (Figs. 1A, 1B, 1C, respectively) were isolated in several water bodies located in Ho Chi Minh city by pipetting and washing [16]. All algae were cultured in Z8 medium [17]. However, for the Z8 medium used to culture the diatom, the initial water solution was a combination of twice distilled water with a portion of microbial filtered sea water to achieve 3 ppt (‰) salinity and Na_2SiO_3 was added (F/2 medium) [18]. The algae were maintained and tested under a photoperiod of 12 h light (3,000 lux) and 12 dark at 27±1°C [19].

(A) (B) (C)

Fig. 1. The test organisms *Scenedemus acuminatus var biseratus* (A), *Scenedemus protuberans* (B), and *Cyclotella* sp. (C). Scale bars=20 µm.

The Cr^{3+} and Cd^{2+} (from $Cr(NO_3)_3$ and $Cd(NO_3)_2$, respectively) at a concentration of 1,000 mgl^{-1} (Merck, Germany) were used as stock solutions for the experiments. The metals Cr and Cd from stock solutions were combined with the algal medium to achieve the proposed concentrations for the experiment. The medium containing the metals was filtered through 0.2 µm filters (Whatman) prior to testing with microalgae. Each algal species was incubated in a 250 ml flask containing 150 ml of test solution and was exposed to either Cr or Cd at three distinct concentrations, ranging from 5-761 μgl^{-1} and 18-667 μgl^{-1} for Cr and Cd, respectively. A control experiment, in which the algae were not exposed to any trace metal, was also conducted. The physical parameters (e.g. pH and temperature) of each treatment, including the control, at the beginning and end of the test days ranged from 6.8-7.2 pH and 29.4-29.7°C, thus did not change significantly. The electrical conductivity (EC) of the test medium for green algae (Z8 solely) varied between 874-884 μScm^{-1}, whereas that of the medium for diatom (modified Z8 with a salinity of 3‰) ranged from 6.52-6.57 $mScm^{-1}$. Similarly, the hardness (characterized by titration [20]) of the Z8 medium ranged from 37-46 mg $CaCO_3$ l^{-1}, and that of the salty (3‰) Z8 medium ranged from 614-628 mg $CaCO_3$ l^{-1}. The large difference between the EC and hardness values of the two media is related to the amount of salt added into the Z8 medium for diatom cultivation. Sub-samples from each test medium, taken at start and end of the experiment, were filtered (with pore size of 0.45 µm - Sartorius, Germany) and acidified with saturated HNO_3 (Merck) prior to the determination of Cr or Cd concentration by electrothermal atomic absorption spectrometry [20]. Both control and treated samples were prepared in triplicates [21, 22]. Over the 14-day experimental period, sub-samples consisting of 2 ml of algal solution were taken from each flask on the starting day and every two days, and preserved with Lugol solution [23] for cell density enumeration.

The growth rate of microalgae (R) was calculated according to Lobban, et al. (1988) [24] with the equation of $R=(\ln X_2-\ln X_1)/(t_2-t_1)$; where X_1 and X_2 are algal density at time t_1 and t_2. Additionally, the following formula was used in order to calculate the metal uptake ratio $(U\%)=100x(M_1-M_2)/M_1$; where M_1 and M_2 are metal concentrations at the beginning and the end of the test. The Kruskal-Wallis test (Sigma Plot 12.0) was used to calculate the statistically significant difference of the growth rate between control and exposures.

Results and discussion

Influence of chromium on growth rate of microalgae

The growth rate of *S. accuminatus var. biseratus* in the control sample and in the samples exposed to 16 µg Cr l^{-1} was 0.27 folds day^{-1}, whereas the growth rate in the samples exposed to Cr at concentrations of 112 and 761 μgl^{-1} was 0.29 and 0.24 folds day^{-1}, respectively. There was no statistically significant difference in the growth rate of this algal strain between the control and all exposures (Fig. 2A). In the experiment with *S. protuberans,* the growth rate in the control sample and exposures to 5 and 80 µg Cr l^{-1} were similar, approximately 0.10 folds day^{-1}. However, the growth rate in the exposure to 660 µg Cr l^{-1} was inhibited and reached only 0.05 folds day^{-1}. Moreover, there was a statistically significant difference in this parameter between the control sample and the sample exposed to Cr at the highest concentration ($p<0.01$ by Tukey test) (Fig. 2B). Compared to *S. acuminatus var. biseratus*, the growth rate of *Cyclotella* sp. in all treatments, including the control, was lower, varying from 0.16-0.18 folds day^{-1}. Nevertheless, they had the same responses when exposed to Cr. Particularly, a statistically significant difference was not found between the control and all treatments (Fig. 2C).

Previous studies demonstrated that the inhibitory effects of Cr on the microalgal growth of *S. protuberans* depended on concentration in a similar manner as observed in this study. Wong and Chang (1991) [25] showed that the growth of *Chlorella vulgaris* was not inhibited when exposed to Cr at a concentration of 250 µgl^{-1}. On the other hand, when the Cr concentration exceeded 500 µgl^{-1}, there would be inhibition of photosynthesis. At concentration higher than 5,000 µgl^{-1}, Cr increased the cell permeability resulting in inhibition of the growth of *C. vulgaris*. Additionally, another study [26] also indicated that the growth of *Chlorella pyrenoidosa* was inhibited upon exposure to 1,000 µgl^{-1} of Cr for 72 hours. On the contrary, some green algae (*Scenedesmus acutus, S. obliquus, Chlorella fusca, C. vulgaris*) and the cyanobacterium *Pseudanabaena mucicola* could grow well at 1,000 µgl^{-1} of Cr [14, 27], which supports this current study. However, there is no compelling evidence to indicate that *S. acuminatus v. biseratus* and *Cyclotella* sp. are unaffected by Cr. These results could be due to the Cr concentrations in this study being below a threshold that would trigger growth inhibition phenomenon in these algae. Moreover, this study showed that *S. acuminatus var biseratus* and *Cyclotella* sp. were relatively tolerant to Cr that could be useful information for further researches on the treatment of Cr pollution in wastewater using these microalgae.

Influence of cadmium on growth rate of microalgae

In Cd exposures, the growth rate of two green algae strains *S. acuminatus var biseratus*, *S. protuberans* and diatoms species *Cyclotella* sp. ranged from 0.06-0.46, 0.02-0.1, and 0.15-0.18 folds day^{-1}, respectively. Interestingly, when compared to the control, there was a statistical decrease in the growth rate of all the green algae except the diatom *Cyclotella* sp. when Cd exposures were at the highest concentration (Fig. 3).

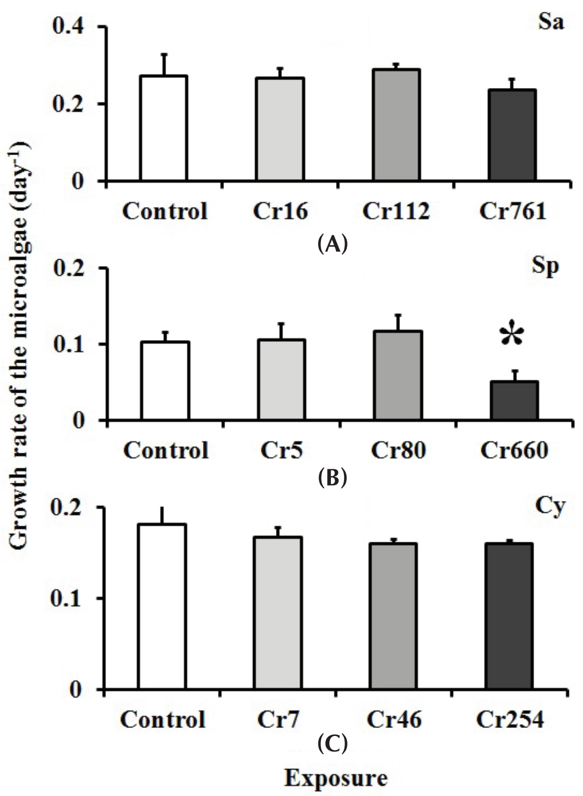

Fig. 2. Growth rate of *S. acuminatus var biseratus* (A), *S. protuberans* (B) and *Cyclotella* sp. (C) in Cr exposures. The asterisk indicates the significant difference ($p<0.05$) between control and Cr exposures by Kruskal-Wallis test.

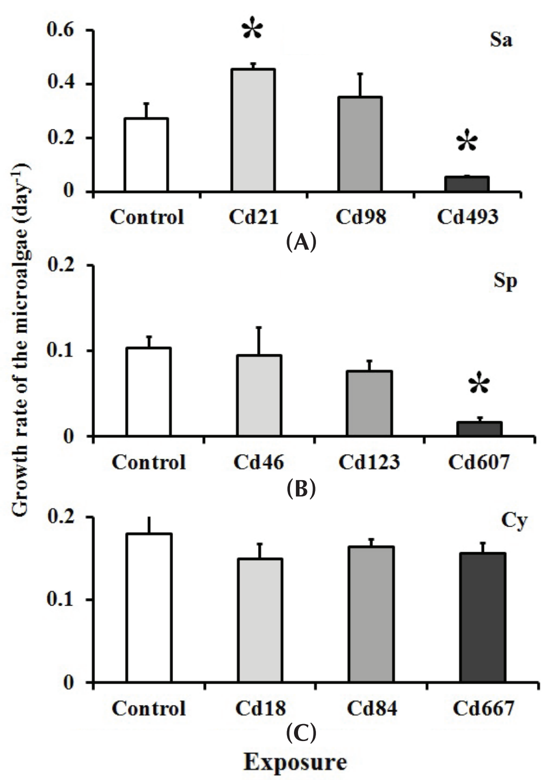

Fig. 3. Growth rate of *S. acuminatus var biseratus* (A), *S. protuberans* (B) and *Cyclotella* sp. (C) in Cd exposures. The asterisk indicates the significant difference ($p<0.05$) between control and Cd exposures by Kruskal-Wallis test.

This result is in total agreement with the findings of previous investigations that revealed the growth inhibition of microalgae could be caused by Cd exposure. For example, Hart and Scaife (1977) [28] showed that Cd at a concentration of 250 μgl^{-1} inhibited the growth rate of the green alga *C. pyrenoidosa*. Similarly, Letty, et al. (2009) [29] determined the detrimental effects of three distinct concentrations of Cd (5, 10, and 20 μgl^{-1}) on cellular viability in the microalgae *Scenedesmus* sp. and *Dunaliella viridis*. Additionally, at Cd concentrations up to 1,000 μgl^{-1} did not impact the growth of diatom *Phaeodactylum tricomutum* [30], which supports our observations of the high tolerance of the diatom *Cyclotella* sp. to Cd exposure (Fig. 3C).

On the other hand, at low concentrations, Cd was demonstrated to significantly stimulate the growth rate of *S. acuminatus var biseratus* (Fig. 3). Although there was no evidence that Cd can stimulate the growth of algae, according to Sbihi, et al. (2012) [10], Cd inhibited the photochemical activity in algae at the concentration of more than 100 μgl^{-1}. The current results show that the diatom species *Cyclotella* sp. was more tolerant to Cd than the other two strains of green algae *S. acuminatus var biseratus* and *S. protuberans*.

Cr and Cd uptake capacity of microalgae

Cyclotella sp. was demonstrated to have a high capacity for both Cr and Cd absorption, reaching 99-100%. This result was similar to the previous investigations of Morin, et al. (2007, 2008) [31, 32], in which diatom species were demonstrated to have a very high potential for metal absorption. On the other hand, a green microalga *Dunaliella* sp. could only uptake less than 10% of Cd in an artificial medium [33]. As it should be, species from different algal classes would have different cell characteristics, for example, diatoms have a frustule made of silicate while green algae does not have this property. Consequently, different species will have a diverse capacity for metal uptake, but this needs further investigations to clarify. Regarding Cr uptake, the capacity of *S. acuminatus var biseratus* and *S. protuberans* were very high, 96 and 90%, respectively. Our findings are strongly supported by a previous study in which *Scenedesmus* sp. removed more than 98% of Cr in a water environment [34]. On the other hand, these green algae showed a poor performance in Cd uptake, only 13% for *S. acuminatus var biseratus* and 6% for S. *protuberans* (Table 1). In contrast, when compared to the Cr uptake potential, *Scenedesmus acutus* and *Chlorella vulgaris* had a higher Cd uptake capacity [35]. This suggests that we can apply different microalgae in order to effectively remove metal contaminants in water bodies.

Table 1. Cr and Cd uptake ratio by the three test microalgae.

Metals	Algal species	Metal concentrations (μgl^{-1})		Uptake ratio (%)
		Initial test	*End test*	
Cr	S. *acuminatus var biseratus*	761	27	96
	S. *protuberans*	660	67	90
	Cyclotella sp.	254	0	100
Cd	S. *acuminatus var biseratus*	493	431	13
	S. *protuberans*	607	569	6
	Cyclotella sp.	667	10	99

Conclusions

This study indicated that *S. acuminatus var biseratus* and *Cyclotella* sp. were relatively tolerant to Cr concentrations up to 761 μgl^{-1} for *S. acuminatus var biseratus* and 254 μgl^{-1} for *Cyclotella* sp. The growth rate of S. *protuberans* was significantly inhibited when exposed to 660 μgl^{-1} of Cr. In the case of Cd exposures, only *Cyclotella* sp. showed to have a high tolerance at concentrations up to 667 μgl^{-1} whereas Cd at the concentrations of 493 μgl^{-1} and 607 μgl^{-1} decreased the growth rate of *S. acuminatus var biseratus* and *S. protuberans*, respectively. Besides, the three tested phytoplankton reduced 90-100% of dissolved Cr in medium. Although the two green algae had a low capacity for Cd absorption, the diatom species showed good performance in removing Cd. Therefore, these algae could be considered as potential candidates for removing metal contaminants in water bodies. Moreover, the mechanisms behind the effects of trace metals on algae and the metal tolerance in algae should be investigated in the future.

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