

# Growth and metal uptake capacity of microalgae under exposure to chromium

*Sự phát triển và khả năng hấp thu kim loại của vi tảo trong phơi nhiễm với crôm*

Research article

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Microalgae play a key function in aquatic ecosystems. Their development and growth are strongly regulated by trace metals as essential elements. However, trace metals could cause negative effects when exceeding certain concentrations in the environment. In this study we tested the development and growth rate of two freshwater microalgae, the cyanobacterium *Pseudanabeana mucicola* and the green alga *Pediastrum duplex*, from Vietnam over the period of 14 days exposing to chromium (Cr) at the concentrations up to 1,936  $\mu\text{g L}^{-1}$ . Besides, the Cr uptake and absorption by *P. mucicola* were evaluated over 7 days incubated in medium containing 422  $\mu\text{g Cr L}^{-1}$ . The results showed that Cr at the concentrations up to 1,078  $\mu\text{g L}^{-1}$  did not inhibit the development and growth rate of *P. mucicola*. Similarly, concentration of 224  $\mu\text{g Cr L}^{-1}$  had no adverse effects on growth of *P. duplex*. The cyanobacterium *P. mucicola* could make a reduction up to 71% of Cr in the test medium, hence become a distinguished candidate for metal phytoremediation. To the best of our knowledge this is the first investigation on the responses and absorption of Cr by freshwater microalgae from Vietnam.

*Vi tảo đóng vai trò quan trọng trong hệ sinh thái thủy vực. Sự sinh trưởng và phát triển của chúng được điều tiết mạnh mẽ bởi kim loại vi lượng như những yếu tố thiết yếu. Tuy nhiên, những kim loại vi lượng này có thể gây ra những ảnh hưởng tiêu cực khi vượt quá nồng độ nhất định trong môi trường. Trong nghiên cứu này, chúng tôi thử nghiệm sự phát triển và tốc độ phát triển của hai loài vi tảo nước ngọt: loài tảo lam *Pseudanabeana mucicola* và loài tảo lục *Pediastrum duplex* có nguồn gốc từ Việt Nam trong thời gian 14 ngày phơi nhiễm với crôm (Cr) tại nồng độ lên tới 1.936  $\mu\text{g L}^{-1}$ . Bên cạnh đó, sự hấp thu Cr của *P. mucicola* cũng đã được đánh giá trong thời gian 7 ngày nuôi trong môi trường chứa 422  $\mu\text{g Cr L}^{-1}$ . Kết quả cho thấy Cr tại nồng độ lên tới 1.078  $\mu\text{g L}^{-1}$  không kìm hãm sự phát triển và tốc độ sinh trưởng của *P. mucicola*. Tương tự, tại nồng độ 224  $\mu\text{g Cr L}^{-1}$  không có bất kì ảnh hưởng tiêu cực đến sự phát triển của *P. duplex*. Loài tảo lam *P. mucicola* có thể làm giảm 71% hàm lượng Cr trong môi trường thí nghiệm, vì vậy được xem là ứng viên sáng giá cho quá trình xử lý môi trường ô nhiễm kim loại bằng thực vật. Theo hiểu biết của nhóm tác giả, đây là nghiên cứu đầu tiên về đáp ứng và hấp thu Cr bởi những vi tảo nước ngọt có nguồn gốc từ Việt Nam.*

**Keywords:** microalgae, chromium, growth rate, metal uptake, phytoremediation

## 1. Introduction

Phytoplankton including algae and cyanobacteria are worldwide distribution and very common organisms in aquatic water bodies. Being primary producers, microalgae play a key role in aquatic ecosystems such as oxygen liberation to the air and vital parts of the geo-bio-

chemical cycle allowing the flow of matter and energy become continuously (Horne and Goldman, 1994).

Many trace metals are known as the essential elements for living things (Andersen, 1981). However, in recent years, the common presence of trace metals (e.g. cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb)) with

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high concentrations in the aquatic and soil environment because the anthropogenic activities have become a potential risk for unintended adverse health impacts to both humans and non-target wildlife (Soares and Soares, 2012). Among trace metals, Cr is widely utilised in tanning factories, steel works, industrial electro-plating, wood preservation and fertilizers (Babchi et al., 2002). It has been found that Cr concentration in wastewater could reach 800 mg L<sup>-1</sup> (Plugaru et al., 2016). There are few studies on toxicity of Cr compared to those of other metals (Wong and Trevors, 1988; Wallen, 1995). Chromium can exist in oxidation state ranging from Cr<sup>2+</sup> to Cr<sup>6+</sup>, however, the trivalent state Cr<sup>3+</sup> and the hexavalent state Cr<sup>6+</sup> are the more readily assimilated forms and most toxic (Schroll, 1978; Towill et al., 1978). Previous studies focused on the toxicity of Cr on animals, especially fish (Mearns et al., 1976; Svecевичius, 2006; Rani et al., 2011; Josefina et al., 2011). Besides, detrimental effects of this contaminant on algae have also been investigated showing that Cr affected the algal photosynthesis and growth (Plugaru et al., 2016; Wium-Andersen, 1974; Fasulo et al., 1983; Horesick et al., 2006).

Recently many researchers have considered using microalgae to remove some toxic substances including trace metals by accumulation, adsorption and metabolism (Mahaemin, 2004; Nacorda et al., 2007; Lim et al., 2010; Priyadarshani et al., 2011). There have been numerous investigations demonstrating high efficiency of algal application for environmental remediation via the high bioaccumulation potential of trace metals such as Pb, Cd, Hg, Cu (Costa and Franca, 2003; Chen et al., 2005; Chojnacka et al., 2005; Lamaïet al., 2005; Al-Rub et al., 2006; Lim et al., 2010; Miranda et al., 2012). Regarding Cr, algae in water bodies have ability to strongly adsorb this pollutant, for example, Doshi et al. (2008) measured the

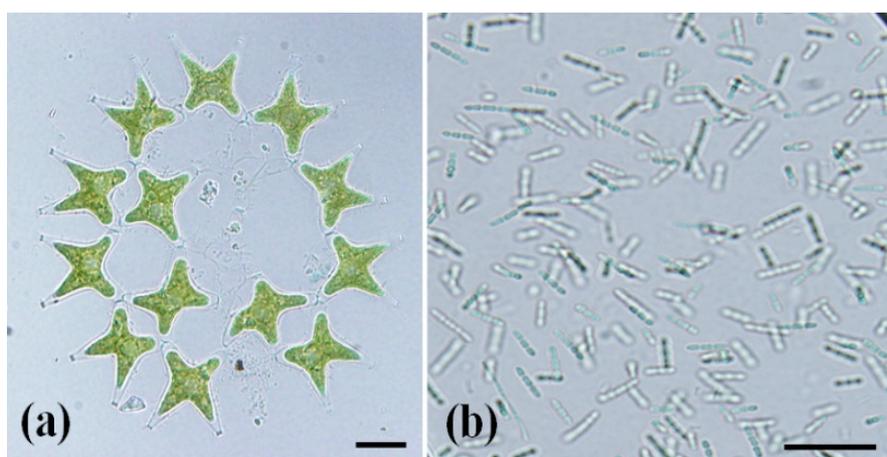
adsorption of Cr<sup>3+</sup> and Cr<sup>6+</sup> on two algal species *Cladophora* sp. and *Spirulina* sp. The authors found that *Cladophora* sp. adsorbed 347 mg g<sup>-1</sup> Cr<sup>3+</sup> and 168 mg g<sup>-1</sup> Cr<sup>6+</sup>, the other species took up to 306 mg g<sup>-1</sup> Cr<sup>3+</sup> and 202 mg g<sup>-1</sup> Cr<sup>6+</sup> (Doshi et al., 2008).

In Vietnam, researches on the potential application of freshwater algae from Vietnam have been conducted e.g. lipid production capacity (Nguyen et al., 2013; Phan et al., 2013), and metal exposures (Dao et al., 2017). However, the information on metal uptake potential of algal is scarce. Therefore, the aims of this study are to assess (i) the growth of two micro-algal species, *Pediastrum duplex* and *Pseudanabaena mucicola*, from Vietnam upon exposure to chromium and (ii) the chromium absorption capacity of *P. mucicola* in the laboratory conditions.

## 2. Materials and methods

The microalgae *Pediastrum duplex* Meyen and *Pseudanabaena mucicola* (Naumann et Huber-Pestalozzi) Schwabe (Fig. 1), isolated from Saigon River and Tri An Reservoir in Southern Vietnam, respectively, were used as the test organisms. These species were cultivated in Z8 medium (Kotai, 1972) in the laboratory conditions of 27 ± 1 °C, light intensity of around 3,000 Lux, light: dark cycle of 12h:12h (Dao et al., 2010).

The Cr solution (Cr(NO<sub>3</sub>)<sub>3</sub>, 1000 mg Cr L<sup>-1</sup>, Merck), a standard for ICP/MS analysis (trace analysis), was used as stock solution for the experiment with algae. Before being used for the tests, the test solutions (Z8, and Z8 added with Cr) were filtered through a filter with the pore size of 0.2 µm (Millipore, England) to prevent any bacterial contamination.



**Figure 1. The two microalgal species used for experiments *Pediastrum duplex* (a), and *Pseudanabaena mucicola* (b). Scale bars = 20 µm**

In the experiment on the growth of microalgae upon exposure to Cr, the two algal species were separately incubated in Z8 medium containing three different Cr concentrations (Table 1). In metal exposures, either *P. duplex* or *P. mucicola* was incubated in a 250 mL flask containing 150 mL of test solution. The incubation in which test solution was not added with Cr was run as the control. Three replicates were prepared for each exposure (Mu-

haemin, 2004). The *P. mucicola* densities at the start of the experiment were 120 (± 6) × 10<sup>4</sup> and 131 (± 32) × 10<sup>4</sup> trichomes mL<sup>-1</sup> in the control and Cr exposures, respectively. The *P. duplex* densities at the start of the experiment were 6 (± 0.4) × 10<sup>4</sup> and 7.1 (± 0.7) × 10<sup>4</sup> colonies mL<sup>-1</sup> in the control and Cr exposures, respectively. At the start of the experiment, a sub-sample from each test solution was filtered through 0.45 µm filter (Sartorius, Ger-

many) and acidified with saturated HNO<sub>3</sub> (Merck). Then Cr concentrations in the sub-samples were determined by the Electrothermal Atomic Absorption Spectrometric Method (PinAACLE 900Z, Perkin Elmer, USA; APHA, 2012). The experiments on the algal growth were run over a period of 14 days. At the beginning of the experiment and every two days, 1 mL of algal solution from the test flasks was taken out, fixed with Lugol solution and counted with a Sedgewick Rafter counting chamber on a microscope (Sournia, 1978).

In the experiment on metal uptake by microalgae, the alga *P. mucicola* was incubated in 1 L flasks containing 500 mL of test solution in the laboratory conditions as mentioned above. The control test solution included Z8 medium containing Cr at the concentration of 566 µg L<sup>-1</sup> (without alga in the control) whereas the exposure, 3 replicates, was prepared with Z8 medium containing the alga and Cr at the concentration of 422 µg L<sup>-1</sup>. This experiment lasted for 7 days. When the experiment termi-

nated, sub-sample (50 mL) of the test solutions (both control and exposure) were filtered (0.45 µg pore size filter, Sartorius), acidified with HNO<sub>3</sub> (Merck) then metallic (Cr) characterized as mentioned above (Perkin Elmer, USA).

The hardness, pH, alkalinity and dissolved organic carbon of the test solutions were analyzed (APHA, 2012) to characterize the basic characteristics of the experiments and the conditions for algal growth.

The growth rate of microalgae (R) was calculated according to Lobban et al. (1988) with the equation of  $R = (\ln X_2 - \ln X_1) / (t_2 - t_1)$ ; where X<sub>1</sub> and X<sub>2</sub> are algal densities at time t<sub>1</sub> and t<sub>2</sub>. Metal uptake ratio was calculated as (%) = 100 x (M<sub>1</sub> - M<sub>2</sub>)/M<sub>1</sub>; where M<sub>1</sub> and M<sub>2</sub> are metal concentrations at the first day and the end of the test (Lobban et al., 1988). Kruskal-Wallis test (Sigma Plot 12.0) was applied for calculation the significant difference of the growth rate between control and exposures.

**Table 1. Chromium concentrations in the test solutions with microalgae. UDL, under detection level of the equipment, 1 µg L<sup>-1</sup>.**

Test species	Chromium concentrations (µg L <sup>-1</sup> )			
	Control	Exposure 1	Exposure 2	Exposure 3
<i>Pediastrum duplex</i>	UDL	8	224	1,936
<i>Pseudanabaena mucicola</i>	UDL	6	118	1,078

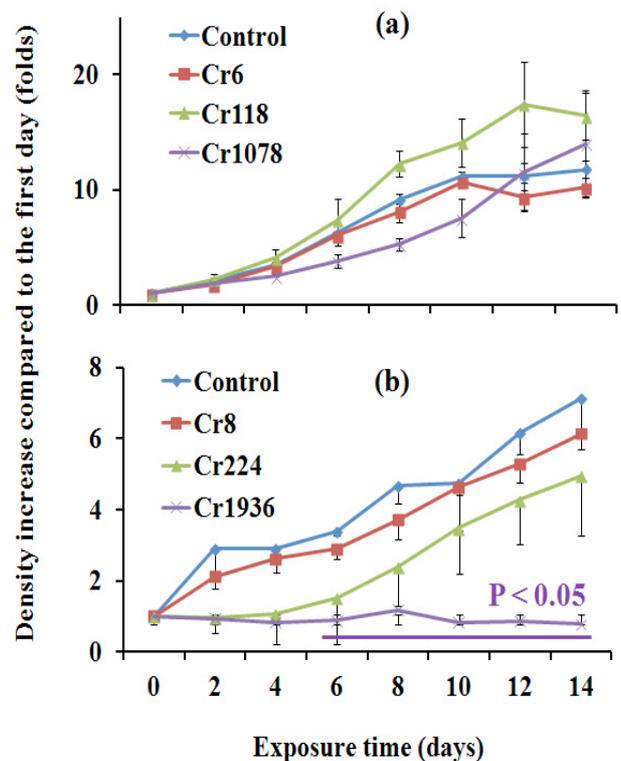
### 3. Results and discussion

The pH of the test solutions ranged from 6.1 – 8.6. The hardness and the alkalinity of the solutions valued from 28 – 35 mg CaCO<sub>3</sub> L<sup>-1</sup> and 8 – 8.5 mg CaCO<sub>3</sub> L<sup>-1</sup>, respectively. The dissolved organic carbon (DOC) concentration of the Z8 medium was 30.5 mg L<sup>-1</sup>. Toxicity of metals to aquatic organisms is regulated by some environmental factors such as pH, alkalinity, hardness and DOC in water. The increase of pH, DOC concentration and the decrease of hardness in the test medium resulted in the decrease of metal bioavailability consequently toxicity decrease to aquatic organisms (Paulauskis and Winner, 1988; Naddy et al., 2015; Taylor et al., 2016). The alkalinity and hardness of this study could be classified as soft water (Villavicencio et al., 2005; Naddy et al., 2015) which enhanced the bioavailability of Cr in the test medium. These physical and chemical characteristics revealed suitable conditions for development of microalgae (e.g. pH) and the high bioavailability of Cr to the microalgae (alkalinity and hardness).

#### 3.1. Development of microalgae under exposure to chromium

The density of *P. mucicola* in control medium and Cr medium linearly increased during the time of incubation (Fig. 2a). After 14 days of experiment, the density of *P. mucicola* in the control medium (density of 1,417 x 10<sup>4</sup> trichomes mL<sup>-1</sup>) was 11.8 times and that in Cr medium (densities of 1,406 – 2,224 x 10<sup>4</sup> trichomes mL<sup>-1</sup>) was 10.2 – 16.4 times higher than the density at the start of the test. However, there was no significant difference be-

tween the *P. mucicola* density increase in control and Cr exposure conditions (p > 0.05, Kruskal-Wallis test).



**Figure 2. Development of *Pseudanabaena mucicola* (a) and *Pediastrum duplex* (b) during the exposure to chromium. Significant different between Cr1936 exposure in *P. duplex* and control (p < 0.05) was tested by Kruskal-Wallis**

In the test with *P. duplex*, by the end of experiment, the density increased in the control medium ( $42.6 \times 10^4$  colonies  $\text{mL}^{-1}$ ) and two lower Cr medium exposures (Cr8 and Cr224; densities of  $36.2 - 41.2 \times 10^4$  colonies  $\text{mL}^{-1}$ ) was slowly raised, reaching around 5 – 7 times higher than that at the start of the test. However, that in exposure to Cr1936 was inhibited and decreased to 0.7 times (or 70% with density of  $5.2 \times 10^4$  colonies  $\text{mL}^{-1}$ ) compared to the start. Consequently, the *P. duplex* density increase of Cr1936 was significantly different from that of the control from day 6 to the end of the experiment (Fig. 2b).

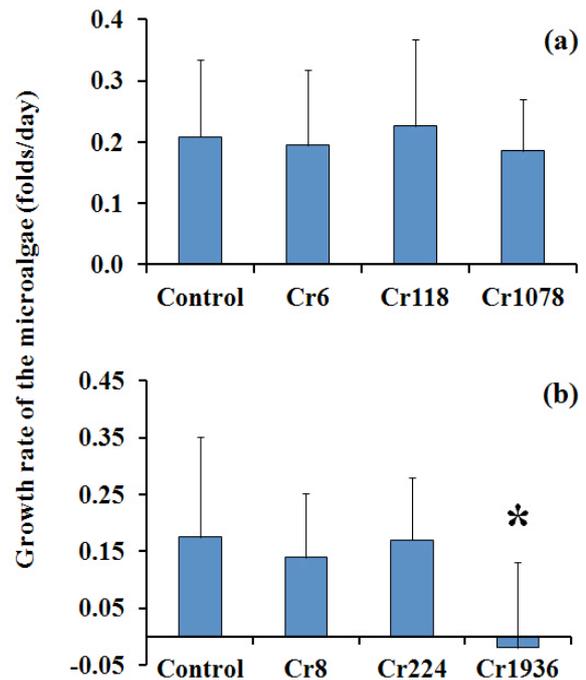
Wong and Chang (1991) reported that Cr at the concentrations of  $250 \mu\text{g L}^{-1}$  did not cause an inhibitory on the growth of *Chlorella pyrenoidosa*. However, the authors found the severe inhibition of  $1,000 \mu\text{g Cr L}^{-1}$  on the *C. pyrenoidosa* growth (Wong and Chang, 1991). However, the green alga *Pseudokirchneriella subcapitata* was more sensitive to Cr than the cyanobacterium *Microcystis aeruginosa* (Rodgher et al., 2012). Besides, two strains of *Chlorella vulgaris* showed different resistance to Cr (Nacorda et al., 2007). Hence, responses of algae to metals should be species and strain specific

### 3.2. Growth rate of microalgae under exposure to chromium

During 14 days of development, the average growth rate of *P. mucicola* in control medium and Cr medium was similar, ranging from 0.18 – 0.22 folds  $\text{day}^{-1}$  (Fig. 3a). No significant difference was found between the control medium and the Cr exposures. Similarly, in the experiment with *P. duplex*, there was no statistical difference ( $p > 0.05$  by Kruskal-Wallis test) between the control and the exposures, Cr8 and Cr224. The average growth rate values of these treatments were from 0.14 – 0.17 (Fig. 3b). However, upon exposure to the highest Cr concentration the growth of *P. duplex* was inhibited. The growth rate of *P. duplex* in the exposure Cr1936 during the experiment was  $-0.02$  folds per day (Fig. 3b).

The metal Cr is a micronutrient that is essential for metabolisms in cells of plants. Growth of algae was not inhibited (Wong and Chang, 1991) at the Cr concentration of  $250 \mu\text{g L}^{-1}$ . However, Cr concentrations exceeded  $500 \mu\text{g L}^{-1}$  inhibited photosynthesis of *C. pyrenoidosa* (Wong and Chang, 1991) and those from  $1,500 \mu\text{g L}^{-1}$  or higher ( $9,000 \mu\text{g L}^{-1}$ ) negatively affected the cell membranes of living things leading to the increase of their permeability consequently inhibit growth (Fasulo et al., 1983). This helped to explain the adverse effect of Cr on *P. duplex* in current study (Fig. 3b). Differently, Horcsick et al. (2006) found the negative effects of  $1,000 \mu\text{g Cr L}^{-1}$  on cell density growth of *C. pyrenoidosa* within 72h of exposure (Horcsick et al., 2006). On the contrary, Plugaru et al. (2016) exposed another strain of *C. pyrenoidosa* to Cr at the concentrations of  $1 - 5 \text{ mg L}^{-1}$  for 72h and recorded no adverse effects of Cr on growth of the alga (Plugaru et al., 2016). However, the later study ran the experiment without replicates (Plugaru et al., 2016) therefore their results lacked of statistical analysis consequently weak convince to readers. Our study showed a long time of exposure (14 days) to the metal whereas previous studies

conducted their experiments in a shorter time, from 3 – 6 days (Plugaru et al., 2016; Horcsick et al., 2006) or up to 12 days (Nacorda et al., 2007). Nacorda et al. (2007) observed the decrease of algal growth with Cr concentration dependence. Growth rate of *C. vulgaris* exposed to  $1 \text{ mg Cr L}^{-1}$  and  $2 \text{ mg Cr L}^{-1}$  was 70% and 50%, respectively, compared to that of the control. Hence, this again indicated that the species *P. mucicola* is less sensitive than the two green algae *C. pyrenoidosa* and *C. vulgaris*. Besides, *P. duplex* in our study is more sensitive than *C. vulgaris* in the investigation of Nacorda et al. (2007). Our finding suggested that *P. mucicola* is a good candidate for



Cr removal investigations.

**Figure 3. Growth rate of *Pseudanabaena mucicola* (a) and *Pediastrum duplex* (b) during the exposure to chromium. The asterisk indicates the significant different ( $p < 0.05$ ) between control and Cr exposure by Kruskal-Wallis**

### 3.3. Chromium uptake by the alga *Pseudanabaena mucicola*

The mean concentrations of Cr in the control (Z8 medium and Cr, without alga) and exposure (Z8 medium and Cr and alga) after 7 days of incubation were  $501 \mu\text{g L}^{-1}$  and  $73 \mu\text{g L}^{-1}$ , respectively. The Cr absorbance of flask in the control was around 11% and that of both flask and alga in the exposure was 82%. Hence the Cr uptake by *P. mucicola* in this study was 71%. This record revealed a very high potential of Cr absorption of *P. mucicola* compared to the Cr absorption capacity of *C. vulgaris*, 21 – 28% (Nacorda et al., 2007). Though the previous study ran for 12 days and did not test the Cr uptake by *C. vulgaris* after 7 days, we believed that the absorption of *C. vulgaris* would not much higher than 28% after 7 days of incubation, because this green alga reached its stationary phase of growth at around 12 days (Nacorda et al., 2007). The much higher Cr absorption capacity of the cyanobacte-

rium in our study should be related to the higher tolerance to Cr of *P. mucicola* than *C. vulgaris*. Therefore, we recommend the species *P. mucicola* as a very promised organism for Cr removal in Cr polluted water.

## 4. Conclusion

Two isolated freshwater microalgae from Vietnam were firstly used as the test organisms for Cr exposure and uptake in the laboratory conditions. The Cr at the concentration up to  $1,078 \mu\text{g L}^{-1}$  slightly influenced on development and growth rate of the cyanobacterium *P. mucicola*. Similar responses of the green alga *P. duplex* were observed upon exposure to  $224 \mu\text{g Cr L}^{-1}$ . However, development and growth rate of *P. duplex* were severely inhibited at  $1,936 \mu\text{g Cr L}^{-1}$ . In the uptake and absorption test, *P. mucicola* could reduce up to 71% of dissolved Cr in water. This cyanobacterium showed a good candidate for phytoremediation in term of metal pollution in water bodies. Further investigations on the effects of metal mixture on microalgae and metal removal by algae are highly suggested.

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