

Article

The Effect of Chromium on Photosynthesis and Lipid Accumulation in Two Chlorophyte Microalgae

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Abstract: Heavy metals have adverse effects on microalgae metabolism and growth. Photosynthesis and lipid profile are extremely sensitive to heavy metal toxicity. The impact of hexavalent chromium – Cr(VI) on photosynthesis and lipid accumulation in *Mucidosphaerium pulchellum* and *Micractinium pusillum* exposed to different concentrations (0 – 500 µg L⁻¹) was investigated for 11 days. A significant ($p < 0.05$) increase in lipid content was observed with increasing Cr(VI) concentration. However, growth was suppressed at higher concentrations exceeding 100 µg L⁻¹. Addition of Cr(VI) in the cell culture medium showed a negative effect on quantum yield (F_v/F_m) and a photosynthetic inhibition of > 65% was noted in both species at 500 µg L⁻¹. However, the lipid gravimetric analysis presented inner cell lipid content up to 36% and 30% of dry weight biomass for *M. pulchellum* and *M. pusillum*, respectively. The fatty acids profiles of both microalgae species showed higher levels of hexadecenoic acid as well as ω3, ω6, and ω7 fatty acids. The effect of Cr(IV) on photosynthesis and lipid accumulation in both microalgae species was concentration and exposure time dependent. This shows that an appropriate concentration of Cr(VI) in culture medium could be beneficial for higher lipid accumulation in freshwater eukaryotic microalgae species.

Keywords: Fatty acids; Freshwater microalgae; Growth kinetics; Heavy metal; *Micractinium pusillum*; *Mucidosphaerium pulchellum*; Photosynthesis; Toxicity

1. Introduction

The use of microalgae in the pharmaceutical, medical and food industry is increasing due to their no reported side effects on human health. Thus, it is necessary to investigate microalgae growth parameters and potential growth inhibitors [1]. Heavy metals, antibiotics and herbicides are toxic to microalgae even at low concentrations however, to a certain extent, microalgae could adapt to a contaminated environment [2–5]. Microalgae, one of the primary producers in the marine ecosystem, change their composition such as fatty acids, lipids, pigments and exopolymers when exposed to heavy metal contamination [6, 7]. Therefore, investigating the adaptation and survival of microalgae species in a contaminated environment is imperative [8–11]. Investigating the effect of heavy metal toxicity on growth and photosynthetic activity of microalgae could substantially influence overall ecological risk evaluation of heavy metals.

Rapid industrialization and urbanization has increased the environmental presence of heavy metals, which is causing water pollution and it is becoming a serious issue worldwide [12]. Several methods have been developed to reduce the concentration of heavy metals from wastewater

[13], however, non-biodegradable heavy metals resist bioremediation and show long environmental persistence times [14, 15], which is posing a serious threat for the biotic life. Heavy metals are abundantly available in nature. Unlike other heavy metals such as zinc (Zn) and copper (Cu), chromium (Cr) is not required for plant respiration or growth. However, due to rapid industrialization, an abundant quantity of Cr is being dumped into the water, causing unfavorable effects on animals and plants [12]. Furthermore, Cr toxicity is form-dependent, with hexavalent chromium – Cr(VI), being the significantly more toxic to humans, than the trivalent chromium – Cr(III) [16]. Trace amounts of Cr(III) are needed daily for adult humans [17]. On the other hand, Cr(VI) has carcinogenic and mutagenic effects on human [17]. Severe acute effects including gastrointestinal disorders, hemorrhagic diathesis, and convulsions could occur when ingesting 1 – 5 g of chromate [17, 18].

The exposure of microalgae to heavy metals could cause inhibited growth, suppressed cell division, reduced photosynthesis, and restrained enzymatic activity [19-21]. Advancement in chlorophyll fluorescence technology has made easier to analyze the photosynthesis processes, which has enabled researchers in finding new factors affecting photosynthesis. Even though the impact of heavy metals on microalgae have gained considerable attention and have been extensively reported, the effects of Cr(VI) toxicity on chlorophyte microalgae species *Mucidosphaerium pulchellum* (formerly *Dictyosphaerium pulchellum*) and *Micractinium pusillum* have not been yet reported.

In the present study, the toxic effects of heavy metal, hexavalent chromium, on growth and modulated fluorescence of freshwater eukaryotic non-model microalgae species *M. pulchellum* and *M. pusillum* were analyzed and compared. Furthermore, the effect of chromium on lipid accumulation and lipid composition was also investigated.

2. Materials and Methods

2.1. Microalgae Culture and Treatment

The eukaryotic freshwater microalgae species *Mucidosphaerium pulchellum* and *Micractinium pusillum* (phylum Chlorophyta, class Trebouxiophyceae) were obtained from the Korea Marine Microalgae Culture Center (KMMCC) Busan, Republic of Korea. Stocks were maintained on modified-AF6 agar slants [22]. The microalgae species were cultured in 250 mL flask bioreactors containing modified-AF6 medium [23], without citrate and EDTA, at a constant light intensity of $50 \pm 2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, $25 \pm 2^\circ\text{C}$ temperature, and 50% humidity. The effects of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$, Yakuri Pure Chemicals, Osaka, Japan), a hexavalent form of chromium – Cr(VI), on photosynthesis and lipid accumulation at different concentrations (0 to $500 \mu\text{g L}^{-1}$) were investigated. All experiments were repeated at least three times.

2.2. Determination of Microalgae Growth Performance

Growth performance was determined by measuring the cell densities, optical densities, and growth rates. Growth rates were calculated using the cell densities. Briefly, on each sampling day,

5 mL sample was collected from each culture flask after a thorough hand mixing and cell densities were determined using hemocytometer (Marienfeld Superior, Germany) under a light microscope at a magnification of 400×. Furthermore, optical density at 750 nm (OD_{750}) was recorded using UV/VIS spectrophotometer (WPA Biowave II, Biochrom, UK) on every alternating day [5].

2.3. Measurement of Toxicity, Modulated Fluorescence and Photosynthetic Inhibition

The sensitivity of *M. pulchellum* and *M. pusillum* in media supplemented with different concentrations of Cr(VI) was evaluated by toxy-PAM dual channel yield analyzer (Heinz Walz GmbH, Effeltrich, Germany) [5]. This toxicity analyzer is extremely sensitive to chlorophyll fluorescence and uses saturation pulse method to determine the effective fluorescence yield of photosystem II (PSII) [24, 25]. To induce an equilibrium state for the photosynthetic electron transport, microalgae samples were dark adapted by placing in complete dark for 30 min before analysis and fluorescence intensity was measured using low intensity modulated light to avoid the reduction of the PSII primary electron acceptor (Q_A) [24].

Fluorescence intensity of microalgae cells excited by toxy-PAM blue light was measured at 650 nm. The minimal fluorescence level (F_0 ; fluorescence measured shortly before the application of a saturation pulse), and the maximal fluorescence level (F_m ; fluorescence measured during a saturation pulse) were recorded and the effective overall quantum yield (Y) of PSII was calculated using the following equation.

$$\text{Quantum Yield (Y)} = (F_m - F_0) / F_m = F_v / F_m \quad [24]$$

Relative photosynthetic inhibition was calculated using the following equations.

$$\text{Relative Photosynthetic Inhibition \%} = 100 (Y_2 - Y_1) / Y_2 (Y_1 < Y_2) \quad [5]$$

$$\text{Relative Photosynthetic Inhibition \%} = 100 (Y_2 - Y_1) / Y_2 (Y_1 > Y_2) \quad [5]$$

2.4. Lipid Extraction

The tested microalgae species were grown in modified-AF6 medium supplemented with different concentrations of Cr(VI) till pre-stationary phase and total lipids were extracted following the Bligh and Dyer method with slight modifications [26]. Briefly, microalgae cells were harvested by centrifugation at 4°C and 5000×g for 20 min. Pellets were rinsed with distilled water and freeze-dried at -85°C. Dried pellets were weighed, dissolved in 10 mL of methanol: chloroform (2:1) solution and homogenized using ULTRA TURRAX Homogenizer (IKA Works. Inc., USA) at 16,000 rpm for 2 min. Samples were incubated overnight in the dark at room temperature. The following day, 6 mL distilled water and 3 mL chloroform was added in each tube, inverted carefully and centrifuged at 5000×g for 10 min. The lower organic phase was transferred to a new 15 mL falcon tube, freeze-dried overnight at -85°C, and dried for another 4 h at 50°C in a dry-oven until all the solvent was evaporated. The tubes were weighed and total lipid content in each sample was calculated.

2.5. Fatty Acid Analysis

Total fatty acids extracted from *M. pulchellum* and *M. pusillum* were analyzed by gas chromatography (GC) of fatty acid methyl esters (FAMES) prepared according to Ichihara and Fukubayashi [27], with slight modifications. Briefly, total fatty acids were trans-methylated with 3 mL of methanolic-hydrochloric acid solution at 90°C for 1 h. Samples were cooled at room temperature. A total of 1 mL hexane and 1 mL distilled water was added to the extracts and mixed well by vortex. The upper fatty acid layer was separated by centrifugation at 3000×g for 6 min and collected in GC vials.

GC analysis of FAMES was conducted using PerkinElmer Clarus 680 Gas Chromatograph (PerkinElmer, Inc., Waltham, MA, USA) equipped with 30 m x 20 µm x 25 mm SP-2380 capillary column (Sigma-Aldrich, Co., St. Louis, MO, USA). The initial oven temperature was set to 140°C and increased at a rate of 4°C to 240°C. Helium (He) was used as a carrier gas at a flow rate of 1 mL/min. Injection temperature and volume were set at 230°C and 1 µL, respectively. Fatty acids were identified by comparison with retention times of 37-component FAME mix standards (Sigma-Aldrich) and were expressed as mg/L. The corresponding fatty acids were further cross checked with the instrument database containing the NIST[®] library [28].

2.6. Statistical Analysis

The statistical significance of the results was calculated using Analysis of Variance via SPSS ver. 27 (SPSS, Chicago, IL, USA). The significance among the samples was assessed using Duncan's multiple-range test and the results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Influence of chromium on microalgal growth

The culture medium, modified-AF6 medium, was supplemented with different concentrations of Cr(VI) ranging from 0 to 500 µg L⁻¹ and their effects on growth parameters was investigated for 11 days. The microalgae species *M. pulchellum* and *M. pusillum* showed significant sensitivities to the tested Cr(VI) concentrations as indicated by the optical density and cell density values. As shown in Figure 1, Cr concentrations, 0 – 100 µg L⁻¹, showed normal cell densities whereas the concentrations exceeding 100 µg L⁻¹ here, 250 µg L⁻¹ and 500 µg L⁻¹, showed a decline in growth after day 7. Both microalgae cultures showed reduced growth as compared to the control; it could be due to the possible toxicity of the Cr(VI). Interestingly, growth measurement study at absorbance of 750 nm (OD₇₅₀) showed similar results with the cell densities (Figure 2).

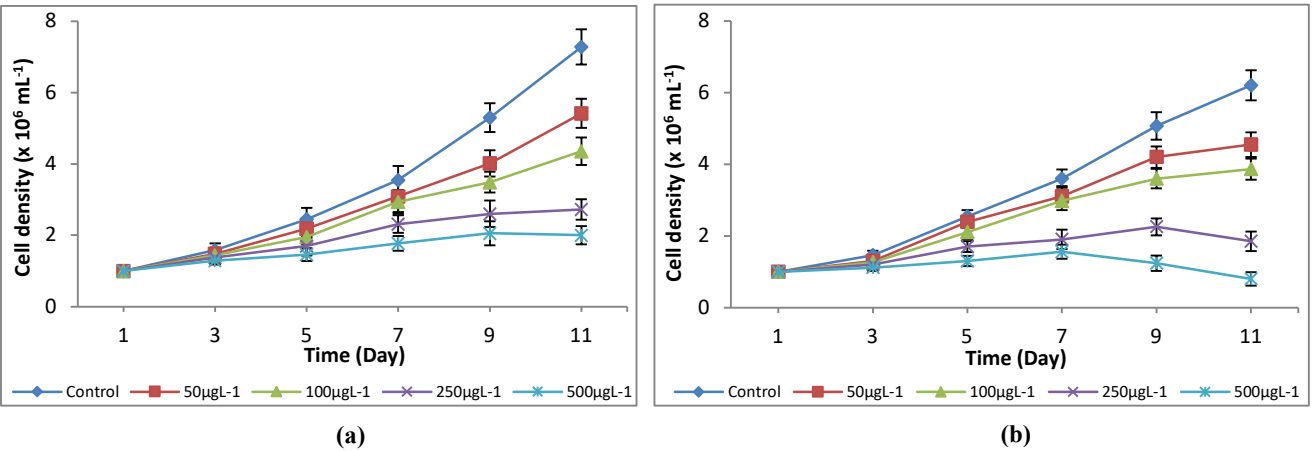


Figure 1. Effect of Cr(VI) on cell density of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents culturing days and the cell densities ($\times 10^6 \text{ mL}^{-1}$) are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

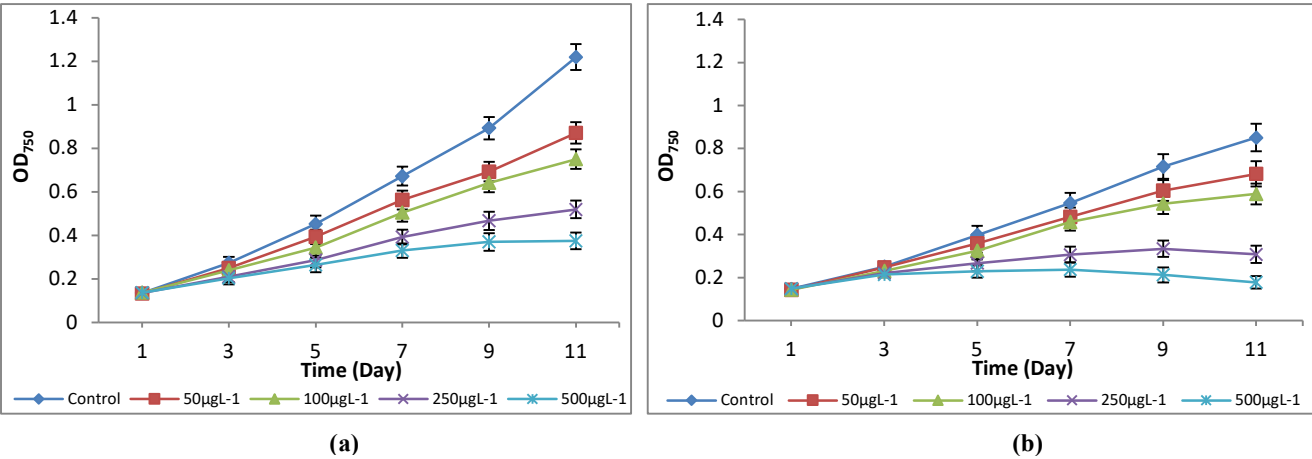


Figure 2. Effect of Cr(VI) on optical density of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents culturing days and the optical density values at 750 nm (OD₇₅₀) are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

Significant ($p < 0.05$) differences in growth rates among chromium treatments for both microalgae species were observed. For *M. pulchellum*, control ($0 \mu\text{g L}^{-1}$) and $50 \mu\text{g L}^{-1}$ chromium treatments showed exponential growth at $\mu = 0.1912$ and $\mu = 0.1605$, while the Cr(VI) induced inhibition of the PSII resulted in a pseudo substrate limited linear growth at 0.3688×10^6 ($100 \mu\text{g L}^{-1}$ chromium), 0.2139×10^6 ($250 \mu\text{g L}^{-1}$ chromium), and 0.1311×10^6 ($500 \mu\text{g L}^{-1}$ chromium) cell per day. Furthermore, a significant reduction in growth rate was observed at Cr(VI) concentrations, $250 \mu\text{g L}^{-1}$ and $500 \mu\text{g L}^{-1}$, after day 10. At the given photon flux density, the control cultivation for *M. pusillum* was substrate limited and showed a linear growth at 0.6007×10^6 cells per day. Increasing concentrations of Cr(VI) decreased growth rates in a similar manner to *M. pulchellum*, with a growth rate of 0.4706×10^6 ($50 \mu\text{g L}^{-1}$ chromium), 0.3937×10^6 ($100 \mu\text{g L}^{-1}$ chromium), 0.168×10^6 ($150 \mu\text{g L}^{-1}$ chromium), and 0.1110×10^6 ($500 \mu\text{g L}^{-1}$ chromium) cells per day (Table 1). The 50, 100 and $250 \mu\text{g L}^{-1}$ chromium treatments showed reduction in growth after day nine, while $500 \mu\text{g L}^{-1}$ chromium treatment showed reduction in growth rate and cell number after day 7.

Table 1. Effect of Cr(VI) on growth rates (cells/day)

Species	Cr(VI) concentration				
	0 µg L ⁻¹	50 µg L ⁻¹	100 µg L ⁻¹	250 µg L ⁻¹	500 µg L ⁻¹
<i>M. pulchellum</i>	µ = 0.1912	µ = 0.1605	0.3688 x 10 ⁶ *	0.2139 x 10 ⁶ *	0.1311 x 10 ⁶ *
<i>M. pusillum</i>	0.6007 x 10 ⁶ *	0.4706 x 10 ⁶ *	0.3937 x 10 ⁶ *	0.1678 x 10 ⁶ *	0.1110 x 10 ⁶ *

* indicates linear growth

3.2. Effect of chromium on modulated fluorescence and photosynthetic inhibition

The variations in quantum yield and photosynthetic inhibition of the tested microalgae cultures supplemented with different concentrations of Cr(VI) were studied for 11 days. Different Cr(VI) concentrations showed a significant difference in fluorescence yield and photosynthetic inhibition. The significant ($p < 0.05$) increases in minimal (F_0) and maximal (F_m) fluorescence values were observed at 0 µg L⁻¹ – 100 µg L⁻¹, however, a decline in F_0 and F_m values was observed at 250 µg L⁻¹ and 500 µg L⁻¹ (Figures 3, 4).

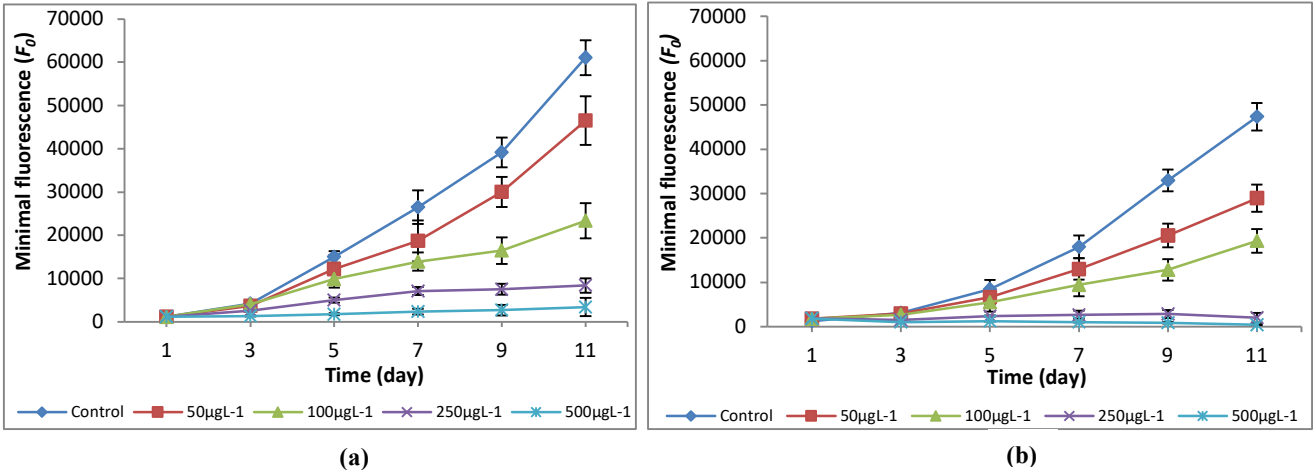


Figure 3. Effect of Cr(VI) on minimal fluorecence of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents the culturing days, and the minimum fluorecence (F_0) values are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

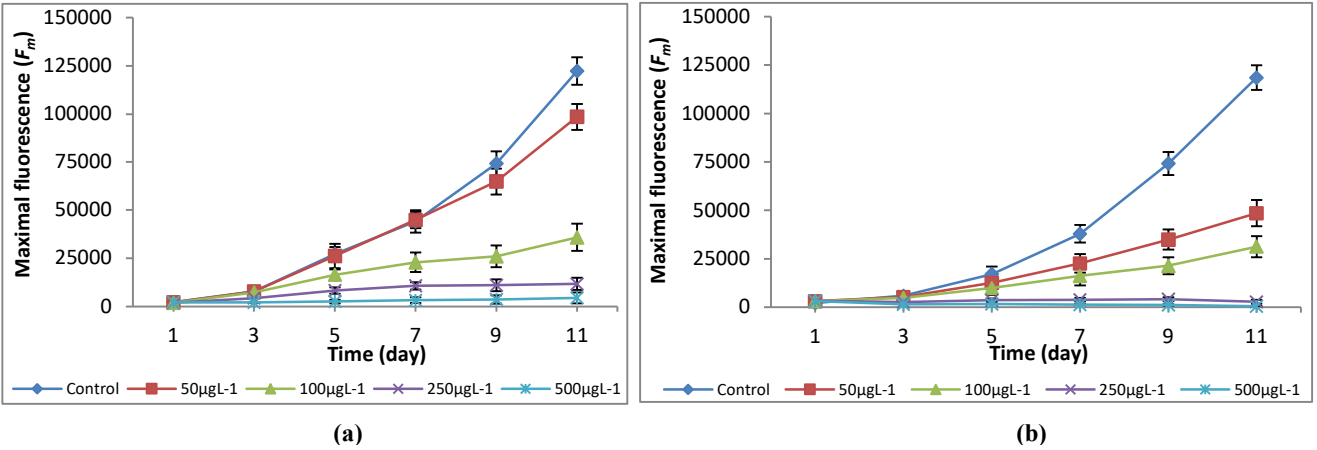


Figure 4. Effect of Cr(VI) on maximal fluorescence of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents the culturing days, and the maximal fluorescence (F_m) values are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

A significant ($p < 0.05$) reduction in quantum yield (Y) was noted in all the tested Cr(VI) concentrations as compared to the control ($0 \mu\text{g L}^{-1}$; Figure 5). It shows that chromium had a negative effect on the tested microalgae growth by interrupting photosynthesis. Both microalgae species showed a similar trend of photosynthetic inhibition at the tested Cr(VI) concentrations after day 3 of culturing and a maximum inhibition of up to 67% and 66% was observed for *M. pulchellum* and *M. pusillum*, respectively, at 500 $\mu\text{g L}^{-1}$ (Figure 6).

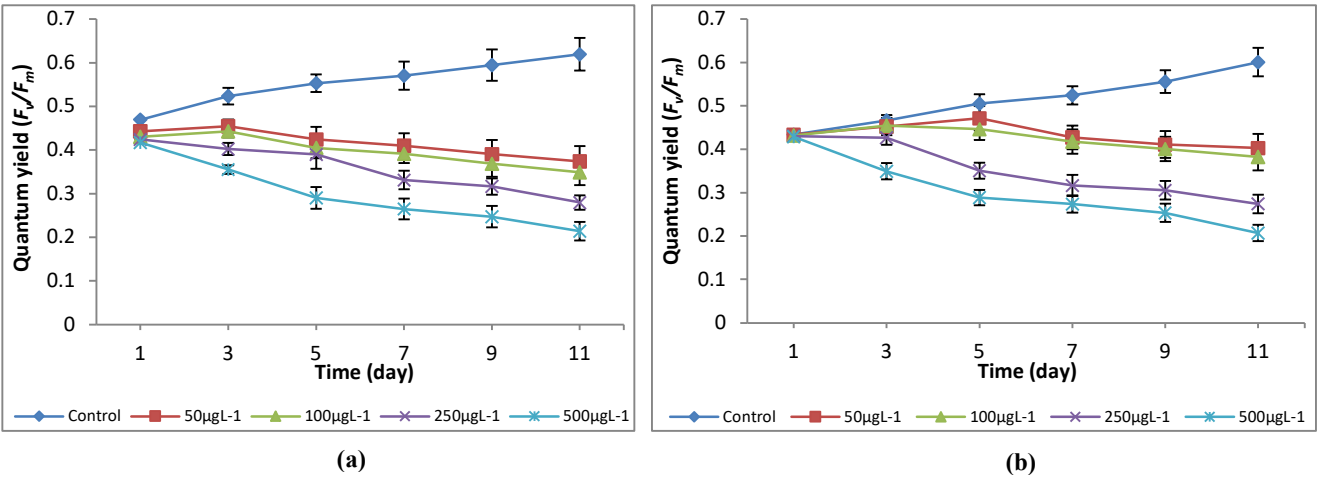


Figure 5. Effect of Cr(VI) on quantum yield of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents the culturing days, and the quantum yield (Y) values are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

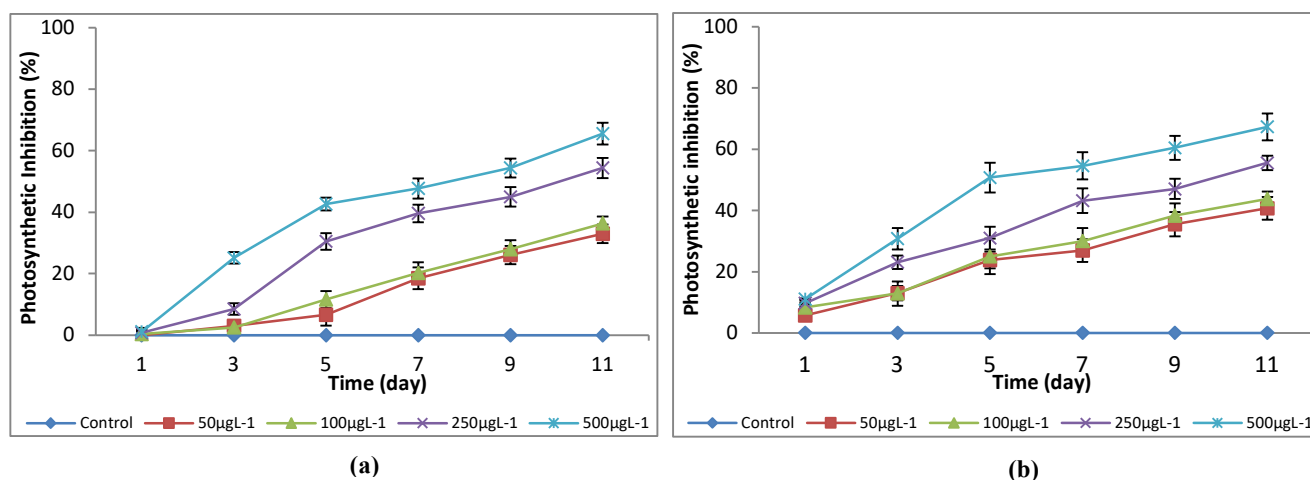


Figure 6. Effect of Cr(VI) on photosynthetic inhibition of (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents the culturing days, and the inhibition (%) values are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

3.3. Effect of chromium on lipid accumulation

The significant ($p < 0.05$) increases in lipid content were observed with the increasing Cr(VI) concentration in the culture medium. A lipid content of up to 36% was observed in *M. pulchellum* cultures at the maximum tested Cr(VI) concentration in this study (500 µg L⁻¹), which was approximately 12 times higher than the control (0 µg L⁻¹). Whereas a maximum lipid content of 30% was observed in *M. pusillum* cultures, which was approximately 3 times higher than the control. A drastic increase in Cr(VI) concentration-dependent lipid content was observed in *M. pulchellum* however, the increase in concentration-dependent lipid content was not consistent in *M. pusillum* (Figure 7). However, both tested microalgae species showed significantly ($p < 0.05$) increased lipid content, which shows that the addition of chromium could significantly enhance the lipid accumulation in the tested freshwater microalgae species.

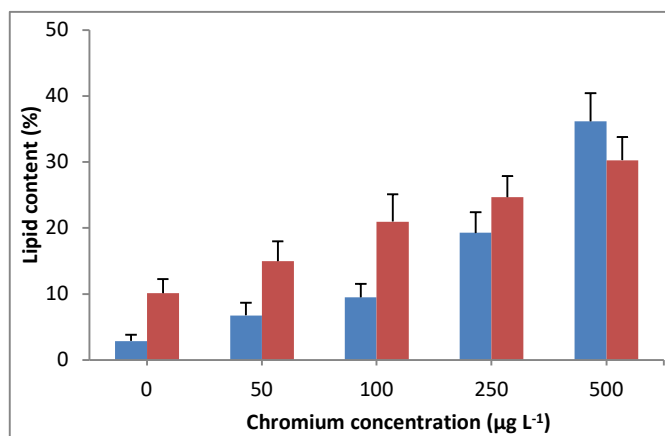


Figure 7. Effect of Cr(VI) on lipid content in *M. pulchellum* (■) and *M. pusillum* (■)

X-axis represents the chromium concentrations (µg L⁻¹) and the lipid content (% g of lipid / g of dry weight biomass) is shown along the y-axis.

Data are means $\pm$ SE, $n = 3$

3.4. Fatty acid composition

The fatty acids were analyzed by GC and the major fatty acid composition of both tested microalgae species included palmitic acid (16:0; hexadecanoic acid), palmitoleic acid (C16:1; ω -7), vaccenic acid (C18:1; ω -7), linoleic acid (C18:2; ω -6), α -linoleic acid (C18:3; ω -3), and arachidonic acid (C20:4; ω -6) as shown in Figure 8. Higher levels of palmitic acid and α -linoleic acid were observed in both microalgae cultures at all the tested concentrations. Higher Cr(VI) concentrations in *M. pulchellum* cultures resulted in slightly higher content of palmitic acid among the major fatty acids. However, all other fatty acids showed a slightly decreasing tendency at higher concentrations. The increasing Cr(VI) concentrations in *M. pusillum* cultures showed a slightly higher content of vaccenic acid and α -linoleic acid. However, a slightly decreasing tendency was observed for palmitoleic acid and arachidonic acid. Furthermore, higher tested Cr(VI) concentrations (250 $\mu\text{g L}^{-1}$ and 500 $\mu\text{g L}^{-1}$) significantly ($p < 0.05$) reduced the linoleic acid content.

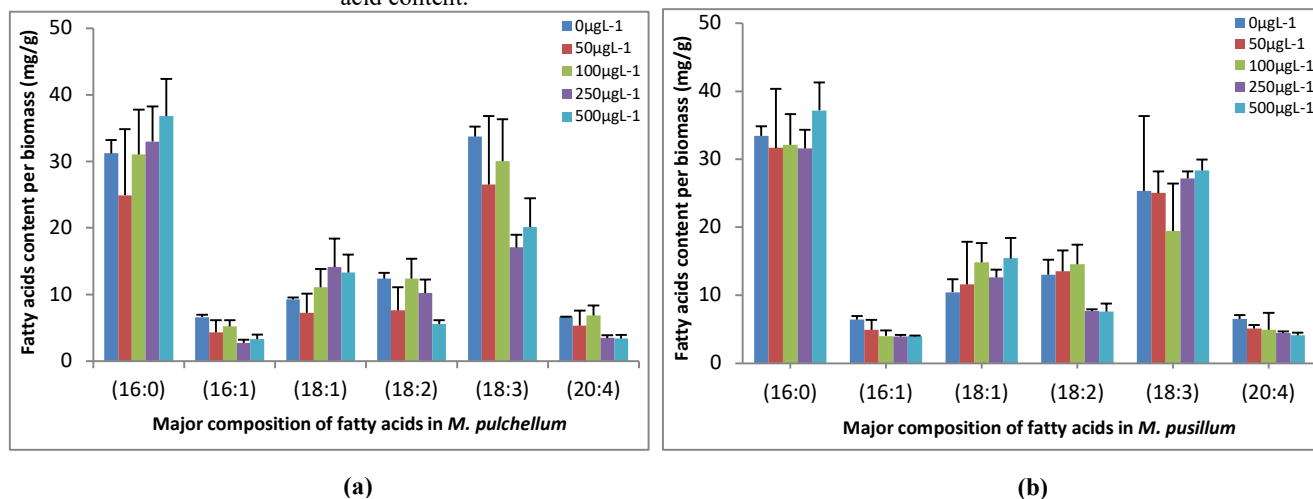


Figure 8. Effect of Cr(VI) on fatty acids composition in (a) *M. pulchellum* and (b) *M. pusillum*

X-axis represents the fatty acids and fatty acid content per biomass (mg/g) are shown along the y-axis. Data are means $\pm$ SE, $n = 3$

4. Discussion

Chromium exists in the environment as trivalent – Cr(III) and hexavalent form – Cr (VI), where Cr (VI) being the highly toxic, carcinogenic and mutagenic [29, 30]. The discharge of chromium from anthropogenic sources such as household, industry, transport, mining, and agriculture increases its concentration several times above normal levels [31]. The aquatic ecosystems are seriously affected by Cr (VI) toxicity, which depends on its physiochemical, oxidation, and structural properties [32]. The Cr (VI) constituents are generally soluble and mobile in the environment [33]. It can easily pass cell membrane due to the structural similarity to inorganic anions which makes Cr(VI) as an alternative substrate in the sulfate transport system (34, 35). The cytotoxic effects of Cr (VI) on living organisms including plants, animals, and human are well reported and it is also a source of a variety of human cancers [32, 36].

Heavy metal toxicity and the effect of heavy metals on microalgae have been extensively reported. However, there is a limited or no literature available on the heavy metal toxicity of economically promising freshwater eukaryotic microalgae species *M. pulchellum* and *M. pusillum*. The freshwater eukaryotic non-model microalgae species, *M. pulchellum* and *M. pusillum* were chosen for this study due to their economic potential as they can grow at low light intensity as well as in CO₂-deficient conditions [37]. They are diverse green phytoplankton species which occasionally inhabit freshwater lakes. Furthermore, the genetic transformation of *M. pulchellum* (formerly *D. pulchellum*) for higher erythropoietin protein accumulation was reported by our group [38], which shows the broad scope of this microalgae species. During this study, the toxicity of hexavalent Cr (0 – 500 µg L⁻¹) on *M. pulchellum* and *M. pusillum* was investigated by comparing variations in cell density, modulated fluorescence yield, relative photosynthetic inhibition, and lipid accumulation. Both microalgae species exhibited significant sensitivities to the tested Cr(VI) concentrations as indicated by the fluorescence kinetics and lipid content.

Both microalgae species showed decreased growth at any Cr(VI) tested concentration when compared to the chromium free control cultivation. This effect has been previously attributed to the PSII inhibition via electron transport inhibition between Q_A⁻ and Q_B/Q_B⁻ by the hexavalent chromium ion [39]. Additionally, higher tested concentrations showed a decline in growth after day 7, likely via the well elucidated genotoxic route, where chromium ions form complexes with nucleic acids, causing strand breaks and the formation of mutagenic Cr-DNA fragments. Similarly, significant increases in minimal (F_0) and maximal (F_m) fluorescence values were observed till 100 µg L⁻¹, however, a decline in F_0 and F_m values was observed at concentrations exceeding 100 µg L⁻¹. Furthermore, 50 µg L⁻¹ chromium treatment showed higher maximal fluorescence in *M. pulchellum* as compared to *M. pusillum*, which shows higher sensitivity of *M. pusillum* to chromium even at low concentration. All tested Cr(VI) concentrations showed a significant decline in quantum yield and photosynthetic inhibition as compared to the control. A maximum photosynthetic inhibition of up to 67% was observed in *M. pulchellum* at 500 µg L⁻¹ whereas, *M. pusillum* showed a maximum photosynthetic inhibition of up to 66%. This shows that Cr(VI) has a negative effect on normal growth of both microalgae species by interrupting photosynthesis.

Interestingly, both tested microalgae species showed quite similar trends for growth and fluorescence yields, this could be because both microalgae are freshwater eukaryotic microalgae species and belong to a same taxon – Chlorophyta and thus showed similar Cr(VI) uptake and a similar PSII structure. Contrary to the present study, a 50% inhibition in growth was observed by Hörtsik et al. [40] when analyzing Cr(VI) toxicity using chlorophyll composition of *Auxenochlorella pyrenoidosa* (*Chlorella pyrenoidosa*) for 72 h in media supplemented with 2 mg L⁻¹ of Cr(VI) [40]. Similarly, in another study, a very minor amount of Cr(VI), 5 µmol L⁻¹, showed up to 40% inhibition in the maximal quantum yield of PSII of *Chlorella vulgaris* when treated for 96 h [41]. The variation in the present study could be due to different microalgae species and different experimental conditions.

The lipid accumulation results were quite interesting, both tested microalgae showed increases in lipid content. A lipid content of up to 36% was observed in *M. pulchellum* at the maximum tested Cr(VI) concentration in this study ($500 \mu\text{g L}^{-1}$), which was approximately 10 times higher than the control. Whereas a maximum lipid content of up to 30% was observed in *M. pusillum*. The fatty acids composition analysis showed higher levels of polyunsaturated fatty acids (hexadecenoic acid) and saturated fatty acids ($\omega 3$, $\omega 6$, and $\omega 7$). However, chromium exposure significantly affected the saturated fatty acids content. In this study, linoleic acid (C18:2; $\omega 6$) and α -linoleic (C18:3; $\omega 3$), were among the mostly affected fatty acids by Cr(VI). This agrees with the previously reported studies of Barsanti et al. [42], and Rochhette et al. [43], which states that chloroplast structure related lipids such as linoleic acid and α -linoleic are mostly affected by chromium. Furthermore, Cr(VI) treated cultures showed no significant differences for the non-photosynthetic structure related fatty acids such as arachidonic acid (C20:4; $\omega 6$), which agrees with Rochhette et al. [43]. This suggests that chloroplasts would be the main target organelle of Cr(VI) toxicity in *M. pulchellum* and *M. pusillum*.

Despite significant decreases in observed saturated fatty acids content at higher Cr(VI) concentration (especially $\omega 3$ and $\omega 6$), total lipid content showed a significant increase. This could be a microalgal defense mechanism to counteract oxidative damage [43]. Both microalgae species showed higher lipid accumulation at higher tested Cr(VI) concentration than the control, this shows that the addition of Cr(VI) could significantly enhance the lipid accumulation in *M. pulchellum* and *M. pusillum*.

The results of this study indicate that Cr(VI) can affect total lipids and fatty acids content, especially affecting the fatty acids related to photosynthetic activity. Changes in fatty acids composition in the treated cells could be due to their defense mechanism to reduce cellular damage caused by Cr(VI) and its route outlined above. Additionally, intracellular Cr(VI) reduction consumes intracellular antioxidants which could induce synthesis of simple and poly unsaturated fatty acids as a defense mechanism. However, further analytical and biochemical analyses are necessary to assist the findings. The present study could aid in aquaculture industry, in maintenance of microalgae stock cultures, and to estimate the possible side effects of using hexavalent chromium in microalgae cultures. It can further aid in the design and construction of biomarkers using eukaryotic freshwater microalgae species.

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