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RESEARCH ARTICLE

Phycoremediation of As(III) and Cr(VI) by *Desmodesmus subspicatus*: Impact on growth and biomolecules (carbohydrate, protein, chlorophyll and lipid) — A dual mode investigation

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Abstract

Microalgae are under research focus for the simultaneous production of biomolecules (e.g., carbohydrates, proteins, pigments and lipids) and bioremediation of toxic substances from wastewater. The current study explores the capability of indigenously isolated microalgae (*Desmodesmus subspicatus*) for the phycoremediation of As(III) and Cr(VI). Variation of biomolecules (carbohydrate, protein, lipid and chlorophyll) was investigated during phycoremediation. *D. subspicatus* survived up to the toxicity level of 10 mg/L for As(III) and 0.8 mg/L for Cr(VI). A 70% decline in carbohydrate accumulation was observed at 10 mg/L of As(III). An increased content of proteins (+ 28%) and lipids (+ 32%) within the cells was observed while growing in 0.5 and 0.2 mg/L of As(III) and Cr(VI) respectively. A decrease in carbohydrate accumulation was noted with increasing Cr(VI) concentration, and the lowest (− 44%) was recorded at 0.8 mg/L Cr(VI). *D. subspicatus* showed an excellent maximum removal efficiency for Cr(VI) and As(III) as 77% and 90% respectively.

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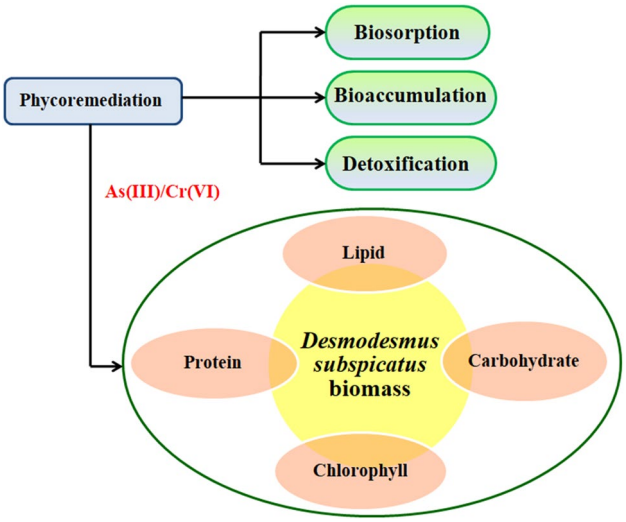
Highlights

- *Desmodesmus subspicatus* is capable to tolerate As(III) and Cr(VI)
- Carbohydrate content decreased by 28% under 5 mg/L As(III) exposure
- Protein content increased by 47% at 0.6 mg/L initial Cr(VI) concentration
- Lipid content increased by 32% at 0.2 mg/L initial As(III) concentration
- Removal efficiency of 77% for As(III) and 90% for Cr(VI)

Extended author information available on the last page of the article

Graphical abstract



Keywords Phycoremediation · Carbohydrates · Proteins · Lipids · Chlorophylls · As(III). Cr(VI)

Introduction

Bioaccumulation of arsenic (As) and chromium (Cr) has become a threat worldwide because of their toxic and hazardous nature (Tripathi and Poluri 2021). As per the standards given by the United States Environmental Protection Agency (USEPA), As and Cr have been listed in group 'A' carcinogenic elements for humans (Biswas and Nag 2021; Elahi et al. 2020). The maximum admitted concentration of As(III) and Cr(VI) in potable water is 0.01 and 0.5 mg/L respectively, as per the guidelines of the World Health Organization (WHO) (Poonia et al. 2021). Inorganic As compounds [As(III), As(V)] are more toxic to humans compared to organic arsenic compounds like dimethyl arsenic acid (DMA) and monomethyl arsenic acid (MMA) (Jomova et al. 2011). Arsenite [As(III)] shows more toxic effects on biota as compared to arsenate [(As(V)] (Hindmarsh et al. 1986). Along with groundwater contamination, other sources of As contamination are wood preservatives, thermal power plants, electronic materials, alloy manufacturing industries, antifouling paints, leather preservatives, pharmaceuticals, glass industries and agricultural industries (Chung et al. 2014). Several health-related problems in humans such as skin disease, bronchitis, malfunctioning of the nervous system and digestive issues are caused by arsenic poisoning (Daneshvar et al. 2019).

Inorganic Cr compounds usually exist in two valency states (hexavalent chromium and trivalent chromium). Chromium in the hexavalent form [Cr(VI)] is almost 100-fold more carcinogenic when compared to trivalent chromium [Cr(III)]. Cr(VI) has high solubility in water and is capable of mutating the DNAs and RNAs in all living organisms. Significant sources of Cr(VI) are domestic sewage and industrial wastewater discharged from leather tanning, mining, electroplating, paints and pigment, textile, steel, metallurgical and alloy industries (Nag et al. 2020; Nag and Biswas 2021b).

The economic constraints of widely used conventional methods for wastewater treatment such as electrochemical treatments, ion exchange methods, evaporation, precipitation and reverse osmosis (RO) have driven researchers to develop more cost-effective and sustainable processes for the remediation of heavy metals. In this direction, modifying adsorbing materials or using biological systems as well as integrated processes are some of the ways to reduce the running costs of a treatment plant, along with producing useful by-products. Bio-based green adsorbents as well as microorganisms (e.g., *Chlorella* sp., *Chlorella vulgaris*, *Scenedesmus quadricauda, Scenedesmus abundans*, *Maugeotia genuflexa*, *Ulothrix cylindricum*), have been used as sustainable and eco-friendly bioremediation methods to overcome these drawbacks (Bahar et al. 2013; Daneshvar et al. 2019; Ganguly et al. 2024; Nag and Biswas 2021a; Sarı et al. 2011; Sibi 2016; Tuzen et al. 2009). Agricultural, forest and green domestic wastes are used as adsorbents for heavy metal removal (Das et al. 2022; Nag et al. 2016). Recently,

several studies have been conducted using microorganisms like fungi, bacteria, yeasts, cyanobacteria and microalgae to adsorb heavy metals from effluents (Cui et al. 2021; Joshi et al. 2011; Saha and Nag 2022; Soares and Soares 2012). The remediation of toxic substances using microalgae as biosorbents has been termed phycoremediation (Moondra et al. 2021). Phycoremediation is a low-cost, sustainable and green process, which produces considerable amounts of biomass, biomolecules and energy (Tamil Selvan et al. 2020). Phycoremediation consists of three sequential steps namely biosorption, bioaccumulation and detoxification (Chaturvedi et al. 2021). Polysaccharides along with proteins and lipids are the building blocks of the microalgal cell wall containing diverse functional groups like thiols, amines, and phosphate (Majhi et al. 2021). There are different ways of surface adsorption between metal ions and cell wall: (i) binding of heavy metal ions with the anionic complexes of polysaccharides, (ii) formation of covalent bonds (hydroxyl, carboxylic) between charged cell wall and heavy metals and (iii) through the ionic exchange between heavy metal cations and the cell wall (Leong and Chang 2020). Bioaccumulation is the process of accumulating chemicals inside the microalgal cell and its organelles while the rate of intake is higher than the rate of excretion. Detoxification is the process by which chelators are secreted by microorganisms that induce the transformation of the toxic substance to non-toxic or less toxic chemical forms (Spain et al. 2021).

Dry biomass of *Maugeotia genuflexa* and *Ulothrix cylindricum* has removal efficiency of ~ 98% and ~ 96% respectively for As(III) (Sarı et al. 2011; Tuzen et al. 2009). Similarly, dry biomass of *Scenedesmus quadricauda* and *Chlorella vulgaris*, has shown adsorption percentages of almost 67% and 81% in remediating Cr(VI), justifying them as efficient, eco-friendly and sustainable adsorbents (Daneshvar et al. 2019; Sibi 2016). Microalgae species like *Chlorella minutissima*, *Scenedesmus* sp. *IITRIND2*, *Scenedesmus abundans* had shown a favourable As(III) removal efficiency of 60%, 70% and 70%, respectively (Arora et al. 2017). Furthermore, living heterotrophic microalgal species like *Botryocossuss* sp. *NJD-1*, *Chlorella* sp. *NJD-9* and *Scenedesmus* sp. *NJD-6* had a Cr(VI) removal percentage of almost 94%, 40% and 65%, respectively from co-contaminated wastewater containing 5 mg/L initial Cr(VI) concentration (Shen et al. 2019). In another study, the Cr(VI) removal capacity from tannery effluents of *Chlamydomonas moewusii*, *Scenedesmus* sp. and *Auxenochlorella pyrenoidosa* was 90%, 65% and 80%, respectively (Venkatesan and Sathiavelu 2022).

Most studies focused on the removal percentage of Cr and As separately by a microalgal species (active and dead). Moreover, studies on heavy metal removal using the genus *Desmodesmus subspicatus* (chlorophyta) are limited in the literature. Most studies using microalgal strains demonstrated the growth of microalgae, biological oxygen demand (BOD), chemical oxygen demand (COD) and removal of total dissolved solids (TDS) along with lipid content while the strains were grown in cassava wastewater or synthetic wastewater (Gressler et al. 2014; Sarfraz et al. 2021). However, analysis of the change in the composition of biomolecules (carbohydrates, proteins, chlorophylls and lipids) with different concentrations of As(III) and Cr(VI) is limited in the literature. Therefore, the present work focused on the variation in biomolecules (lipids, proteins, chlorophylls and carbohydrates) while remediating As(III) and Cr(VI) using isolated *D. subspicatus*. In this study, emphasis has been given to understanding the mechanisms involved in the change of lipid, protein, chlorophyll and carbohydrate molecules. Furthermore, the growth kinetics of *D. subspicatus* when grown in As(III) and Cr(VI) containing media were investigated along with their removal percentage.

Materials and methods

Microalgal strain and chemicals

Desmodesmus subspicatus, an isolated microalgae collected from the Deopani River in Arunachal Pradesh (India) was used in the current study (Sarkar et al. 2023). The BG-11 media was used for the growth of the microalgal species. The isolated microalgae was preserved at 19° C, 14–10 night-day ratio and 29 µmol photons/(m^2 s) of light intensity. All the chemicals were purchased from Merck (India) and the media used in the experiments were purchased from Hi-media laboratories (India). As(III) and Cr(VI) solutions were prepared by adding a calculated amount of potassium di-chromate ($K_2Cr_2O_7$) and sodium arsenite ($NaAsO_2$) salts in distilled water.

Experimental outline

250 ml of BG-11 media was prepared for seed culture and the media was inoculated with 2% stock culture. The seed culture was allowed to grow for 15 days at 28° C, 14–10 night-day ratio and 29 µmol photons/(m^2 s) of light intensity. This seed culture was used as the inoculum for all the experiments. The experiments were executed in two sets for both As(III) and Cr (VI). In this first set of experiments, *D. subspicatus* was cultivated at varying As(III) and Cr(VI) concentrations. In 100 mL conical flasks, 4% seed culture was inoculated in 50 mL of BG 11 culture with various concentrations of As(III) and Cr(VI). The concentrations of As(III) and Cr(VI) were varied within 0.5–15 mg/L and 0.2–1 mg/L, respectively. At the end of the 15 days of cultivation, the biomass pellet was obtained after centrifugation (10,000 rpm, 10 min)

of samples. The measurement of growth, carbohydrate, protein, total chlorophyll and lipid was performed from the biomass pellet collected through centrifugation. The collected supernatant was used to estimate the remaining concentrations of As(III) and Cr(VI).

In the second experimental set, the kinetics and adsorption efficiency of *D. subspicatus* grown at 10 mg/L of As(III) and 0.6 mg/L of Cr(VI) were investigated. Here, *D. subspicatus* were cultivated in 250 ml culture media [containing As(III) and Cr(VI)] for 30 days in triplicate. Subsequently, samples were collected at 3-day intervals for the determination of the growth of *D. subspicatus* and concentration of As(III) and Cr(VI).

Measurement of growth

The growth of *D. subspicatus* was measured by centrifugation of 2 ml of sample at 10,000 rpm for 10 min using a centrifuge (Eppendorf, USA, Model 5424). Afterwards, the supernatant was separated from the biomass pellet and collected for metal ion estimation. The biomass pellet was mixed with 2 ml of distilled water and vortexed to evenly mix the sample solution. Subsequently, biomass growth of all the samples was measured at an absorbance of 680 nm using a UV–visible spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA) (Sarkar et al. 2022b).

Measurement of biomolecules (carbohydrates, proteins, chlorophylls and lipids)

Biomolecules (carbohydrates, proteins, chlorophylls and lipids) were estimated using the procedures described in previous studies (Ansari et al. 2017; Ghosh et al. 2017a, 2017b; Herbert et al. 1971; Lichtenthaler 1987; Lowry et al. 1951; Sarkar et al. 2023, 2022a, 2022b). Carbohydrate content was estimated using the phenol–sulfuric acid method (Ghosh et al. 2017a, 2017b; Herbert et al. 1971; Sarkar et al. 2022a). Protein accumulation was estimated as per Lowry's method (Ansari et al. 2017; Ghosh et al. 2017b; Lowry et al. 1951; Sarkar et al. 2022a). Total chlorophyll content of *D. subspicatus* was determined using the procedure detailed in Lichtenthaler et al. (Lichtenthaler 1987; Sarkar et al. 2022a, 2022b). Briefly, a biomass pellet was obtained on centrifugation (10,000 rpm for 10 min) and an equal amount of 99.9% methanol was mixed with the pellet. Extraction of chlorophyll pigments was carried out in the dark by shaking at 150 rpm for 24 h at 4 °C in a sealed container using a shaking incubator (Daihan Labtech, India). The absorbances of the samples were measured at 652.4, and 665.2 nm. Chlorophyll a and b contents were determined using the following formulas:

$$Chlorophyll\ a\left(\mu g\ mL^{-1}\right) = 16.72A_{665.2} - 9.16A_{652.4} \quad (1)$$

$$Chlorophyll\ b\left(\mu g\ mL^{-1}\right) = 34.09A_{652.4} - 15.28A_{665.2} \quad (2)$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ biomass)} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (3)$$

where, $A_{665.2}$ and $A_{652.4}$ were the absorbances of the sample at 665.2 and 652.4 nm, respectively.

Lipid estimation was conducted by the sulfo-phosphor-vanillin (SPV) method (Sarkar et al. 2023). Phospho-vanillin reagent was prepared by dissolving 0.15 g of vanillin in 2.5 ml ethanol and 22.5 ml of deionized water while being stirred constantly. Subsequently, 100 ml of pure phosphoric acid was added to the mixture. The biomass obtained after centrifugation was dissolved in distilled water. Afterwards, 100 µl of dissolved biomass was kept in a water bath for 10 min at 100 ℃ after adding 2 ml of 98% pure sulfuric acid to the sample mixture. After adding 5 ml of SPV reagent to the sample mixture, the absorbance at 530 nm was measured using a spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA).

As(III) quantification

The supernatant obtained after the microalgal biomass separation (described in the previous sections), was used for As(III) quantification. 15.2 ml of sample after suitable dilution was taken in a test tube and concentrated HCl (0.8 ml) was added. The calibration curve was obtained using known As(III) solutions prepared from sodium arsenite ($NaAsO_2$). Total As(III) concentration was determined using an Atomic Absorption Spectrophotometer (AAS, Model-iCE 3000 Series, Thermo Scientific, USA). Subsequently, the removal percentage and metal uptake (mg/g-biomass) were calculated using the equations given as follows:

$$\text{Removal } \% = [(C_0 - C_i)/C_0] * 100\% \quad (4)$$

$$\text{As(III) uptake } (mg/g - biomass) = (C_0 - C_i)/w \quad (5)$$

where, C_0 and C_i are the initial and final concentrations of As(III) in mg/L, respectively, and w denotes the dry weight of biomass (g) obtained after cultivation time.

Cr(VI) quantification

The collected supernatant obtained after centrifugation of the microalgal culture was used for the estimation of the Cr(VI) concentration that remained in the solution. The carbazide-acetone solution has been used as an indicator for measuring the absorbances of the samples at 540 nm in a spectrophotometer (Basak et al. 2018; Nag et al. 2016).

The known Cr(VI) solutions prepared from potassium dichromate ($K_2Cr_2O_7$) salt at different concentrations were used to obtain the calibration curve. Thereafter, final concentrations, removal percentage of *D. subspicatus* and Cr(VI) uptake (mg/g-biomass) were determined utilizing the Eqs. (4) and (5).

Statistical analysis

Experiments were performed in triplicate. Microsoft Excel 2007 software was used for statistical analysis of the data using a two-tailed *T*-test. A *T*-test was executed to find the significance between values. Calculated *P*-value less or equal to 0.05 was considered as significant.

Results and discussion

Variation of *D. subspicatus* growth at different As(III) and Cr(VI) concentrations

Figure 1 represents the biomass of *D. subspicatus* after 15 days of growth under exposure of 0.5–15 mg/L initial concentrations of As(III) (Fig. 1a) and under exposure of Cr(VI) within the range 0.2–1 mg/L in the growth media (Fig. 1b). The pH of the growth medium varied within 8–8.8 during the experiments. Biomass growth declined consistently with increasing As(III) concentrations and the lowest growth was recorded at 1 mg/L (40% decrease compared to control). Afterwards, the growth increased again until 10 mg/L and finally the growth ceased at 15 mg/L initial As(III) concentration. The growth of microalgal species was likely affected by the generation of reactive oxygen species (ROS) which might have been triggered in the presence of As(III) (Arora et al. 2017). The 'U' shaped growth response of *D. subspicatus* with increasing As(III) concentrations could be the result of the increasing response of malondialdehyde and antioxidant enzymes like superoxide dismutase (SOD), and catalase (CAT) (Rai et al. 2013). SOD is an important enzyme for the anti-oxidative defence process, capable of changing O_2 to H_2O_2 at a very fast rate. Generally, the CAT enzyme is a major ROS-scavenging enzyme that promotes the disproportion reaction of H_2O_2 in H_2O and O_2 when H_2O_2 concentration is high at the cellular level (Rai et al. 2013). However, ascorbate peroxidase (APX) has a higher affinity towards H_2O_2 and therefore, APX could scavenge trace amounts of H_2O_2 present within the cells. Moreover, it could be noted from Fig. 1a, that the protein/carbohydrate ratio was less than '1', while microalgal cells were not under exposure to As(III) depicting carbohydrate

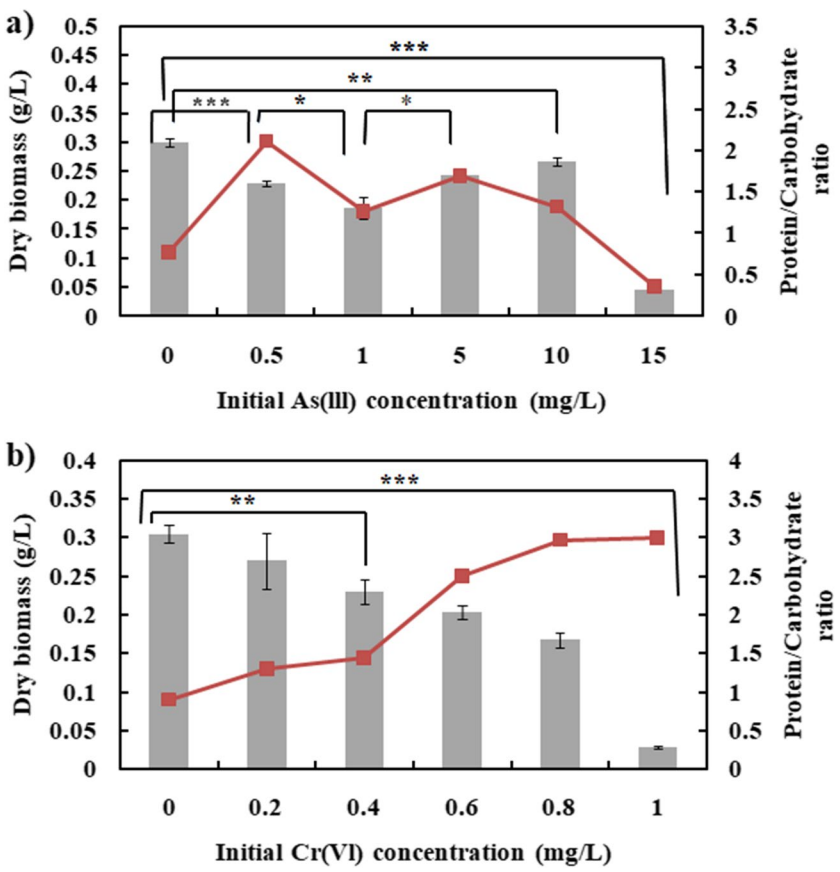


Fig. 1 Change in the growth of *D. subspicatus* and protein/carbohydrate ratio with varying (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates ($p^{***} < 0.005$, $p^{**} < 0.01$, $p^{*} < 0.05$). The bar graph represents the growth and the protein/carbohydrate ratio is represented by the line plot

content was more than protein content. However, this ratio was higher than '1' under exposure of As(III) (0.5, 1, 5, 10 mg/L), depicting that protein synthesis is more than carbohydrate within the microalgal cells. At 15 mg/L initial As(III) concentration, the protein/carbohydrate ratio was less than 0.5, showing an increased carbohydrate content, required to maintain the cell structure.

It could be noted from Fig. 1b, that the biomass formation of *D. subspicatus* remained almost unaffected in the presence of Cr(VI) at low (0.2 mg/L) concentrations compared to the control (biomass grown at BG-11 media). At low concentrations (0.2 mg/L of Cr(VI)), the metal removal might occur primarily by the process of biosorption and couldn't influence the intracellular metabolic activities within the cells of *D. subspicatus* (Danouche et al. 2020). However, with increased initial Cr(VI) concentrations, its toxicity might accelerate the oxidative stress within the cells, which influenced the intracellular metabolism by generating reactive oxygen species (e.g., H_2O_2, O_2^-) (Danouche et al. 2020). Therefore, it resulted in a descending trend of microalgal growth with increasing Cr(VI) concentrations. Moreover, the growth ceased at a Cr(VI) concentration of 1 mg/L and declined by almost 90.7% compared to the control ($p^{***} < 0.0005$). Enzymes like chromium reductase can inhibit oxidative stress by reducing harmful Cr(VI) compounds to less toxic Cr(III) compounds that were secreted by the microalgal species (Kamaludeen et al. 2003). Moreover, as reported in Fig. 1b, the protein/carbohydrate ratio in the control was less than '1'. Therefore, the carbohydrate content was higher than the protein content. But from 0.2 mg/L initial Cr(VI) concentration, a higher ratio value (more than 1) was observed with increasing initial concentration.

Variation in carbohydrate accumulation with initial concentration of As(III) and Cr(VI)

The consequences of initial concentrations of As(III) and Cr(VI) imparted on the carbohydrate accumulation within microalgal cells were investigated by measuring their carbohydrate content at different initial As(III) and Cr(VI) concentrations after the 15 days of microalgal growth (Fig. 2). The carbohydrate content within the microalgal cells

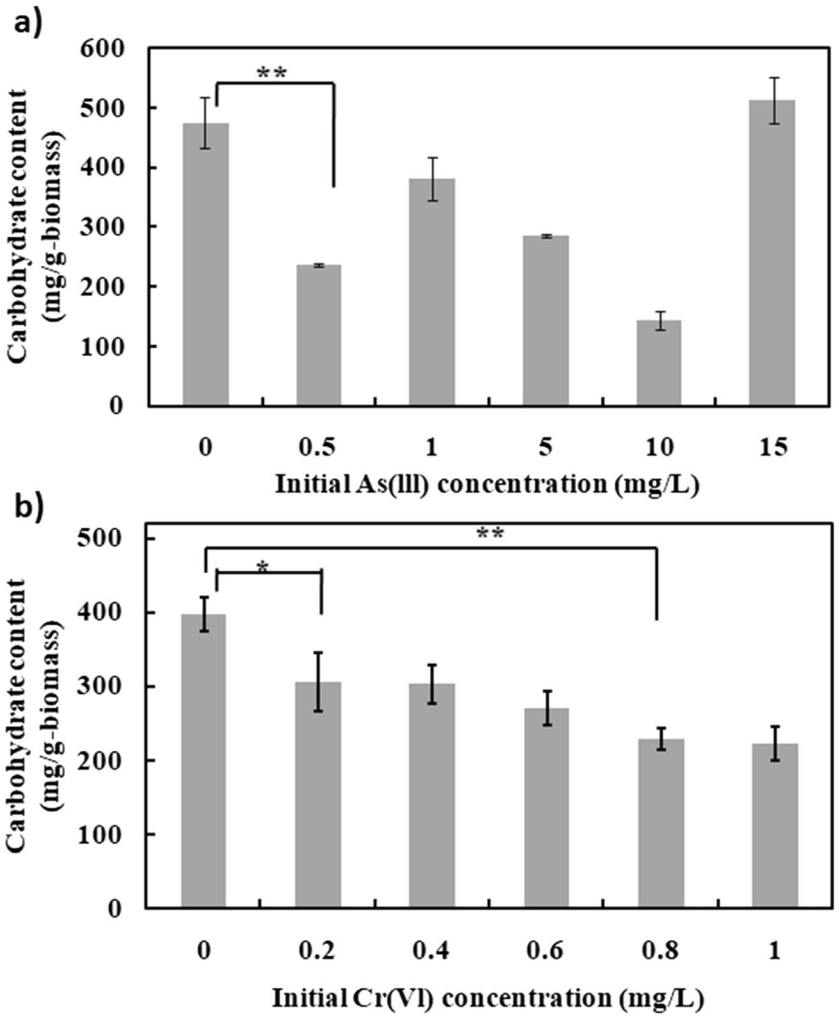


Fig. 2 Variation in carbohydrate content of the cells of *D. subspicatus* with varying (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates ($p^* < 0.05$, $p^{**} < 0.01$)

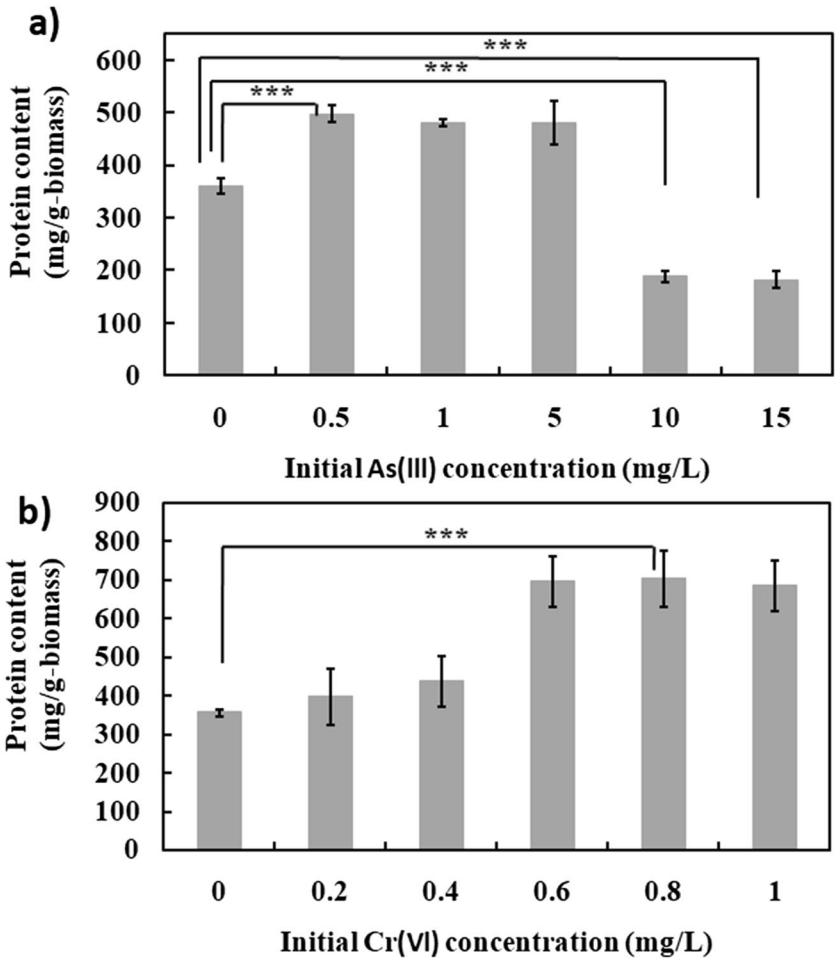


Fig. 3 Variation in protein content of the cells of *D. subspicatus* with varying (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates ($p^{***} < 0.005$)

declined with increased initial As(III) concentration from 0 to 0.5 mg/L. Carbohydrate production of the microalgal cells was affected by the oxidative stress within the cells which might have resulted in the synthesis of reactive oxygen system (ROS) like superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO) (Mahana et al. 2021). The required energy for ROS degrading processes is supplied from the breakdown of carbohydrates. Therefore, the synthesis of ROS depletes the carbohydrate content of the cells. In our experiments, the exposure to increasing concentrations of As(III) might have increased the synthesis of ROS resulting in the oxidative stress of the cells. This enhanced ROS activity could decrease the carbohydrate content by almost 50% at 0.5 mg/L of As(III) concentration. Microalgae possesses ROS-depleting mechanisms that include the generation of malondialdehyde and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione peroxidase (GPOX) (Danouche et al. 2020). Studies have shown a bell-shaped response to As(III) concentration 0.5–10 mg/L, Fig. 2a, in the production of malondialdehyde, ascorbate peroxide and superoxide dismutase (SOD) when microalgal cells are exposed to heavy metals (Rai et al. 2013). The initial decline in carbohydrate accumulation (at 0.5 mg/L) might be due to the oxidative stress generated by As(III) toxicity. At a higher initial As(III) concentration (1 mg/L), As(III) toxicity might have stimulated the increased synthesis of anti-oxidant enzymes that inhibit the synthesis of ROS resulting in less requirement of energy from carbohydrates. Increased content of anti-oxidant enzymes depletes ROS synthesis and thus less carbohydrate was used for maintaining cell structure. Therefore, carbohydrate content within the cells was higher. Afterwards, a decline in carbohydrate content at 5 and 10 mg/L initial As(III) concentration could be the result of increased ROS synthesis. Higher carbohydrate production (15 mg/L initial As(III) concentration) by microalgal cells could be a defensive strategy of *D. subspicatus* against the As(III) stress.

Interestingly, the trend of carbohydrate accumulation on the microalgal cells showed a declined carbohydrate content with an increasing initial concentration of Cr(VI). The highest carbohydrate accumulation was noted when *D. subspicatus* was grown in BG-11. At the exposure of 0.2 Cr(VI) concentration, carbohydrate accumulation declined

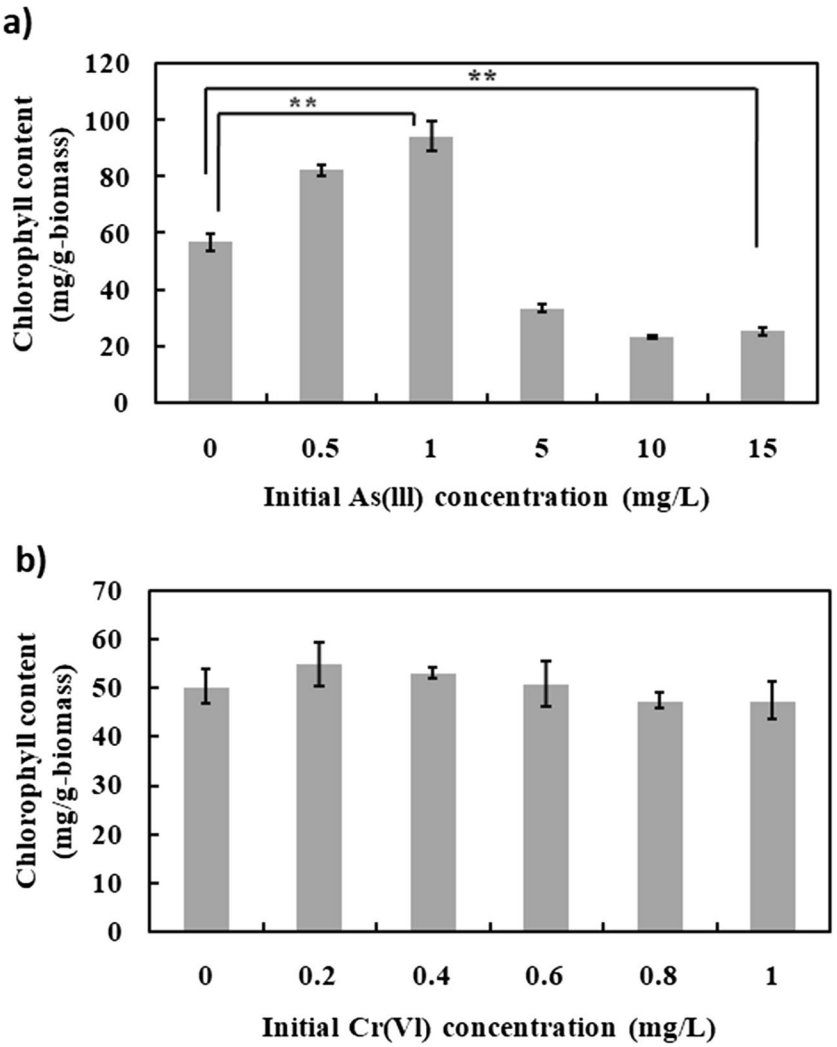


Fig. 4 Variation in chlorophyll content of the cells of *D. subspicatus* with varying (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates ($p^{**} < 0.01$)

by almost 23% compared with the control. The highest decrease (43%) in carbohydrate accumulation was noted when *D. subspicatus* was grown at 0.8 mg/L Cr(VI) concentration. The oxidative stress produced by the cells due to the increasing toxicity of Cr(VI) could be the probable reason for declining carbohydrate accumulation. Carbohydrate is the primary source of energy required for various steps of bioremediation mechanism (membrane transport, sequestration). Carbohydrates could be incorporated simply by initiating biochemical reactions to create energy in the form of adenosine triphosphate (ATP) (De Coen et al. 2001).

Variation in protein accumulation with initial As(III) and Cr(VI) concentration

A maximum surge in protein accumulation (~27% compared to control) after 15 days of microalgal growth was observed at 0.5 mg/L As(III) concentration ($p^{***} < 0.005$) (Fig. 3a). Thereafter, the protein content was the same with increasing As(III) concentration up to 5 mg/L As(III) concentration. This increase in protein content with increased As(III) might be the self-protecting mechanism of microalgal cells by forming protein-As(III) complexes, synthesizing thiol-rich proteins and phytochelatins to fight the stress generated by the toxicity of As(III) (Gómez-Jacinto et al. 2015; Priatni et al. 2018). At 10 and 15 mg/L As(III) concentrations, a declined protein accumulation was observed (~50% compared to control) ($p^{***} < 0.005$). The decrease in protein accumulation might be the outcome of the oxidative stress, triggered by the toxicity of As(III). This stress might result in the generation of ROS, which had given rise to the depletion of cells and proteins (Pawlik-Skowrońska et al. 2004).

The protein content of the *D. subspicatus* (after 15 days of biomass growth) grown at different Cr(VI) concentrations showed a similar trend (Fig. 3b). It was noted that with increasing Cr(VI) concentration, the protein content in the cells has shown a positive growing trend. Further, the change in protein accumulation (at 0.6, 0.8, 1 mg/L Cr(VI)) was almost negligible. Maximum protein accumulation (~1.5 times) was observed at 0.8 mg/L Cr(VI) concentration

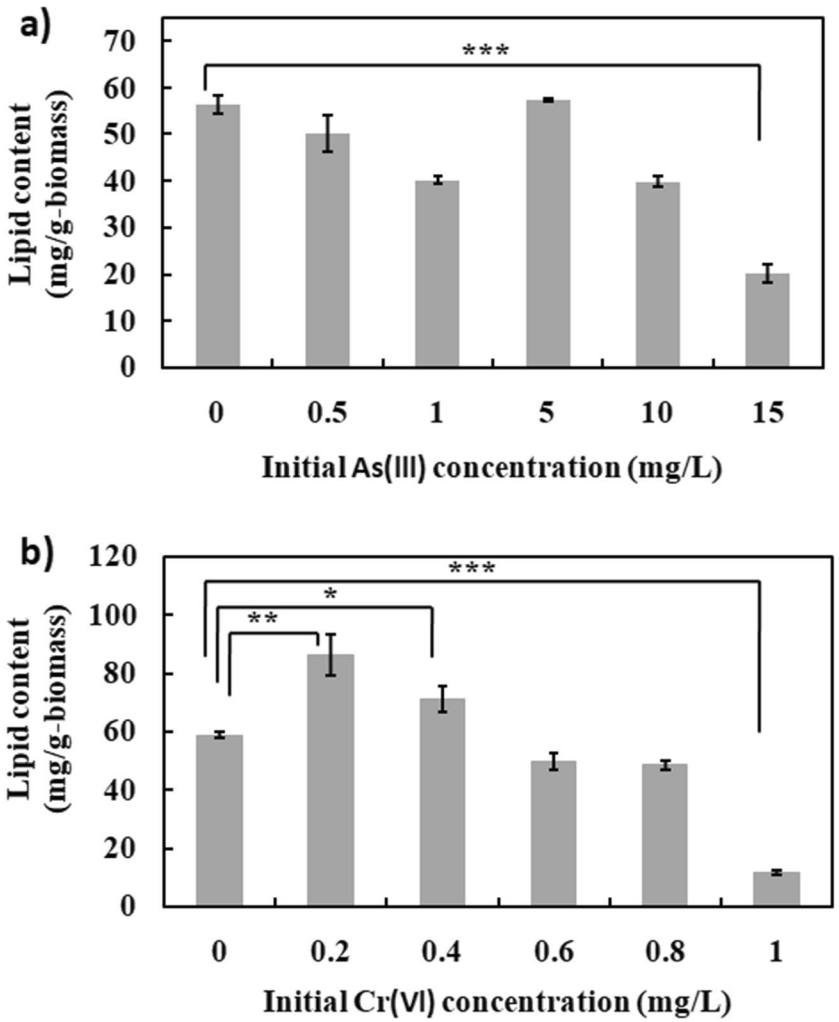


Fig. 5 Variation in lipid content of the cells of *D. subspicatus* with varying (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates ($p^* < 0.05$, $p^{**} < 0.01$, $p^{***} < 0.005$)

compared with the control. Therefore, increasing the synthesis of proteins (such as phytochelatins, heat-stable proteins and heat-denaturable proteins) by the cells of *D. subspicatus* could be the possible action of the microalgal species to decrease the toxic effects of Cr(VI) by forming complexes (Aharchaou et al. 2017; Gómez-Jacinto et al. 2015; Priatni et al. 2018). Some proteins (phytochelatins) might convert toxic metal ions into harmless metal complexes by forming protein-bonded metal complexes, called chelation (Arora et al. 2017). Overall, it seems that the cells started to produce more protein to encounter excessive toxicity.

Variation in chlorophyll accumulation with initial As(III) and Cr(VI) concentrations

The chlorophyll content of *D. subspicatus* (after 15 days of growth) grown at varying metal concentrations [As(III) and Cr(VI)] was evaluated and represented in Fig. 4. It could be noted that chlorophyll content increased up to As(III) concentration of 1 mg/L ($p^{***} < 0.005$). The enhanced chlorophyll accumulation within the microalgal cells at higher As(III) concentrations might be due to the inhibition of chlorophyllase. This enzyme is responsible for the degradation of chlorophyll by catalyzing the process of converting chlorophyll to phytol and chlorophyllide. Therefore, inhibition of the chlorophyllase enzyme could prevent chlorophyll degradation and could result in higher chlorophyll content in microalgal cells (Zhang et al. 2013). However, at higher As(III) concentrations (from 5 to 15 mg/L), the chlorophyll content declined by almost 41–66%. The heavy metal's toxicity affected photosynthesis activity of microalgal cells. The ROS synthesized due to stress caused by toxic heavy metal probably resulted in the accumulation of H_2O_2 and degraded the chloroplast of the microalgal cells (Arora et al. 2017).

The chlorophyll content of the cells after 15 days of growth at different initial concentrations of Cr(VI) did not vary significantly (Fig. 4b) attributing chlorophyll content was least influenced by the toxicity of Cr(VI). Peroxidation of chloroplast is the primary reason for the decline in

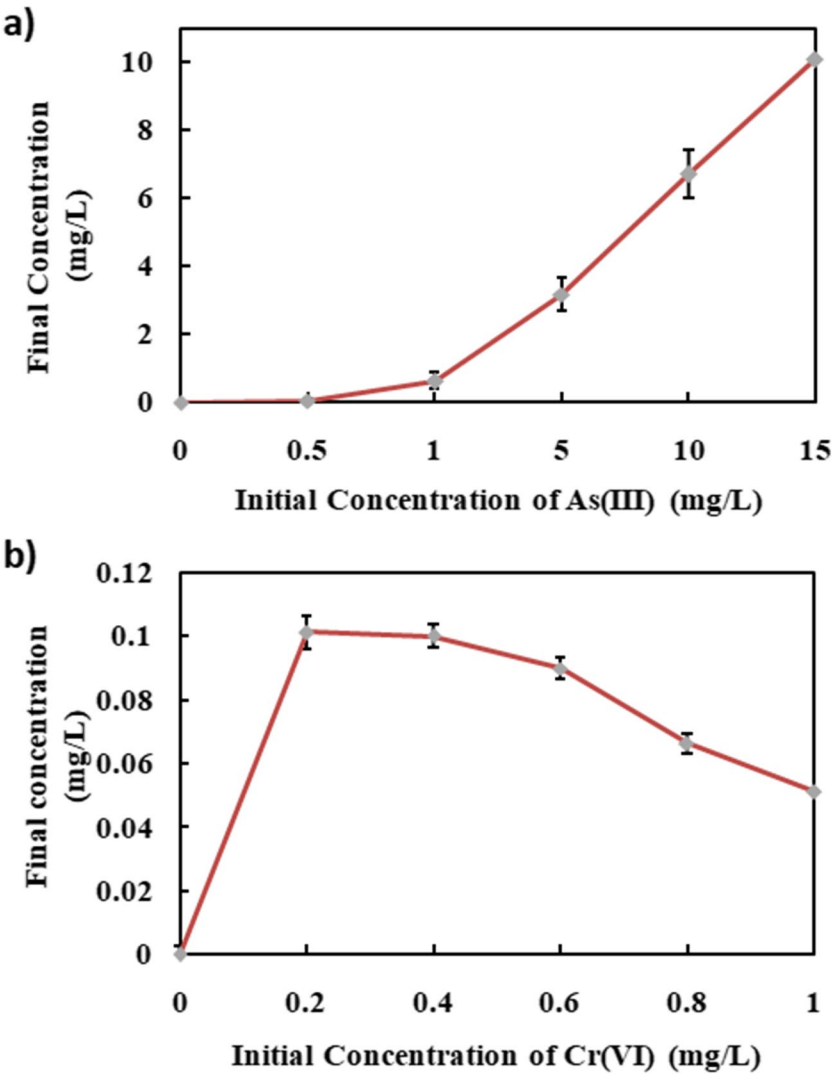


Fig. 6 Final concentration of metal ions after cultivation for 15 days with different initial concentrations. (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates

chlorophyll content within the cells due to increased toxicity of Cr(VI). APX acts as a scavenger to H_2O_2 produced primarily in chloroplast and to maintain the cell's redox state (Rai et al. 2013). Also, the negligible change in chlorophyll content could result from higher protein production by the microalgal cells at higher concentrations of Cr(VI). Some proteins may act as a binding complex (phycochelatin) for binding heavy metals and act as an antioxidant to reduce the toxicity of heavy metals (Arora et al. 2018).

Variation in lipid accumulation with initial As(III) and Cr(VI) concentrations

The lipid content of *D. subspicatus* grown for 15 days in different As(III) concentrations was depicted in Fig. 5a. It has been observed that with ascending concentration of As(III), the lipid content on the cells initially showed a declining trend. Minimum lipid content was recorded at 15 mg/L initial As(III) concentration (~64.2% compared with the control). Oxidative damage of lipid biomolecules caused by toxicity of As(III) could be the significant reason for declining the lipid content at 10 and 15 mg/L initial As(III) concentration (Xiao et al. 2023).

Notably, lipid accumulation increased (~32% compared with the control) at an initial Cr(VI) concentration of 0.2 mg/L ($p^{**} < 0.01$) (Fig. 5b). Afterwards, lipid accumulation within the cells of *D. subspicatus* declined with increasing Cr(VI) concentration. Minimum lipid accumulation was recorded at a Cr(VI) concentration of 1 mg/L and decreased by ~80% compared with the control. Lipids consist of fatty acids and their increased amount within *D. subspicatus* could be included as their self-protection strategy to overcome the cellular distress created by Cr(VI) exposure (0.2 mg/L) (Bashir et al. 2021). Thereafter, the decrease in lipid accumulation might be the result of the peroxidation of lipid biomolecules caused by the oxidative stress generated by Cr(VI) toxicity. It may be noted that lipid peroxidation occurs from the free radicals (ROS) as they react with the

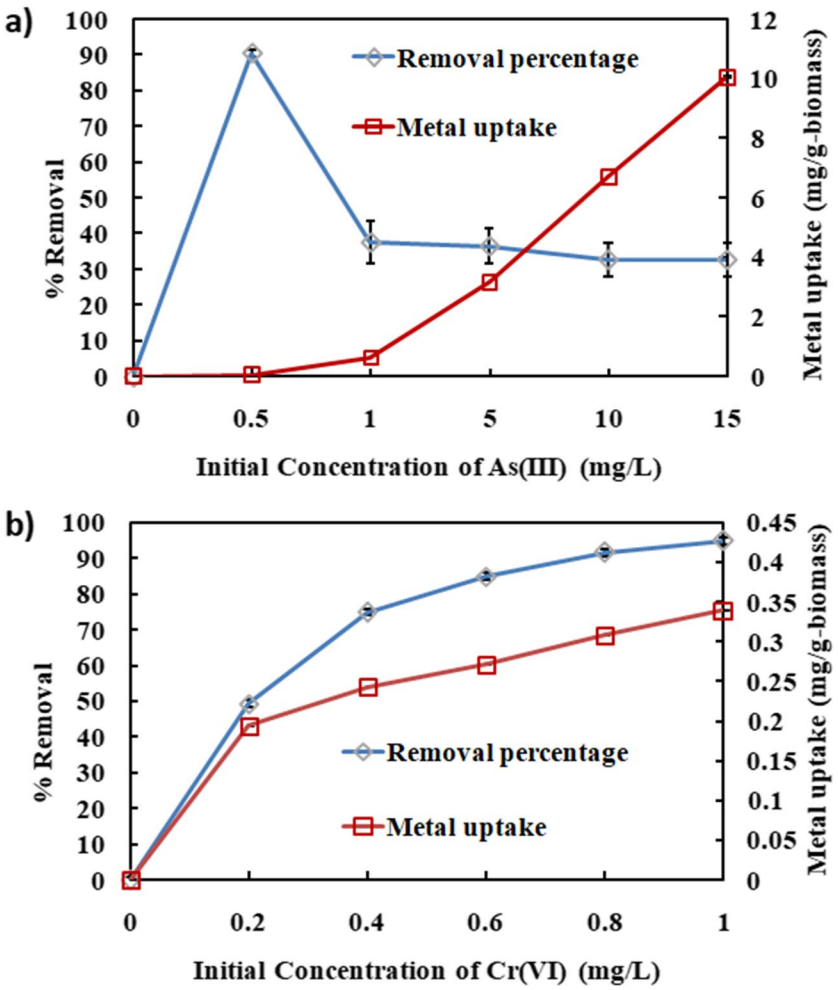


Fig. 7 Variation of metal uptake by *D. subspicatus* and removal percentage with varying initial concentrations of (**a**) As(III), (**b**) Cr(VI). The data are mean ± S.D. obtained from triplicates

carbon–carbon double bonds (C = C) present in polyunsaturated fatty acids (PUFAs) (Xiao et al. 2023).

Removal efficiency and metal uptake capacity by *D. subspicatus*

Figure 6 represents the final concentrations of As(III) and Cr(VI) after 15 days of microalgae cultivation with different initial As(III) and Cr(VI) concentrations. It could be noted that approximately 10 mg/L of As(III) remained in the cultivation media (BG-11) after phycoremediation (for 15 days) when the microalgae was grown in 15 mg/L initial As(III) concentration (Fig. 6a). Removal efficiency and metal uptake of As(III) and Cr(VI) by *D. subspicatus* after 15 days of microalgal growth was represented in Fig. 7. From Fig. 7a, it was observed that with increasing initial As(III) concentration, the removal efficiency of *D. subspicatus* declined to minimal values (~32%) at an As(III) concentration of 15 mg/L. Interestingly, the removal of As(III) was maximum (almost 90%) and the least concentration remained in the media at 0.5 mg/L initial As(III) concentration. Moreover, the metal uptake by *D. subspicatus* in remediating As(III) showed an increased trend with increasing initial metal concentration. It can be observed that the maximum As(III) uptake was 10.07 mg/g-biomass, at an exposure of 15 mg/L initial As(III) concentration (Fig. 7a). Biomass content was declining upto 1 mg/L of initial As(III) concentration (Fig. 1a) and therefore, the metal uptake was slow initially upto 1 mg/L initial As(III) concentration. Moreover, beyond 1 mg/L initial As(III) concentration, biomass quantity was increasing resulting in a sharp increase in metal uptake. As(III) primarily exists in the form of H_3AsO_3 at a pH ranging between 8 and 8.8, which was the pH range of the present study (Arora et al. 2017). Intracellular metabolism of As(III), includes As(III) oxidation, complex formation with thiol compounds and sequestration into vacuoles, biomethylation of As(III) methylarsenicals (MA, DMA, and TMA) and delivery from cells are the probable detoxification mechanisms (Cullen et al. 1994; Rahman and Hassler 2014; Rahman et al. 2014). Therefore, at higher As(III) concentrations, the stress generated within the microalgal cells due to the toxicity of As(III) damaged various cell

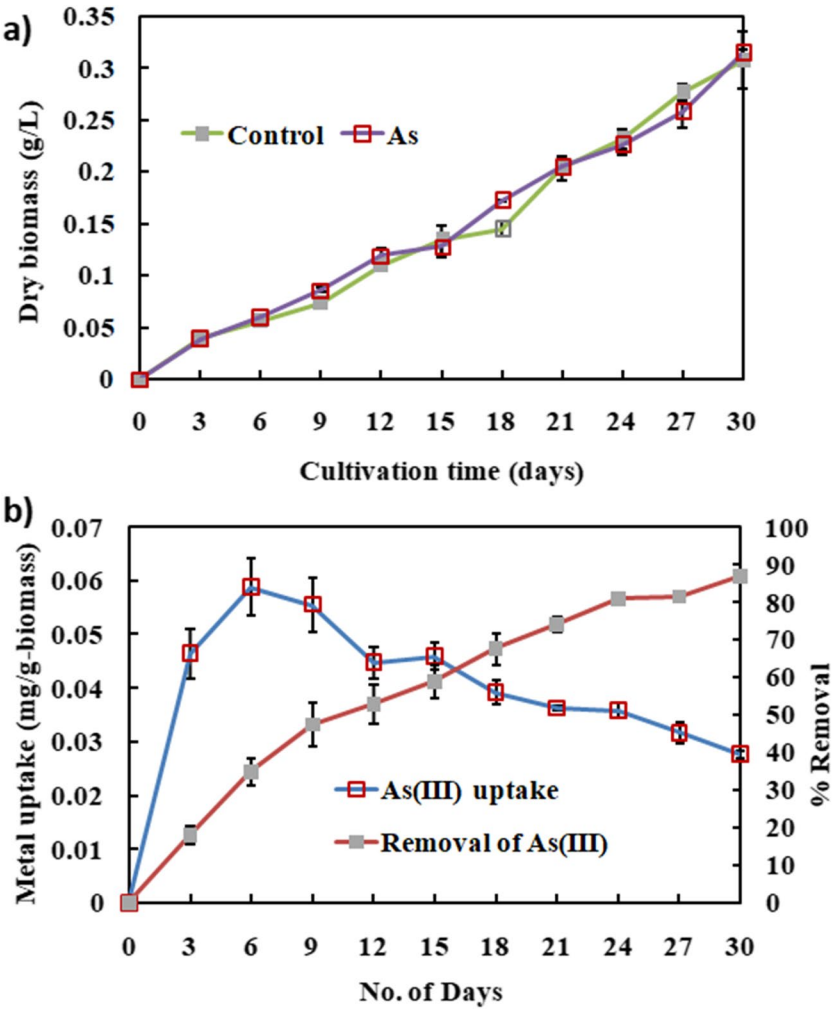


Fig. 8 **a)** Growth kinetics of *D. subspicatus* when *grown* in BG-11 (Control) and in As(III) (10 mg/L) containing BG-11, **b)** Removal percentage and metal uptake of As(III) with cultivation days. The data are mean ± S.D. obtained from triplicates

organelles (mitochondria, chloroplast) which in turn imbalanced the stability of microalgal cells. The surface adsorption of As(III) might have declined with increasing initial concentration since biomass quantity was declining.

Final Cr(VI) concentration was highest at 0.2 mg/L initial concentration of Cr(VI) and thereafter decreased with increasing initial Cr(VI) concentration (Fig. 6b). Notably, the percentage removal of Cr(VI) was maximum (~95%) at a Cr(VI) concentration of 1 mg/L and least (~50%) at a Cr(VI) concentration of 0.2 mg/L. It may be noted that Cr(VI) uptake by *D. subspicatus* increased with increasing initial Cr(VI) concentration (Fig. 7b). Interestingly, biomass content decreased with increasing initial Cr(VI) concentration (Fig. 1a) while metal uptake increased with increasing initial Cr(VI) concentration (Fig. 7b). Functional groups that were majorly found accountable for chromium adsorption were amine, amide, alcoholic groups, carboxylic groups, aldehydes complexes, halide compounds, sulfoxide and phosphate (Leong and Chang 2020). In a study, it was observed that with ascending values of initial Cr(VI) concentration (10.6 to 21.2 mg/L), the removal efficiency of *Botryocossuss* sp. NJD-1 increased, when grown in co-contaminated (organics and chromium) wastewater (Shen et al. 2019).

Growth kinetics of *D. subspicatus* and removal efficiency of As(III)

The kinetics and removal percentage of As(III) by *D. subspicatus*, were performed and recorded for 30 days in a media containing an initial As(III) concentration of 10 mg/L (Fig. 8). At 10 mg/L initial As(III) concentration, similar growth parameters were recorded both for treated and control samples. It has been observed that the influence of As(III) on the growth of *D. subspicatus* was much less since the growth curves of control and under the exposure of As(III) were almost the same (Fig. 8a).

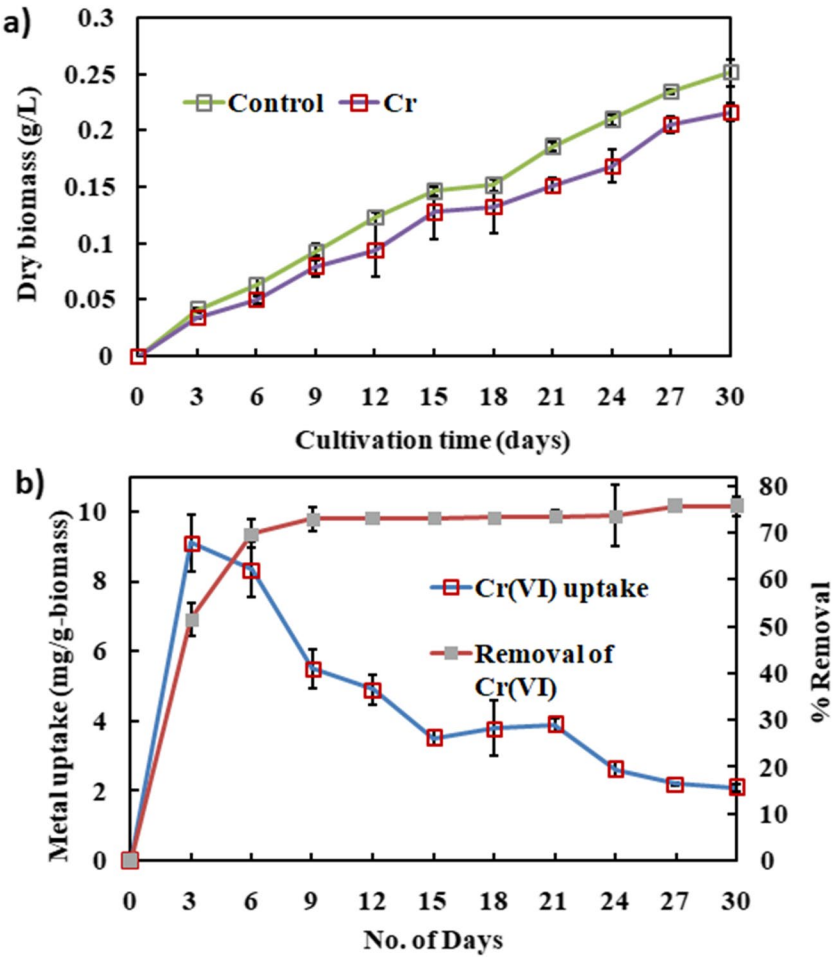


Fig. 9 **a** Growth kinetics of *D. subspicatus* when *grown* in BG-11 (Control) and in Cr(VI) (0.6 mg/L) containing BG-11. **b** Removal percentage and metal uptake of Cr(VI) with cultivation time. The data are mean ± S.D. obtained from triplicates

Moreover, biomass production and removal percentage of As(III) was increasing with time. It could be predicted that a significant amount of As(III) diffusion into the cell cytoplasm was not happening through the microalgal cells. Alternatively, to nullify the toxic effects of As(III) and balance the structure of the cells, protective actions were executed by the microalgal cell by producing more proteins (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase) at higher As(III) concentrations. These expressed proteins might act as chelators by forming complexes with metal ions and help in decreasing the carcinogenic effects of As(III) (Aharchaou et al. 2017; Gómez-Jacinto et al. 2015; Priatni et al. 2018).

Percentage removal and metal uptake of *D. subspicatus* were evaluated and presented in Fig. 8b. It could be observed that with increasing cultivation time (days), the percentage removal of As(III) increased steadily and reached 87% after 30 days of cultivation. Metal uptake increased along cultivation time and was at its peak on the 6th day of cultivation. Thereafter, it declined steadily with days and the least uptake was recorded on the 30th day. The decrease in the metal uptake rate could be the result of the reduction of available binding sites for As(III) and less pronounced diffusion of As(III) within cell cytoplasm with cultivation time.

Growth kinetics of *D. subspicatus* and Cr(VI) removal

Similar to As(III), the growth kinetics of *D. subspicatus* cultivated in 0.6 mg/L of initial Cr(VI) concentration was compared with the control (Fig. 9a). The growth of *D. subspicatus*, grown in a Cr(VI) environment was less (~ 18%) as compared to control. The removal percentage and metal uptake of Cr(VI) by *D. subspicatus* were represented in Fig. 9b. It could be noted that around 51% of the removal of Cr(VI) happened within the first 3 days and reached around 70% within 6 days of microalgal cultivation. Thereafter, it reached to steady state and the maximal removal value (75%) was achieved after 30 days. Moreover, the metal uptake was maximum (~ 8.5 mg/g-biomass) within 3–6 days of cultivation followed by a decreasing trend. This result may be due to the availability of functional groups, which are saturated

after 6 days of cultivation. Afterwards, the rate of bioremediation decreased with the number of days, resulting in a steady slow variation in removal percentage (73% in 15 days and 75.7%) in 30 days with the cultivation time.

Conclusion

The current study demonstrated the capability of *D. subspicatus* for the remediation of As(III) and Cr(VI) and change in the accumulation of biomolecules within the microalgae cells. Also, the removal efficiency of *D. subspicatus* at various concentrations of Cr(VI) and As(III) was investigated. The isolated *D. subspicatus* survived the toxic exposure of up to 10 and 0.8 mg/L of As(III) and Cr(VI) concentration respectively. The toxicity stress caused by Cr(VI) and As(III) stemmed from remodeling the biomolecule compositions within the cells (carbohydrates, proteins, chlorophylls, lipids). Carbohydrate content decreased under the exposure to Cr(VI) and As(III) while protein, chlorophyll and lipid content increased. This study provided insights into the increased accumulation of proteins and pigments at higher As(III) and Cr(VI) concentrations using *D. subspicatus*. Therefore, this strain could be used for dual benefits: (i) the removal of harmful metals [As(III) and Cr(VI)] from wastewater sources and (ii) high yield of proteins and pigments. Large-scale cultivation of microalgae in wastewater contaminated with heavy metals can serve as an efficient, eco-friendly and sustainable approach to removing pollutants. Moreover, upon completion of bioremediation, the biomass of microalgae can be collected for extracting and producing bio-based products. In this direction, the current study holds a strong scope for large-scale cost-effective phycoremediation of wastewater along with the production of value-added substances like proteins and pigments.

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Author contribution Anisha Ganguly and Kalyan Gayen contributed to the conception and design of this work. Experiments, data collection and analysis were performed by Anisha Ganguly and Kalyan Gayen. The first draft of the manuscript was written by Anisha Ganguly. Soma Nag, Tridib Kumar Bhowmick and Kalyan Gayen reviewed the manuscript. All authors read and approved the final manuscript.

Declarations

Consent for publication We undertake and agree that the manuscript submitted to your journal has not been published elsewhere and has not been simultaneously submitted to other journals.

Conflict of interest The authors declare no competing interests.

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