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MORPHOLOGY, PHENOTYPIC PROFILE, CYTOTOXICITY AND UPTAKE FLUX OF THE MARINE MICROALGA *Tetraselmis gracilis* DURING Cr (VI) EXPOSURE IN CONTROLLED LABORATORY CONDITION

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ABSTRACT

The wide range of industrial usage of chromium is a potential threat to the environment in specific to aquatic organisms. In the present study, we report the toxicity of Cr (VI) upon marine microalga *Tetraselmis gracilis*. Effluents from the industries, for instance leather, textile, steel, etc. release Cr (VI) which ultimately reaches to seawater, is potential route for Cr (VI) assimilation and toxicity. To comprehend the mechanism of toxicity, cells of *T. gracilis* was exposed in various concentrations of Cr (VI) upto 96 h. The detailed cytotoxicity assay revealed a substantial reduction of growth rate in a dose dependant manner. Significant decline in chlorophyll-a pigment and biomass was noted with increasing concentration of Cr (VI). The FTIR study suggested the surface chemical interaction of Cr (VI) with cells of *T. gracilis*. Further, micrometric study, photo micrographic (SEM and LM) study revealed the substantial changes in cellular morphology and cell wall damage.

KEYWORDS: Cr (VI), FTIR, Micrometry, Morphology, SEM, *Tetraselmis gracilis*.

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INTRODUCTION

Metal pollution is creating one of the prime threats to the aquatic ecosystem.[1, 2, 3, 4] At the onset of the industrial era, an accelerated rate in the heavy metal accumulation has been documented from various environmental sampling in an alarming manner.[4, 5] The ingress of heavy metals in the aquatic ecosystem takes place from various autochthonous and alochthonus sources due to several environmental consequences, anthropogenic activities, etc.[2] The plethora of metal xenobiotics get accumulated in the aquatic ecosystem due to the excessive discharge of metal xenobiotics clubbed with prolonged half-life and nondegradable property of metal and builds up in concentration.[5, 34] Though controlling of heavy metal release into the aquatic ecosystem is challenging, yet several technologies, for instance, chemical treatment, mechanical separation, flocculation, biological treatments, etc. are being applied to combat the heavy metal pollution. Among the treatment methodologies, biological treatment is reported to be the most suitable technology as it is cost effective in terms of efficiency, energy and economy.[2, 6] Microalgae, which belong to the phytoplankton community, recently have gained substantial attention worldwide with a new tempo as a natural tool to quench and sequester insidious metals leading to bioremediation and biotransformation.[34] However, the cellular uptake of heavy metal by microalgae is optimal and takes place solely under homeostatic control. Environmental parameters play a great role in regulating the phenotypic plasticity of microalgae which in turn effects on the production of secondary metabolites, biomarkers, etc.[5] Setback arises when the concentration of nonessential (Cr, As, Pb, etc.) metal nutrient overtakes or substitutes the essential (Cu, Mg, Co, etc.) ones or the essential metal ions concentration gets exalted beyond the threshold limit instead of extensive phenotypic plasticity of the microalgae.[6] As a result of this, free radical generation, morphological disorder of cells, fluctuation in the concentration of biochemical biomarkers, physiological instability including acclimatization, stress tolerance, growth inhibition, etc. are experienced by the microalgal cells. Monitoring of the physicochemical parameters of the aquatic ecosystem reflects the status of the aquatic environment but it fails to forecast about the adverse impact upon the health of the microalgae. Because microalgae form the base of the food chain, hence any impairment at the base has potential to affect the next trophic level.[30,31] In this aspect, laboratory cultures are important tools to investigate the biology, ecophysiology, cytotoxicity and morphology of the microalgae, allowing the experiments under controlled conditions and simultaneously ruling out the probable synergistic or antagonistic effect which may contribute stress upon the microalgae due to the associated biotic and abiotic factors.[8,9,10] The conventional ecophysiology studies are based on growth rate, growth inhibition, chlorophyll a endpoints which provide an insight of toxicity at population level of the species.[11] Whereas, micrometric analysis, Scanning Electron Microscopy (SEM) micrograph, etc. are potential techniques to investigate the impact upon morphology of the microalgal cell due to metal xenobiotics at cellular level. To achieve a clear picture of the toxicity, the analysis needs to be conducted at population level and at the cellular level as well. The population level provides the information of toxicity on community through food chain while cellular level toxicity provides insight about the heterogeneity in the bulk.[12] Chromium is one such metal xenobiotic which is ubiquitously present in the aquatic ecosystem and naturally prevails in the earth's crust. It exists in several oxidation states. However, Cr (VI) is reported to be the most toxic due to its high solubility, mobility and oxidation state.[13, 35] Cr (VI) has a wide range of industrial applications, for instance, electroplating, tanning, textile, dyeing, corrosion inhibition, etc. Globally, India is the second runner up as the largest leather producing country and collectively the states of Tamilnadu, West Bengal and Uttar Pradesh are sheltering 88% of tannery units of the country.[6] The subsequent Cr (VI) containing industrial effluent gradually contaminates the marine environment ecosystem by means of surface run-off, precipitation, etc. Upon ingress into the marine ecosystem the plethora of this insidious non-essential metal ion ultimately interacts with *in situ* biochemical pathways of the microalgae. In our study, the marine microalga *Tetraselmis gracilis* (NIOT- 16, NCBI Accession No: KU351743) has been exposed to the different concentrations of Cr (VI). During the study, morphology, micrometry, Cr (VI) uptake flux, phenotypic profile of *T. gracilis* has been evaluated. The results presented over here are mean ± S. D. of triplicates and considered significant at $p < 0.05$ upon processing through one way Analysis of Variance (ANOVA).

MATERIALS AND METHODS

Microalgal Culture and growth media[14]

The axenic monoculture of the marine microalga, *Tetraselmis gracilis* (NIOT-16, NCBI Accession No: KU351743) was received from microalgae culture collection bank of NIOT and explored as a model organism because of its exalted tolerance (as recorded from the range finding test conducted earlier) towards Cr (VI). The culture was maintained in seawater enriched with f/2 media under cool white fluorescent light with 54 µMol m^{-2} s^{-1} photosynthetically active radiation (PAR) intended for 12 h light and 12 h dark photoperiod at 24 ± 2°C. The initial pH and salinity was 8.0 and 35 psu, respectively. A constant number of cells (10^6 cells/mL) from the exponential phase were inoculated during each subculture and the microalga was allowed to acclimatize upto 5th generation in the experimental biotic and abiotic conditions. To prevent cell clumping, the cultures were swirled gently thrice daily.

Preparation of Cr (VI) toxicant

Potassium dichromate ($K_2Cr_2O_7$) was procured from Merck and used as Cr (VI) toxicant. A stock solution of 5000 µg/mL of Cr (VI) was prepared and used for the experiments. The required lower concentrations of Cr (VI) xenobiotics were prepared through proper dilution of the stock solution using sterile Milli Q water.

Experimental method[15]

The experiment was performed in triplicate based on the protocol of the Organization for the Economic

Cooperation and Development. During the experiments the *T. gracilis* cells from the exponential phase were exposed in five different concentrations (19 – 272 µg/mL) of Cr (VI) along with control in triplicate. A standardized initial inoculum of the *T. gracilis* was inoculated in culture flasks (500 mL) which contained 200 mL of seawater enriched with f/2 media. The Cr (VI) concentrations were initially added as nominal concentration and then measured in ICP-OES as the 0^{th} h metal concentration. Further, upon finding the IC_{50} value of Cr (VI), the culture was exposed to the finalized concentration in triplicate for 96 h. Samples were aliquoted for morphological, physiological, micrometric, and cell viability analysis at the end of 96 h while for metal analysis both biomass and media were aliquoted during 0^{th} hour and at the end of 96^{th} hour at all stages of experiment. Throughout the experiment the abiotic conditions were kept uniform as that of maintained during acclimatization. Optical density (OD) and cell count were carried out at 24 h regular interval to monitor the growth and probable contamination all through the experiment.

***Measurement of growth parameters*[16, 17]**

Microalgal cell counting was continued through conventional method using haemocytometer by visualizing under a microscope (Karl Zeiss Axioscope2, 400X) upon fixing 1 mL of the sample aliquot from each sample with Lugol's iodine and expressed as number of cells/mL, whereas optical density (OD) was measured in a spectrophotometer (Shimadzu) at 660 nm. Biomass was measured according to the protocol prescribed by Zhu and Lee. Data were expressed as µg/mL of algal suspension. Chlorophyll a was measured following the protocol of Jeffrey and Humphrey. Trichromatic equation was applied to calculate the chlorophyll-a content. The pigment content was calculated (µg chla/ mL of microalgal suspension) under consideration of the dilution factors. To calculate growth rate (GR) of the microalga Nichol's equation was applied: $K\ (/day) = [(Ln\ (N_2/N_1))/\ (t_2-t_1)]$; where, N_2 is the OD value at time t_2 and N_1 is the OD value at time t_1; while the percent of growth rate inhibition (GI) was calculated based on the equation: $GI\ (\%) = [(GR_{control} - GR_{concentration})/(GR_{control})]*100$ and successively doubling time (DT) was calculated as DT(h) = (ln2/K).

***Metal Analysis*[18]**

Metal analysis was carried out for seawater, culture media and biomass. Samples were collected from each test flask at 0^{th} hour and at the end of 96^{th} hour. To determine the metal concentration in biomass, the samples were harvested, lyophilized, weighed and then digested with concentrated ultra pure HNO_3 and H_2O_2 (30%) (1:4) in 100 mL pre-cleaned teflon vessel with lid; whereas for culture media, an aliquot of samples were preserved in 2 % ultra-pure HNO_3 till analysis. The metal concentrations of the samples were determined in ICP-OES (VARIAN 725-ES). Also, the corresponding media and matrix were analyzed as prescribed by Grasshoff *et al.* with necessary modification. Blanks and spikes were analyzed to validate the digestion process of spectroscopic analysis, obtaining 95% recovery. In addition, 0.5 µg/mL of multi-elemental standard was analyzed upon every 10 samples to monitor the matrix effects of the analytes and for quality assurance and quality control. The entire analysis was carried out under uniform instrumental conditions viz., power (1.2 KW), plasma flow (15.0 L/min), replicate (3) and sample uptake delay (5 s).The nominal concentrations of Cr (VI) which were measured in ICP-OES were found to match well with the measured values. During data analysis only measured concentrations of Cr (VI) were considered wherever applicable, unless otherwise specified.

***Calculation of BCF*[19]**

The BCF was calculated as defined by Brooks and Rumsby which is the concentration ratio of an element in dry biomass and in the water (culture growth media).

Calculation of % uptake of Cr (VI) by biomass

The % uptake in the respective exposure metal concentration was calculated as [total weight of Cr (VI) in the biomass (µg)/total weight of biomass (µg)] multiplied by 100.

Uptake flux

The transport of trace metal nutrients in the microalgae is mostly dependent upon the fastening and freeing of molecules or ions to the active site of the transporter biomolecules, for instance proteins, phytochelateins, etc. To understand the regulation of transport of metal ions, kinetic studies were done. The uptake flux of Cr (VI) by the microalgal cell was calculated as: Uptake flux [{µg Cr (VI)/g dry wt.}/day] = [Concentration of Cr (VI) in cells {µg Cr (VI)}/g dry wt. of biomass]/ Exposure duration (day).

Cell viability and microscopic analysis

The sample was pelletized at 6000 rpm for 15 min at 4ºC. Followed by the pellets were resuspended in 100 µL of 0.1% of Trypan blue (SIGMA). The mixture was incubated at room temperature for 5 min. The % of cell viability was calculated as the ratio of the number of viable cells to the total number of cells and multiplied by 100. The bluish compromised cells were noticed and counted through microscopic (Karl Zeiss Axioskope2, 400X) observation. The images were captured through immersion oil technique upon smearing the sample onto a glass slide and sealed with cover slip (Blue Star, No. 0) at 1000X ((Karl Zeiss Axioskope2).

***Field Emission Scanning Electron Microscopy (SEM Analysis)* [20, 21]**

Field emission scanning electron microscope (Fei Netherlands, IITM, Chennai) was used to observe the morphology of the pristine and treated alga. Both pristine and treated samples were processed following the protocol of the Huck Institute of Life sciences and Elumalai *et al.* Optimization and modifications were carried out during sample preparation to suit the best for our sample of interest. An aliquot (1 mL) of the samples were fixed in buffered glutaraldehyde for overnight at 4°C. The samples were incubated in 4% of OsO_4 and subsequently kept at room temperature in dark. The fixed samples were then dehydrated with series of ascending grade (%) alcohol and subjected to gold sputter-coating for approximately 30 min. Finally, the mounted samples were visualized under HR-SEM (Fei

Netherlands, voltage 20 KeV and spot size 3) at high vacuum mode and significant images were captured for documentation using XP Microscope Server software.

Analysis of surface chemicals through Fourier Transform Infrared (FTIR) spectroscopy

Fourier Transformed Infra Red (FTIR) spectroscopy provides valuable information for understanding the involvement of surface charge-based attachment of metal ions upon the microalgal cells, leading to the membrane damage and aggregate formation as well. To investigate the involvement of the functional groups responsible for the metal binding on the microalgal cell surface, FTIR analysis was carried out. To process the samples, an aliquot (5 mL) from control and treated *T. gracilis* after 96 h of exposure were pelletized (6000 rpm,15 min, 4ºC), freeze-dried and were subjected to FTIR analysis by KBr method using PerkinElmer Spectrum 1 equipped with deuterated triglycine sulfate detector (DTGS). The pulverized KBr powder was mixed with the dried algal sample separately for control and treated samples. The samples were subjected to pressure in a KBr press and the pressed discs containing samples were removed carefully. To avoid the moisture absorption till analysis the sample discs were stored in desiccator. Finally, the sample pellets were placed on a gold mirror into the sample holder and determined in transmission mode, baseline corrected, smoothed and normalized using PerkinElmer Spectrum software within the wave number range 450 – 4000 cm^{-1} (4 cm^{-1} resolution , 3 number of scans, and air as background).

Statistical analysis

Results were tested by one-way Analysis of Variance (ANOVA). ANOVA effects and treatments were considered significant when $p < 0.05$. Statistical analysis were carried out using Microsoft excel in Windows 7 Ultimate while charts are prepared in Ms. Excel and GraphPad Prism 5. All the dataset presented over here are Mean ± S.D. of triplicate unless otherwise specified.

RESULTS

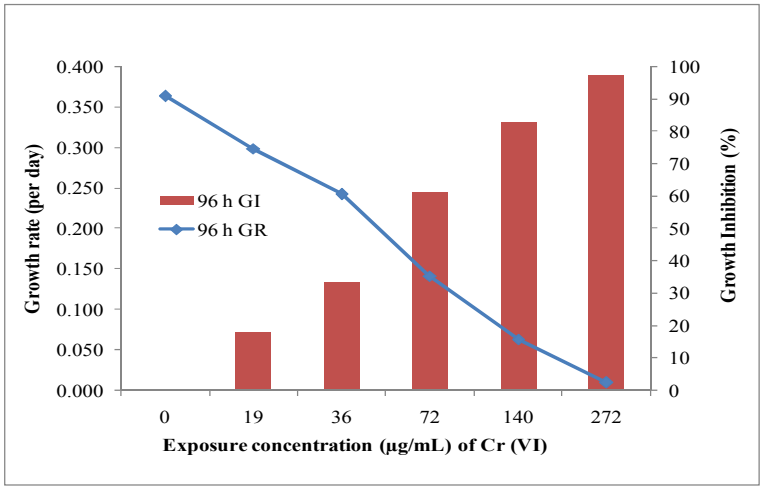

Figure 1
Growth rate (GR) and Growth inhibition (GI) curve of T. gracilis at the end of 96 h

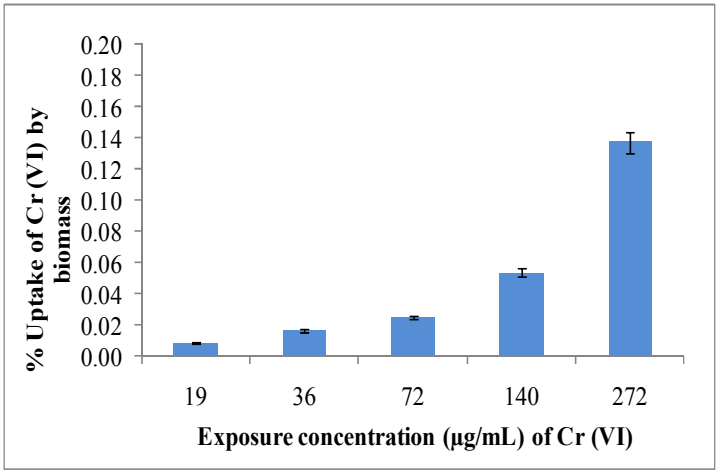

Figure 2
% uptake (w/w) of Cr (VI) by T. gracilis biomass at different concentrations of metal during 96 h.

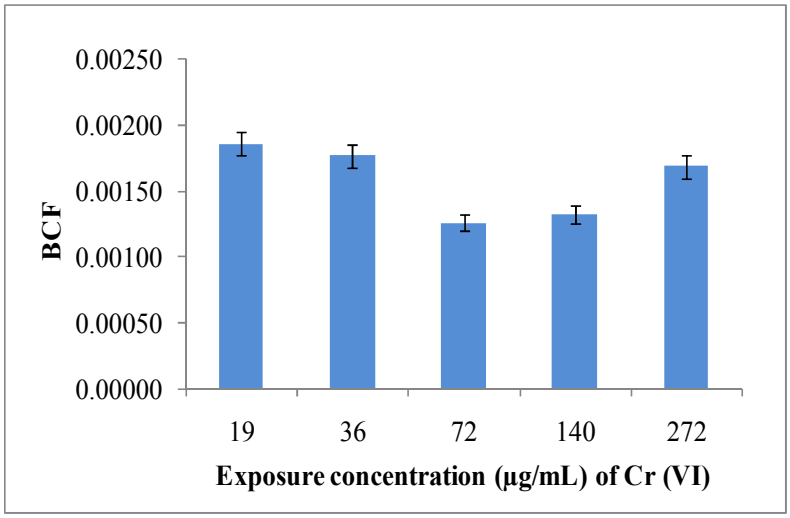

Figure 3
BCF of T. Gracilis at different exposure concentration of Cr (VI) during 96 h.

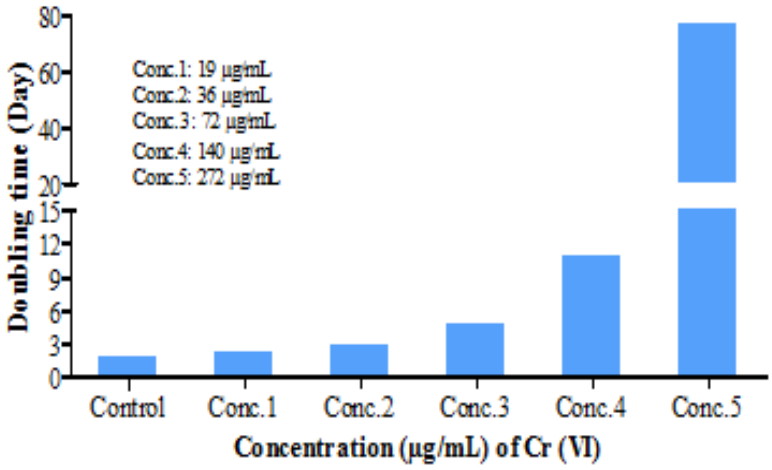

Figure 4
Variation in doubling time of T. gracilis at various concentrations of Cr (VI) during 96 h study

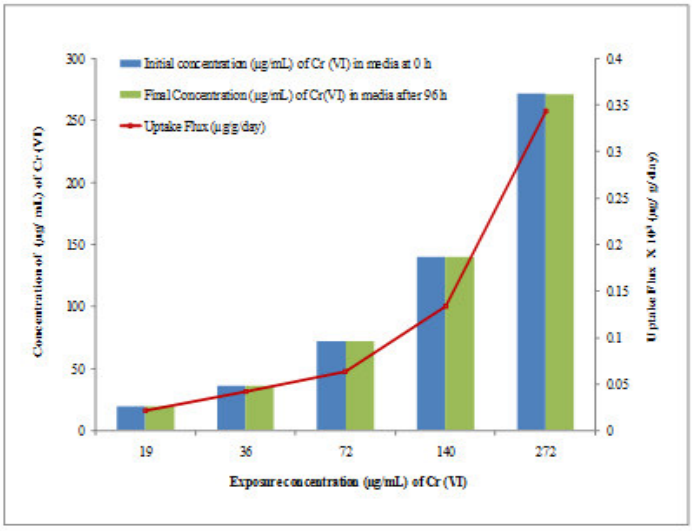

Figure 5
Initial (0 h) and final (96 h) Cr (VI) concentration (µg/mL) in the culture media (primary Y axis) and uptake flux of treated T. gracilis during (96 h) of Cr (VI) exposure (secondary Y axis).

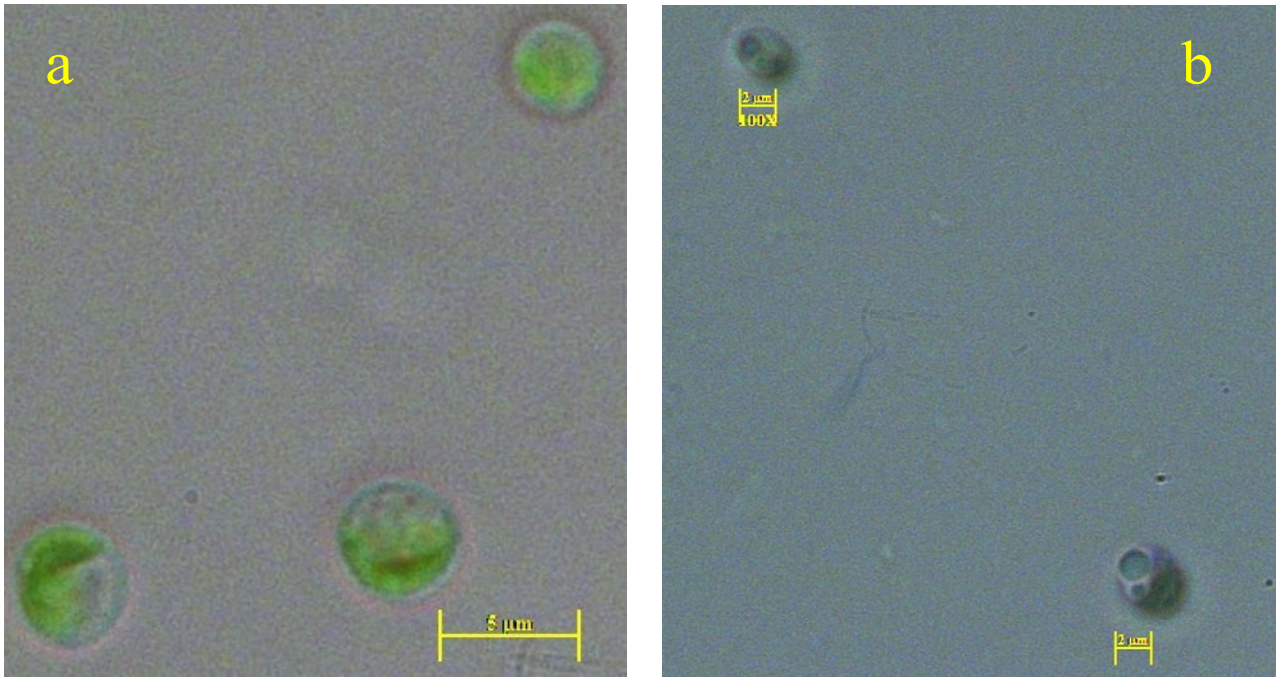

Exhibit 1
LM photomicrograph of T. gracilis at 1000X,
a. Control, b. Exposed to IC_{50} of Cr (VI).

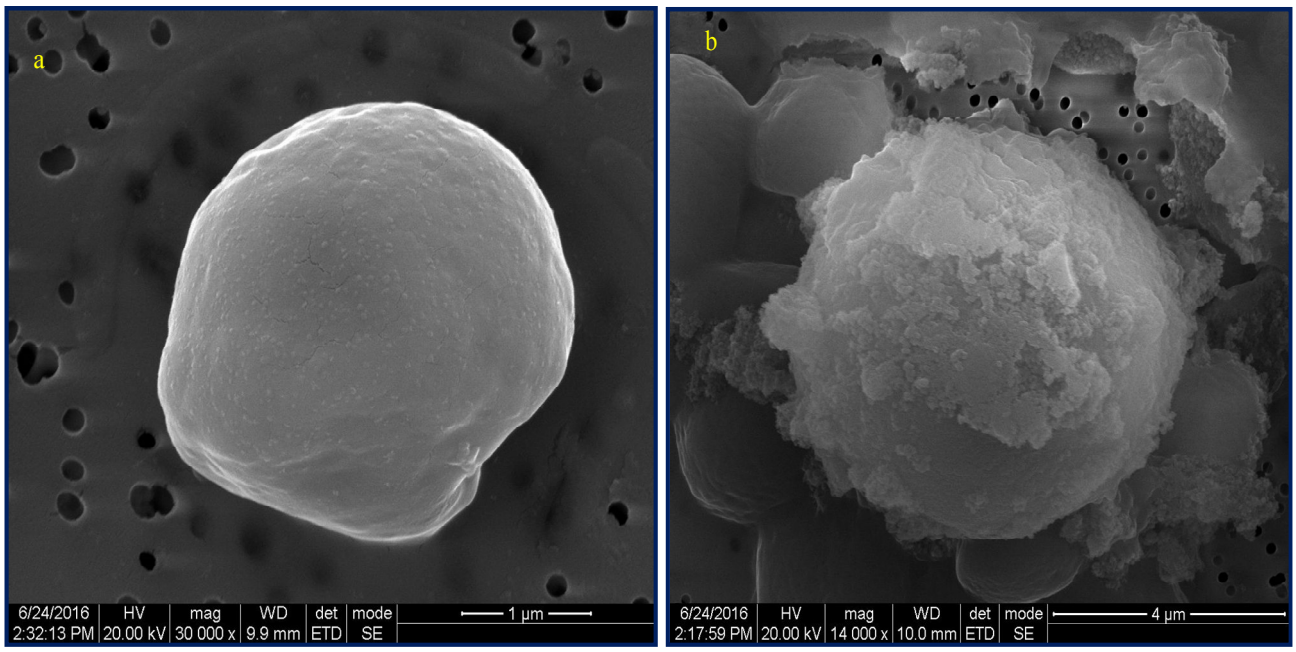

Exhibit 2
SEM photomicrograph of T. gracilis
a. Control, b. Exposed to IC_{50} of Cr (VI).

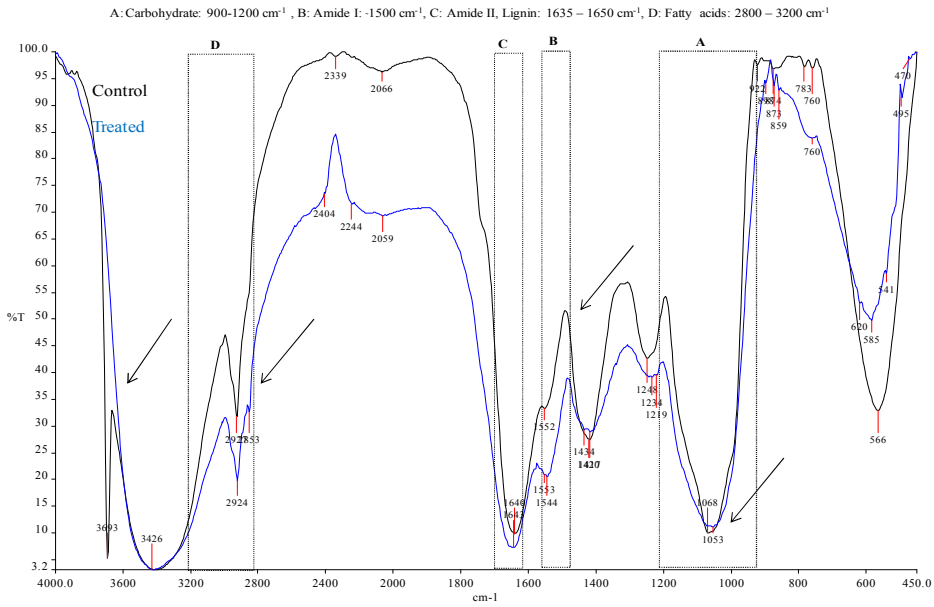

Figure 6
FTIR spectrum of the control and Cr (VI) treated T. gracilis at harvest (96 h).

DISCUSSION

Phenotypic profile

Microalgal phenotypic characteristics, such as optical density, cell count, biomass, chlorophyll a, growth rate, growth inhibition were observed in significantly declined trend with the increasing concentration of Cr (VI) exposure. The results during our investigation revealed that the exposure duration and exposure concentration of heavy metals leads to retardation in growth profile of the exposed microalga. The alga was found to tolerate high concentration (272 µg/ mL) of Cr (VI). The phenotypic changes observed were found to be significant ($p < 0.05$) at all concentrations (Figure 1). The changes in phenotypic characteristics observed during Cr (VI) exposure can be attributed to the oxidation state of the chromium. Owing to the oxidation state +6, the chromium ions initially reacts with the biomolecules present on the cell membrane and further entering into the cell interacts with nucleic acids, antioxidant enzymes, proteins, lipids, etc. through free radical generation. In this experiment marine microalgae *T. gracilis* has been subjected to five different concentrations of Cr (VI) to evaluate the uptake of chromium (VI) by the microalga from seawater matrix enriched with f/2 media. The five concentrations of Cr (VI) were selected to conduct the experiment were 19 µg/mL, 36 µg/mL, 72 µg/mL, 140 µg/mL and 272 µg/mL. These five concentrations were selected based upon the range finding test conducted earlier. The results were found congruent with the results shown by Fathi *et al.*, Namita *et al.* and also with our earlier studies. [30, 31, 33, 34] The GR and GI values were calculated from the OD values and 50% inhibition of growth (IC_{50}) was derived from the interpolation of the growth-response curve as 54.37 µg/mL (Figure 1). It is also observed that % uptake of the metal in microalgal cell is directly proportional to the increasing concentration of Cr (VI) in the culture media and with increasing exposure duration (Figure 2). However, the % uptake at five concentrations, viz. 19 µg/mL, 36 µg/mL, 72 µg/mL, 140 µg/mL and 272 µg/mL was found as low as 0.01%, 0.02%, 0.03%, 0.05% and 0.14% respectively. The highest % uptake was recorded at 272 µg/mL exposure concentration which is also the highest concentration the microalga *T. gracilis* can tolerate during the experimental condition. The low BCF (0.0013 - 0.0019) profile (Figure 3) of the microalga *T. Gracilis* supports its high resistance towards Cr (VI). The highest BCF (0.0019) was recorded at 19 µg/mL of Cr (VI) exposure. Due to the uptake of Cr (VI) metal ions various abnormalities for instance, growth inhibition, reduction in chlorophyll a pigment, inhibition in cellular multiplication and cellular functions, etc. were observed as compared to that of the control.[33, 34] Doubling time was found to increase drastically (1.9 – 77.0 days) with increasing concentration of Cr (VI) from 19 – 272 µg/mL (Figure 4). The uptake flux of *T. Gracilis* was found to be directly proportional with the increasing exposure concentration of Cr (VI) and duration as well (Figure 5). Chlorophyll-a was found to be reduced by 37% at IC_{50} of Cr (VI) as compared to that of the control. This data supports the fact that with the increase in % uptake of Cr (VI) the photosynthesis process is getting inhibited leading to the retarding growth rate and ruptured morphological integrity of the cells.

Light Microscopic observation and cell viability

From the light microscopic (LM) observations spherical healthy cells (Exhibit 1a) were observed in control whereas the exposed cells (Exhibit 1b) are found with distorted morphology. The viability assay revealed that the number of existing cells were in live condition though there was gradual reduction in cell numbers with increasing concentration of Cr (VI) as compared to the control. Around 81% of cells were reduced at IC_{50} of Cr (VI) during 96 h exposure as compared to that of control. The increase in cell length and cell area

supports the fact that the cell enlargement occurs during exposure of Cr (VI) as compared to that of control. During Cr (VI) exposure of *T. gracilis* at IC_{50}, it is found that there is an increase in cell length (27.40%) and in cell area (62.26%) as compared to the respective control. Further, analysis of the area to length (diameter) ratio [$(4A/D^2)$; where A: Area and D: diameter revealed that cells are not perfectly spherical or round rather slightly elliptical. Though, the ratio, $(4A/D^2)$ in the control cells remains unaltered but in the exposed cells the ratio was found to be increased which substantiates the fact of expansion of the cells occurred owing to the Cr (VI) exposure.

SEM micrograph

To examine the surface topology and morphology of the microalga, SEM analysis was carried out to obtain the photomicrograph of the microalga. In Exhibit 2a during control condition an irregular smooth surface topology of the cell was witnessed. The corresponding Cr (VI) exposed cells (Exhibit 2b) are witnessed with deposition of metals on the surface depicting an uneven rough surface topology with enlarged cell size indicating the loss of cell membrane integrity.[22] As stated by Melchor, the heavy metals are adsorbed passively and assimilated actively from their surrounding niche.[23] It is also mentioned that during the stress, release of organic ligands takes place into the media. These ligands are efficient to bind metal ions. Microalgal cell surface is heterogeneous and owing to this property, microalgae bind metal ions through various functional groups, for instance, carboxyl, amino, carbonyl, hydroxyl, etc. The efficiency of metal binding property with various functional groups is revealed through SEM surface micrograph (Exhibit 2b) as compared to its respective control (Exhibit 2a). During the exposure into the metal solution either heavy metal ions get attached on to the surface primarily or they enter into the cell through cell wall during passive transport of the metal ions. The adherence of metals on the surface of the microalgal cell is devoid of cellular metabolism whereas the ingress of metal into the cell and subsequently accumulation into the cell is dependent on cellular metabolism as reported by Sen and Ghosh Dastidar.[22] Chromate ions cross the cell membrane via sulphate transport system as reported by Silvia *et al.* and many others. The onset of spontaneous reaction takes place due to free radical generation upon entry of the Cr (VI) ions into the cell. [24] The intracellular reductant for instance, ascorbate and glutathione transform the Cr (VI) into Cr (III) passing through intermediate free radical generation viz. Cr (V) and/or Cr (IV). Consequently cellular morphology gets injured and damaged. The high oxidation potential of Cr (VI) leads to mutagenic and carcinogenic effects on the microalgal cell. Microalgal cells contain a variety of functional groups, for instance amino, carboxylic, hydroxo, hydroxo-carboxylic, etc. with which heavy metals interact. Though there are a variety of functional groups but the total number of functional groups present on the biosurface is constant leading to a maximum proton adsorption capacity.[23] From the SEM photomicrograph formation of nanoparticles is also detected. Similar observation was found by Radhika *et al.* in *Chlorella vulgaris*.[25] Formation of nanoparticles, cell entrapment can be attributed to the reduction of Cr (VI) to Cr (III) during the interaction with the functional groups of cell surface.

FTIR Spectroscopy

FTIR spectroscopy is a simple, well-established and non-destructive technique to study the biotic and abiotic stress induced response in microalgae.[26] The spectrum of the pristine sample i.e. control, was found to be different as compared to that of the treated one, which provides the insight about the involvement of the bio molecules, present on the cell wall to bind and/or interact with the metal ions. These bio molecules exert a whole negative charge and bind the metal ions through their binding sites. A distinct change was observed in the spectrum of the treated one as compared to that of the control (Figure 6). The surface of the microalgae contains a number of electron dense functional group, for instance, carboxyl (–COOH), hydroxyl (-O-H), carbonyl (-C=O), etc.[27] These functional groups pose high affinity towards positively charged metal ions. These groups act as the binding sites of the metal ion/s and further transport the metal ions across the cell wall. Literature reveals that two distinct phases are involved during the ingestion of metal ions into the cells. Primarily, a rapid assimilation of metal ions occurs and equilibrium is established between the intracellular and extracellular metal ion pool. Followed by, a slower and facilitated ion transport into the cytoplasm takes place.[28] The FTIR spectrums of control and treated one were overlapped (Figure 7) for easy of understanding and interpretation. From the overlapped spectrums (Figure 7) it is clearly observed that the peak at 3693 cm^{-1} depicting free O-H stretch in control which is missing in the treated one. This can be attributed to the involvement of O-H group/ polysaccharide moiety in binding the Cr (VI) upon the cell surface. The variation of peaks was also observed at 3200 cm^{-1} to 2800 cm^{-1} wave number region. This variation can be attributed to the involvement of the –C-H stretch and =C-H stretch. Further, variation of peaks was detected in the spectrums within the 2700 cm^{-1} to 1800 cm^{-1} wave number region which depicts the participation of alkynes to interact with Cr (VI). The peak at 1248 cm^{-1} of the control represents the stretching vibration of C-O. The same region of the treated one shows variation which indicates the interaction of C-O group with Cr (VI). An extensive variation of peaks in fingerprint region (1500 $cm^{-1}$to 500 cm^{-1}) of the spectrums was also observed. Metal ion adsorption on the microalgal cell surface provides an insight about the initial toxicant loading of the cells. The loading of toxicant into the cells is also a function of the microalgal cell surface area and the nature of the binding sites.[29, 32] Also, the modulation of the peaks in the region of carbohydrate (900-1200 cm^{-1}), amide (1635 – 1650 cm^{-1}), fatty acids (2800 – 3200 cm^{-1}) in the treated one as compared to control, can be attributed to the interaction of the biomolecules while accumulating Cr (VI) ions.

CONCLUSION

In conclusion, the experiment revealed that the alga is efficient enough to withstand up to 272 µg/mL of Cr (VI) which is a relatively high concentration for the survival of phytoplankton. The reason could be attributed to the low

BCF profile of the alga due to the strong resistance of the alga towards cellular uptake Cr (VI). The fact is also evidenced by the FTIR studies where the peak intensity of the functional groups were completely absent or reduced in the treated one as compared to that of the control and by SEM surface micrograph where deposition of chromium was detected on the cell surface. However, the tolerance efficiency is subjected to the biotic and abiotic conditions.[36] The study also reveals that the uptake of metal is not only a function of metal concentration but also of the duration. It is also affirmed this *T. gracilis* strain has been exploited in Cr (VI) for the first time since ever. The growth profile was found congruent with several other research works. Morphological studies at IC_{50} were found with loss of cellular integrity. Interestingly formation of nanoparticles was detected which may be beneficial as green synthesis to convert waste to wealth which requires further optimization in terms of energy, economy and efficiency. Authors are optimistic in this aspect.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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