Identification of a New Microalgal Strain From Chromite Mine Wastes for Detoxification of Hexavalent Chromium for Sustainable Crop Growth

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Research Article

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Abstract

Sukinda chromium mine is well known for its chromium (Cr) reserve in India. It accounts for 97% of Cr production in the country. The open cast mining results in the seepage and accumulation of chromium in the nearby paddy fields through soil runoff. Deposition of high concentrations of toxic Cr^{6+} adversely affected the growth and productivity of rice plants. It was studied that Cr^{6+} toxicity can be counteracted by the microbes especially algae. Hence, an attempt has been made for the exploration of an indigenous micro-algal strain for the detoxification of Cr^{6+} in the rice fields. Three different micro-algal strains were isolated from the waterlogged regions of the mine waste area and tested against Cr^{6+}. The average concentration of Cr^{6+} in the soils of rice fields and its surrounding regions was estimated around 40ppm. *In vitro* study was conducted to determine the optimal growth parameters for the growth of the algal strains. The concentration of total chromium availability was determined by using ICP-OES (Inductively coupled plasma atomic emission spectroscopy. It showed that all the algal-stains were able to detoxify Cr^{6+}, but the best result (89.63%) was observed in one strain 'SM3'. SEM-EDX study also showed that there was no Cr adsorbed on the surface of the algal strain. Raman Spectroscopy study confirmed the reduction of Cr^{6+} to Cr^{3+} in algal strain. The strain was identified as *Fischerella* sp. (Accession no. MK422171) through morphological and molecular characterization. This algal strain can be used for the bioremediation of chromium contaminated crop fields.

1. Introduction

Naturally available chromium in the earth's crust has immense industrial applications, especially in stainless steel, tannery, paper pulp, and electro-plating industries (Monograph on chromite, 2013; Katircioglu et al. 2012). In nature, chromium occurs in several oxidative forms, of which hexavalent Cr and trivalent Cr are the most stable and available forms for use (Dwivedi et al. 2010). Chromium in trivalent form is considered as non-toxic or less toxic while its hexavalent form is more toxic and carcinogenic (Singhvi and Chhabra, 2013), hence, requires environmental cleansing especially in chromium mine waste. Odisha, is rich in its mineral reserve and it has the lion's share of 98% in the context of total chromium (Cr) reserve in India (Mishra and Sahu, 2013), mostly concentrated in the Sukinda Valley of Jajpur district which holds 97% of total share of the state (Monograph on chromite, 2013).The Cr extraction in the mining site follows open cast mining procedure, which leads to seepage of toxic Cr^{6+} to the nearby areas through soil run off and causes contamination to local biota especially in the paddy fields (Hayat et al. 2012). It has been reported that chromium accumulates in different plant parts such as shoot, root, leaves and grains (Ahmad et al. 2011) and results in abnormal growth and reduced the crop yield (Shanker et al. 2005). Pigment content of the leaves, rate of photosynthesis, nitrogen, potassium, phosphorus content in the root and shoot get reduced due to the high concentration of Cr^{6+} deposition in soil and hence, leads to less growth and productivity (Panda and Chaudhury, 2005). It is also subjected to bio-magnifications in the food chain and ultimately reaches to human being (Chatterjee and Abraham, 2015). The consumption of rice grains containing good amount of hexavalent chromium invites health hazards like irritation in lungs and stomach, damage in respiratory tract, inflammation, dermatitis, bronchitis, ulcers and cancer that leads to death (Abdel-Ghani et al. 2014). Hence, environment clean-up of Cr^{6+} is of utmost essential in the chromite mine waste and its adjoining regions. The toxicity of the Cr^{6+} can be reduced by various conventional methods like physical and chemical process (Elhaddad and Abeer, 2015), but are very expensive and also appears to unsafe (Belattmania et al. 2015). Keeping in view of these afore referred facts, development of novel approach like detoxification of Cr^{6+} using biological methods seems to be the need of the hour (Esmaeili et al. 2010). Micro-organisms help in detoxification of Cr^{6+} through the process of biotransformation or biosorption (Pagnanelli et al. 1997). Among the micro-organisms, algae draw a lot of attention for the detoxification of various metal pollutants as reported (Brahmabhatt et al. 2012). These photosynthetic autotrophic nitrogen fixing organisms grow abundantly in waterlogged conditions (Banerjee et al. 2004). Rice cultivation requires a standing water level in the field for most of its life cycle (Bhattacharyya et al. 2005) and provides the natural condition for algae to grow. Algae can withstand against various concentrations of toxic metals and are potential source of heavy metal remediation (Davis et al. 2003; Volesky et al. 2007). It has also been proved that algae are capable of detoxify heavy metals through biosorption (Gupta et al. 2008) and bio-transformation (Bender et al. 1995). Apart from that algae can also bring in noticeable improvements in the soil texture and quality (Paudel et al. 2012), hence, enhancing the soil fertility through atmospheric nitrogen fixation (Padhy et al. 2016). As per our observation, it is imperative to mention that many chromium resistant local micro-algal strains are available in the water logged places of the Sukinda mining area and they can be used for the bioremediation of chromium contaminated soil of rice fields (Sundarmoorthy et al. 2010). Hence, an attempt has been made in the present study to characterise a potential micro-algal strain is capable of detoxification of Cr^{6+} in the chromium contaminated rice fields.

2. Material And Methods

2.1. Physicochemical properties of rice field soil

Soil samples were collected rice field of four different locations in Sukinda chromite mine in Odisha, India. Fig.1 showed the sample collection sites where S1, S2, S3 and S4 referred to four different paddy fields from where the soil samples were collected and the red marked region was the mining area from where the algal samples were collected. About 250gms of soil samples were collected from each location a time and kept in zipper bags, taken to the laboratory. The pH and temperature of the soil samples were recorded by using pH meter and thermometer on their sampling sites. The samples were mixed together to estimate their Cr content and other physicochemical parameters such as moisture content, electrical conductivity, total dissolve solid etc (Pattnaik et al. 2017).

2.2 Collection of algal samples and maintenance of pure cultures

The algal samples along with water and soil were collected in different zipper bags from the water logged places of the mining sites. Different algal samples were thoroughly washed (4–5 times) through running tap water and then with distilled water. The samples were cultured and sub-cultured repeatedly in their specific growth medium to obtain the pure cultures of three algal strains named as SM1, SM2 and SM3 respectively. SM1 and SM2 were grown in BG11 medium (Stainer et al. 1971) but SM3 was cultured in nitrate free Allen and Arnon medium (Allen and Arnon, 1955). The algal samples were maintained at

28±2^{0}C with 42µmol photons $m^{-2}s^{-1}$ light intensity using cool white fluorescent lamps under laboratory conditions (Kiran et al. 2008, Majhi and Samantaray, 2021).

2.3. Growth of algal isolates at different concentrations of Cr^{6+}

The isolated algal strains were allowed to grow in 100ml of respective media containing varying concentrations of Cr^{6+} (0 to 200mg/L) for 30 days. Cell growth was determined by taking the dry cell weight of all the strains at 4 days interval. The cultures were centrifuged by taking them in pre-weighed 60 ml centrifuge falcon tubes at 16,000rpm for 10min. Then, the supernatant was discarded and the pellets formed were placed in a hot air oven at 65^{0}C (until the final weight became constant). Now, the dried pellets along with the tube were weighed and the final weight was calculated by subtracting the initial weight of the centrifuge tube from the final one containing the cell pellets (Bottomley and Van-Baalen, 1978; Razi, 2006).

2.4. Growth optimization of the algal isolates

Growth conditions such as pH, inoculum size and photoperiod of the three algal strains were optimised at 40ppm Cr^{6+} containing medium (according to Majhi and Samantaray, 2020a, 40ppm is the average concentration of chromium in the rice fields of Sukinda mining area) to observe their growth and to find out the maximum biomass producing algal strain (Majhi and Samantaray, 2021).

2.5. Analysis of total chromium and hexavalent chromium

The chromium reduction assay was carried out by growing the algal isolates in 1lit of culture medium supplemented with 40ppm of $K_2Cr_2O_7$ solution. Then, the culture (log phase) was centrifuged at 10,000rpm for 30min to obtain the crude cell free extract. The amount of total Cr, Cr^{6+} and Cr^{3+} was determined by following Majhi and Samantaray, 2021. The organism showing maximum Cr^{6+} removal was selected for further study.

2.6. Effect of agitation time

The effect of agitation time on Cr^{6+} removal by the selected organism was determined by taking 5 numbers of conical flasks, each containing 50mL of medium (distilled water having 40mg/L concentration of Cr^{6+}) and were placed in an orbital incubated shaker at 28±2^{0}C with different agitation times (24, 48, 72, 96 and 120 hours). These samples were centrifuged separately and then filtered through Whatman filter papers grade no. 41. These filtrates were tested for the final concentrations of chromium remaining in the aqueous solution by DPC method (Kavitha et al. 2016).

2.7. Study on chromium adsorption

The chromium adsorption by the algal isolates was studied through scanning electron microscope-energy dispersive X-Ray (SEM-EDX) and Fourier transmission infra-red spectroscopy (FTIR) analysis (Majhi and Samantaray, 2021).

2.8. Study on chromium absorption

2.8.1. TEM analysis

For the TEM analysis, the control and hexavalent chromium treated cyanobacterial cultures were centrifuged at 10,000rpm for 10min, washed thrice with saline phosphate buffer solution (PBS, pH 7.4). Then, it was re-suspended in 1mL of fixative solution (2.5% glutaraldehyde and 2% formaldehyde) and kept for 6 hour at 4ºC. The post fixation and fixative solutions were separated by centrifugation. The cell pellet was then washed five times with PBS and again re-suspended in 1mL of sodium phosphate buffer. Aliquots of the cell suspension were mounted on 400 mesh Cu grids and allowed to dry overnight in a desiccator. Microscopic images of these cells were taken on a Tecnai Transmission Electron Microscope operated at 200kV accelerating voltage (Das et al. 2014).

2.9. Study on chromium reduction

2.9.1. Raman spectroscopy

Raman spectroscopy was carried out for the analysis of hexavalent and trivalent chromium in the Cr^{6+} treated media and inside the cell of treated organism (Majhi and Samantaray, 2021).

2.10. Identification of the algal isolate

2.10.1. Microscopic observation

The potent algal isolate was identified under light microscope and phase contrast microscope following the standard algal monograph (Desikachary, 1959).

2.10.2. Molecular identification

Molecular identification of the algal isolate was carried out by 16S rDNA sequencing. The extraction of DNA was conducted by DNeasy Plant Mini Kit (Qiagen).

Amplification of 16S rDNA and sequencing

The extracted DNA was sent to SRM-DBT, Tamilnadu, India for 16S rDNA sequencing. The extracted DNA quality was evaluated on 1.0% TAE agarose gel electrophoresis using 1kb DNA ladder. Fragment of 16S rDNA gene was amplified and a single discrete PCR amplicon band resolved on agarose gel. Then, the amplified product was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with CYA106F, CYA359F and CYA781R (a+b) using Big Dye Terminator v3.1 Cycle sequencing kit on Applied Biosystem™SeqStudio™ Genetic Analyzer. Consensus sequence of 16S rDNA was analysed by Bio-Edit software v7.0.5.3 and submitted to NCBI for generation of accession number.

Evolutionary analysis

The submitted16S rDNA sequence of the cyanobacterial isolate was subjected to multiple sequence analysis using ClustalW v1.6. Then phylogenetic tree was constructed using neighbour joining method (Saitou and Nei, 1987) of MEGA v 7.0 (Molecular Evolutionary Genetic Analysis) software (Tamura et al. 2013). The resultant phylogenetic tree topologies were evaluated by bootstrap analysis of neighbour- joining data sets based on 1000 resampling.

2.11. Effect of the selected alga on the growth performance of Cr^{6+} treated rice plants

2.11.1. Selection of rice seeds for the experiments

Certified healthy seeds of *Oryza sativa* (Var. Lalata) were procured from the Department of Plant Breeding and Genetics, College of Agriculture, Odisha University of Agriculture & Technology, Bhubaneswar, India for the experiment during the Kharif and Rabi season of the year 2019. This crop matures in about 120 days.

2.11.2. Pot culture experiments under axenic conditions

Plant growth experiments were conducted in 12” plastics pots for six different treatments. Each pot contained air dried sterilised garden soil along with different proportion of Cr^{6+} rich mine spoil. The experiment was performed in triplicates for each of the six treatments. Three inoculated seedlings were transplanted with uniform distance in each pot aseptically. The experiment was carried out under normal environmental condition during the Rabi and Kharif season (Two seasons) during the year 2018 & 2019. Before and after transplantation, all the physico-chemical parameters of the soil were measured. The six different treatments of the experiments were as follows:

Treatment no. 1 (T_1): 500 gm of Cr^{6+} containing chromite mine spoil collected from paddy field (100%) + 1 litre Cyanobacterial inoculums

Treatment no. 2 (T_2): 75% Cr^{6+} containing chromite mine spoil collected from paddy field + 1 litre Cyanobacterial inoculums+ 25% normal garden soil.

Treatment no. 3 (T_3): 250 gm of Cr^{6+} containing chromite mine spoil collected from paddy field (50%)+ Cyanobacterial inoculums+ 50% normal garden soil

Treatment no. 4 (T_4): 150 gm of Cr^{6+} containing chromite mine spoil collected from paddy field (25%) + Cyanobacterial inoculums+ 75% normal garden soil

Treatment no. 5 (T_5): Normal garden soil (control)

Treatment no. 6 (T_6): Cr^{6+}mine soil collected from paddy field (100%)

2.11.3 Physico-chemical analysis of the plant samples

Number of tillers

The number of functional tillers were counted and recorded at each 30 days interval.

Number of leaves

The number of functional leaves were counted and recorded at each 30 days interval.

Shoot and root height

The shoot and root height was measured non-destructively using a meter scale at an interval of 30 days and expressed in centimetres.

Seed vigour index

Seed vigour index (SVI) was calculated by the protocol given by Majhi and Samantaray, 2021.

Shoot and root dry weight

For the determination of dry biomass, the shoot and root were put in labelled paper bags and placed in an oven at 65°C for 48 hours. Then the weight was measured using an electronic weighing balance.

Total leaf area

The length and breadth of each and every leaf was measured and the total leaf area was calculated by using the formula: L x B x Correction factor x No. of leaves

It is expressed in cm^2/plant where, L and B are the length and breadth, respectively and the correction factor was taken as 0.73.

Number of grains/ panicle

Total number of grains per panicle was counted manually from each panicle which were selected randomly from each pot. The mean of ten randomly selected panicles from pot were used to determine the number of grains per panicle.

Weight of 1000 seeds

Thousand healthy grains are randomly selected from the treated and control plants, weighed by the help of an electronic weighing balance.

2.12. Biochemical analysis of the plant samples

2.12.1 Chlorophyll estimation

Five hundred mg leaf from each treatment were cut from the composite leaves and were immersed in 50ml of 80% (v/v) acetone in a conical flask and kept in dark for 24hour for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were centrifuged at 10000rpm for 10 min. and the supernatant was taken for chlorophyll content analysis. Absorbance of the chlorophyll extract was measured at 645nm and 663nm using a colorimeter. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated in mg/g fresh weight according to the following equations.

i) Chlorophyll –a (mg/g fresh weight basis)

$$= 12.7 \text{ x } (OD_{663}) - 2.69 \text{ x } (OD_{645}) \times \frac{V}{1000 \times W}$$

ii) Chlorophyll-b (mg/g fresh weight basis)

$$= 22.9 \text{ x } (OD_{645}) - 4.68 \text{ x } (OD_{663}) \times \frac{V}{1000 \times W}$$

iii) Total chlorophyll (mg/g fresh wt. of basis)

$$= 20.2 \text{ x } (OD_{645}) + 8.02 \text{ x } (OD_{663}) \text{ x } \times \frac{V}{1000 \times W}$$

Where

OD_{645} = Optical density at 645nm

OD_{663} = Optical density at 663nm

V = Final volume of 80% acetone chlorophyll extract in ml

W = Fresh weight in g of fresh leaves used in the extraction of chlorophyll

2.12.2 Estimation of crude protein content

Cells in the late exponential phase were taken out from culture flasks and centrifuged at 12,000 rpm for 15min. and at 4°C. The cell pellets were homogenized in liquid N_2 and suspended in extraction buffers (50mM Tris-HCl buffer (pH 7.5) + 0.1mM PMSF + 1mM EDTA). The homogenate was then centrifuged at 13000g at 4°C and supernatant was collected as crude enzyme extracts for assay of enzymatic activity. Estimation of protein was done following modified Bradford assay (1976). Briefly, variable concentration of crude proteins were mixed with 1ml Bradford reagent and incubated in dark for 15-20min. Absorbance ratio was then taken at 590nm and 450nm and only Bradford reagent was taken as control. Standard graph was prepared for the Bradford against bovine serum albumin (BSA).

2.12.3 Catalase activity

Activity of catalase was measured following methods of Aebi (1983). The enzyme catalase (CAT; EC 1.11.1.6) catalyzes the decomposition of H_2O_2 to H_2O and O_2. The reaction mixture was prepared by adding 50mM potassium phosphate buffer (pH 7.2) and enzyme extract, 10mM H_2O_2 and decrease in absorbance at 240nm (due to decomposition of H_2O_2) was recorded using UV-VIS spectrophotometer.

2.12.4 SOD activity

Assay of super oxide dismutase (SOD; EC 1.15.1.1) activity in the crude protein was performed using spectrophotometric analysis (Beauchamp and Fridovich, 1971). The extracted crude protein sample was thawed and then kept for some times on ice. All the reagents required for the experiment were pre-warmed to room temperature. The reagents were mixed gently and were taken in duplicates. The reaction mixture was prepared in 50mM potassium phosphate buffer (pH 7.2) containing 2.45mM NBT, 1.8mM xanthine and a suitable concentration of xanthine oxidase (for which a linear curve with slope 0.021 absorbance per

min in time scan at 550nm was obtained). One unit of SOD activity was calculated as the amount of protein that reduces the reaction rate of NBT by 50% in the reaction mixture.

2.12.5 Estimation of total carbohydrate content

Dried plant sample (100mg) was weighed and kept in a boiling tube. The sample was added with 5ml of 2.5N HCl, hydrolysed by keeping it in boiling water bath for 3hours and cooled to room temperature. Then it was neutralize with solid carbonate until the effervescence ceased. The volume was made to 100ml after transferring the sample in to a 100ml volumetric flask. 10ml of the diluted sample was taken in a centrifuge tube and centrifuged for 10min. at 10,000rpm. The supernatant was then collected from which 0.5ml and 1ml of aliquots was taken for further analysis. A standard was prepared by taking 0, 0.6, 1.2, 1.6, 2.4 and 3ml of stock solution where 0 (distilled water) was served as the blank. The volume of all the working solution along the sample were made up to 3ml with distilled water and finally 12ml of anthrone reagent was added to each tube. The mixture was then heated for 8min. in a boiling water bath, cooled rapidly to the room temperature and the absorbance was read at 630nm after the conversion of green coloured sample in to dark green colour. The standard graph was plotted by taking the O.D. values of the standard solutions and the amount of carbohydrate was calculated by the following equation (Sadasivam et al. 1992).

Calculation of total amount of carbohydrate present in 100mg of sample

(mg of glucose/ volume of the test sample) x 100

2.13. Elemental analysis

The dried algal sample was taken to analyse by CHNS(O) elemental analyser (Elementar, UNICUBE) to study the organic carbon, hydrogen and nitrogen amount (Tasic et al. 2016). Total carbon, hydrogen and nitrogen content are expressed in terms of mass fractions of the dried sample. The contents of inorganic elements were analysed by ICP-OES in triplicates. (Godlewska et al. 2016, Majhi and Samantaray, 2021).

2.14. Statistical analysis

The data were analysed using one way ANOVA in SPSS software version 21. Mean value of the samples were compared using least significant difference (LSD) at $P< 0.05$.

3. Results

3.1. Physicochemical parameters analysis

Table 1 showed the physicochemical properties such as pH, temperature, moisture content, electrical conductivity (EC), total dissolved solid (TDS), chromium content in the soil samples collected from the chromite mine as well as associated rice fields. The maximum pH (7.4), conductivity (73µS/cm), moisture content (27.43%) and Cr^{6+} concentration (47.5 ppm) were observed in the soil from mining area. The Cr^{6+} content of rice field in the periphery of mining area was 40ppm.

3.2. Growth of algal isolates at different concentrations of Cr^{6+}

Table 2 shows the growth of algal isolates at different concentrations of hexavalent chromium (0 to 200mg/L). SM3 isolate exhibited the highest growth at 40ppm of Cr^{6+} concentration among all the algal isolates while other two isolates SM1 and SM2 exhibited maximum growth at 60ppm and 100ppm, respectively.

3.2. Growth optimization of the algal isolates

The growth parameters like incubation period, photoperiod, pH and inoculum size of the three algal strains were optimised keeping the Cr^{6+} concentration constant (40ppm) in the media (Fig.2. a, b, c and d.). Maximum algal growth (SM3) was observed in pH 5, 18 hour photoperiod and maximum 2mg of algal inoculums within 18 days of growth period.

3.3. Total chromium and Cr^{6+} analysis

The percentage of Cr^{6+} reduction by SM1, SM2 and SM3 isolates presented in Table 3. The results showed that the SM3 algal isolate exhibited the maximum removal capability up to 89.63% at 40ppm. Therefore, this isolate was taken for further studies.

3.4. Effect of contact time on Cr^{6+} removal

The Cr^{6+} content in the medium was observed after each 24hours for five days. The concentration was found to be decreased until 72hour while after that the rate of removal almost came to a constant point. The initial Cr^{6+} concentration (40ppm) was decreased to 1.79ppm within 24h and to 1.057 ppm within 72h after which the amount became constant (Fig.3).

3.5. *Microscopic and Molecular identification*

Microscopic observation (Fig.4a.) revealed that SM3 is blue green in colour and beaded filamentous in structure. It was characterized by 16S rDNA consisting of 20 different closely related strains of *Fischerella* sp., *Nodularia* sp., *Nostoc* sp., *Anabaena* sp., *Kryptousia*sp., *Cyllindrospermum* sp., *Cronobergia* sp., *Iphinoe*

sp., *Brasilonema* sp., *Phyllonema* sp. and *Chlorogloepsis* sp. (Fig.4.b). The optimal tree with the sum of branch length is 0.010. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test presented next to the branches. The evolutionary distance was computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The phylogenetic tree suggested that this algal isolate belongs to the genus *Fischerella* sp. with Gene Bank accession numbers (MK422171).

3.6. Scanning Electron Microscope and EDX and FTIR analysis

The scanning electron micrographs of SM3 algal isolate (Cr^{6+} treated and untreated) were presented in Fig. 5a, b. No significant variations were found on their cell surfaces. EDX report (Fig.6) inferred that there is no chromium attachment on the surface of SM3 (Cr^{6+} treated) sample. Hence, it is confirmed that there is no adsorption of chromium by the algal strain. FTIR spectra of Cr^{6+} treated and untreated algae are shown in Fig. 7. The FTIR spectral analysis showed that there was a slight shift in the major functional groups. Mostly the alcohols, phenols, primary, secondary amines at 3268.15, N–H bond at 1624.19, carboxylic, esters, aliphatic amines at 1024.02 and alkyl halides at 554 cm^{-1} were slightly shifted to 3268.70, 1626.24, 1016.82 and 543.21 cm^{-1} respectively.

3.7. TEM analysis

The chromium absorption by the algal isolate was studied through TEM. The transmission electron micrographs of *Fischerella* sp. grown as SM3 (Cr^{6+} treated) and untreated medium was presented in Fig. 8a and b. The inner cell surface has shown an electron dense layer along with some accumulation of starch surrounding the pyrenoids. The treated cells contain some intracytoplasmic inclusions which were not found in the control cells.

3.8. Raman Spectroscopy

Raman Spectroscopy analysis (Fig.9 a, b) revealed that the reduction of Cr^{6+} in to Cr^{3+} in cell free medium and SM3 cells, because of the presence of high intense characteristic peak at 600cm^{-1}.

3.9. Physico-chemical analysis of the plant samples

The physiological characteristics of the rice plants such as number of tillers, number of leaves, shoot and root length and dry weight of root and shoot of all treated and control plant samples were presented in Table 4 and Table 5 respectively. Total leaf area, number of grains per panicle and weight of 1000 seeds were presented in Table 6.

3.9.1 Number of tillers

The number of tillers was gradually increased till 90 days and from 90 to 120 days it was found to be almost constant in both the seasons. The highest number of tillers were found in the treatment T_3 (15) where as the lowest was observed in T_6 (2.6) in Rabi, 2018. The number of tillers in Rabi, 2018 was 9.2 in control (T_5) while T_1, T_2 and T_4 had 10.3, 11.3 and 14.4 numbers of tillers, respectively. In Kharif, 2019, the number of tillers in T_1, T_2, T_3, T_4, T_5 and T_6 were 11.6, 13.6, 14.2, 13.6, 11.5 and 5.3 respectively.

3.9.2 Number of leaves

The number of leaves depended upon the number tillers. So the highest number of leaves was observed in T_3 (48.3) treatment because of highest number of tillers during Rabi, 2018 (Table 4). The remaining treatments such as T_1, T_2, T_4, T_5 and T_6 were found to have 30.2, 33.2, 40.1, 28.2and 8.3 leaves respectively during their harvesting period in Rabi, 2018. Similarly, in Kharif, 2019 T_4 has numbers of leaves (41.4) and lowest in T_6 (15.6) (Table 5).

3.9.3 Root and shoot height

In Rabi season, the highest root length (20.6 cm) and shoot length (112.1 cm) were observed in T_4. During the Kharif season, 2019, the highest root and shoot length were 21.9 cm and 106.7 cm in T_4 treatment respectively. Lowest root and shoot length were observed in the T_6 treatment in both the season. (Table 4 and 5)

3.9.4 Shoot and root dry weight

The dry weight of the shoot and root were found to be lower in the T_6 treatment. There was no significant variation in the dry weight of both the season. The highest dry weight of root and shoot were 13.8 gm and 20.5 gm in Rabi season while in Kharif season, 2019, the dry weight of root and shoot were 14.1 gm and 20.5 gm, respectively in T_4 (Table 4 and 5)

3.9.5 Total leaf area

The total leaf area was calculated on the basis of length and width of the selected healthy leaf. The leaf area was ranged between 25.8 to 40.2cm^2in Rabi, 2018 and 25.2 to 40.3 cm^2 in Kharif, 2019. Both in Rabi and Kharif season T4 treatment (40.2 and 40.3 cm^2) has shown larger surface area while T6 treatment (25.8 and 25.2 cm^2) has lesser leaf area like other parameters (Table 6).

3.9.6 Number of grains/ panicle

The highest number of seeds were observed in T_4 (94) of Rabi season, 2018 and Kharif season, 2019. The lowest number of seeds were observed in T6 (60.6) of Rabi season, 2018 and 62.3 in Kharif season, 2019. Overall number of grains was more in Kharif season than the Rabi season but the difference was not

significant in some treatment (Table 6).

3.9.7 Weight of 1000 seeds

The total weight of 1000 numbers grains was 28.3 and 29.05gm in case of control plants of Rabi and Kharif season. In other hand, the total weight of 1000 numbers of grains was significantly less in T_6 (12.9 and 13.1) rice plants. The weight of 1000 seeds was more in T_3 (48.8 gm) treatment of Kharif season and in T_4(45.9) rice plants in Rabi season, 2018 (Table 6).

3.10. Biochemical analysis of the plant samples

The amount of Chl a, Chl b & total chlorophyll content, total protein, carbohydrate content, catalase and superoxide radical of each treatment both in Rabi, 2018 and Kharif season, 2019 were presented in Table 7 and 8.

3.10.1 Chlorophyll estimation of leaf

The chlorophyll content were ranged between 1.5 to 2.8µg g^{-1}, 1.7 to 4.5µg g^{-1} and 3.2 to 7.3µg.g^{-1} for Chl a, Chl b and total chlorophyll respectively during their post harvesting period in the Rabi season, 2018. The amount of pigment was higher in Rabi season, 2019 than Kharif season, 2018. In both the season T_1 had highest chlorophyll content while T_6 had lowest amount of chlorophyll content.

3.10.2 Estimation of protein content

The amount of protein content was more in Kharif season than Rabi. The highest protein content was observed in T_4 treatment of Kharif season (0.9mg g^{-1}). In Rabi season the protein content was ranged between 0.2 to 0.9 mg g^{-1} while in Kharif season it was laid between 0.2 and 1.05 mg g^{-1}. In both the season, the protein content was lowest in T_6 treatments (Table. 7 and 8).

3.10.3 Estimation of carbohydrate content

The analysis of carbohydrate content was found to be high with high amount of chromium along with the alga. But the Cr^{6+} treated plants have shown very measurable amount of carbohydrate content. This content was increased consequently with increased amount of Cr^{6+}. The carbohydrate content was higher in Kharif season (7.6mg g^{-1}) when compared to the same of Rabi season (6.7mg g^{-1}). Control plants were also produce good amount of carbohydrates in both the season (Table 7 and 8).

3.10.4 Estimation of catalase activity

The amount of catalase enzyme was found to be more in Kharif crops as compared to Rabi crops. During Rabi season, this activity ranged from 3.2 to 7.2U/ mg of protein while in Kharif season it was between 3.4 and 8.8U/ mg of protein. The activity of enzyme catalase was significantly increased with increase in the concentration of Cr^{6+} in presence of the alga. Maximum catalase activity was studied in T_1 treatment while minimum in T_5 of both Kharif and Rabi season (Table. 7 and 8).

3.10.5 Estimation of superoxide dismutase activity

SOD activity was increased with increasing concentration of hexavalent chromium. SOD activity was remarkably high during Kharif season, 2019 than Rabi, 2018. In presence of the alga, increase in chromium concentration in soil, enhance the production of SOD. Hence, maximum amount of SOD was noticed in T_1 both in Kharif (9.9 U/ mg of protein) and Rabi season (9.1 U/ mg of protein). SOD activity of T_5 indicated 3.5 U/ mg of protein in Kharif and 2.2 U/ mg of protein in Rabi. The SOD activity of T6 was lower than that of all other treatments (Table 7 and 8).

3.11. Elemental analysis

The elemental analysis confirmed that the algal biomass had a good content of nutrients. The chemical composition of the alga was presented in Table 9. As per the findings, the biomass had some organic compounds like 13.8% of carbon, 1.54% nitrogen, 5.39% hydrogen and 0.15% sulphur. Similarly, the inorganic compounds like Ca, Cu, K, Mg, Mn, Ni, Zn, Fe, S and P were measured as2.61, 0.031, 64.1, 4.54, 0.171, 0.06, 0.03, 0.006, 0.15±0.1 and 14.83mg/l. Though this organism was able to produce such components hence, it can be considered as a bio-fertilizer as well.

4. Discussion

The soil sample collected from mining areas showed slightly alkaline pH which may be due to the manifestation of Cr^{6+} and mostly it is stable at alkaline pH (Pattnaik et al. 2017; Das et al. 2013a; Mishra et al. 2010). The control soil sample is slightly acidic in nature (Jena et al. 2008; Dey et al. 2010; Sahoo et al. 2016). The Cr^{6+} concentration was more in soil sample of mining areas as compared to the soil of rice fields which may be due to the soil runoff of the mining area carries some amount of chromium to rice field. Therefore, the average chromium content of the rice field soil was found to be 40ppm. However, the mine waste soil having high concentration of chromium as reported by several authors (Pattnaik et al. 2017; Dubey et al. 2001; Dhakate et al. 2008).

Three collected algal strains have been tested for maximum growth. Among the three algae, maximum growth was observed in SM3 isolate at 40ppm. The other strains were also resistant to Cr^{6+} at a range of 0 to 200ppm Cr^{6+} concentration because they were isolated from Cr^{6+} enriched water sample of mining area and exposed for several years to the chromium contaminated regions. This finding was in agreement with the outcomes of Anjana et al. 2007. On the

basis of dry weight, the maximum growth was obtained in SM3 isolate at pH 5.0, 18h photoperiod within 18 days of incubation period with maintaining the Cr^{6+} concentration at 40ppm. Anjana et al. 2007 reported that the Cr^{6+} reduction in case of *Nostoc calcicola* was optimal at pH 3.0. The growth peak for *Nostoc* sp. was found on the 15th day of inoculation reported by Kiran et al. 2008. These data are quite similar to the findings of the present study. Maximum removal of chromium (89.63%) was observed in SM3 isolate. This result corroborated with the result of Das et al. 2013b. The percentage of Cr^{6+} removal by all the isolated strains within 72h were found to be significant (87- 89%). Biosorption of Cr^{6+} studied by Anjana et al. 2007 who revealed that two native strains of cyanobacteria (*N. calcicola* HH-12 and *Chroococcus* sp. HH-11) were found to be potent for the reduction of Cr^{6+} from the contaminated soil from textile mill. Among which *N. calcicola* was proved as more suitable for biosorption. Kiran et al. (2008) has also observed removal of $Cr^{6+}$by *Nostoc linckia*.

SEM- EDX and FTIR analysis confirmed that adsorption had no significant role in the removal of hexavalent chromium by SM3 isolate. Similar trend of result was observed by Shukla et al. 2012 and reported that the cyanobacterial mat remained unchanged before and after Cr^{6+} biosorption. Zincovscaia et al. 2014 reported that the functional groups (such as OH, NH-CH_2, NH_2, NHC(O) amid, CH=CH) present on the surface of *Nostoc linckia* which was proved as one of the best studied cyanobacteria for the process of bioadsorption. Although the present study showed the presence of similar functional groups but due to the lack of shifting in the major functional group, it was confirmed that adsorption was not taking place.

The Raman spectroscopy analysis revealed that the reduction of Cr^{6+} into Cr (III) by SM3 isolate was quite similar as reported earlier by Das et al. 2013b who have used *Bacillus amyloliquefaciens* collected from the chromite mine. Due to the maximum reduction of Cr^{6+} to Cr^{3+}, SM3 isolate was further selected for identification. Microscopic study and molecular characterisation confirmed that the SM3 isolate was *Fischerella* sp. It was well known for its efficiency in nitrogen fixation (Soltani et al. 2007) and increasing soil fertility (Singh et al. 2014). Hence, this organism can be used in soil, both for the reduction of Cr^{6+} and the enhancement of soil fertility. The present study revealed that the inner cell surface of *Fischerella* sp. has an electron dense layer along with some accumulation of starch surrounding the pyrenoids. Andosch et al. (2015) has also studied the severity of Cr^{6+} along with some other metals through TEM analysis in *Desmidium swartzii*. They have shown that those metals are readily taken up into the cells, sequestered in intracellular compartments and cell walls hence, affecting the cell ultrastructure, photosynthetic activity and biomass production. Similar ultrastructural changes were also observed by Mota et al. 2015 in *Cyanothece* sp. and *Synechococcus sp.* PCC 7942 respectively treated with heavy metals. According to them the ultra structural modifications were mostly included the disintegration and disorganization of thylakoid membranes, increase of the intrathylakoidal space and the presence of inclusions. Goswami et al. 2019 reported that the structural changes that involved the widening of inter membrane space and appearance of noticeable amount of polyphosphate bodies upon the treatment of 20mg/l Cu. Hence, from the above study, it was confirmed that removal of Cr^{6+} was taken place through the reduction (hexavalent Cr to trivalent Cr).The present study is the first report that *Fischerella* sp. has ability to convert the hexavalent chromium reduced to trivalent chromium having less toxic. Cr^{6+} was also detected in different plant parts of the rice grown in the rice fields surrounding the mining area. Chromium can be easily transported to different plant parts during the process of absorption and translocation of nutrient from soil.

The amount of Cr^{6+} was 11.50, 0.289, 0.119 and 0.6mg/L in root, stem, leaf and grain respectively (Majhi and Samantaray, 2020b). Mohanty et al. 2011 reported that the higher deposition of chromium observed in leaves, stem and grain. Johanto et al. 2018 had also found significant amount of Cr in root, shoot and rice grains (6.35, 2.32 and 6.65mg/kg) present in chromium polluted paddy fields. Tariq and Rashid, 2013 had observed high Cr content in rice plants (4.28 to 15.54mg/kg) and their grains (4.65 to 12.21mg/kg). In another experiment, the rice seed germination percentage was found to be high (97%) in seeds treated with Cr^{6+} and *Fischerella* sp. (MK422171) as compared to Cr^{6+} alone. Sundaramoorthy and Ganesh, 2015 showed rice seed germination was 74% in Cr^{6+} as compared to control. According to Joshi et al. 2019, the reduction in germination was due to the increase in protease activity and depressive effect of Cr^{6+} which affect the successive transportation of sugar to embryo axis. The length of rice seedlings amended only with Cr^{6+} were shorter than that of the control and seedlings treated with Cr^{6+} amended in presence of the cyanobacterium. Joshi et al. 2019 opined that the length of root become shorter because of the accumulation of Cr^{6+} in the root vacuoles and hence, collapse the root hairs, decreases root numbers and ultimately, translocates to the shoot and shortens its length. The seed vigour index was more in plants amended with Cr^{6+} in presence of the alga than that of the positive and negative control plants. Sharma et al. 2018 has reviewed that the seed vigour is an important trait that indicates the seed quality, germination percent, seedling growth & its longevity and ultimately, it increases the resistance to adverse environmental conditions. Seeds with higher vigour can improve the crop growth and yield. The seed vigour index completely depends upon the shoot and root length of rice seedlings and the seed germination percentage. It means the higher metal concentration is inversely proportional to the seed vigour index. Joshi et al. 2019 has also shown lower seed vigour index in Cr^{6+} treated seeds than the control.

The present study revealed that the addition of *Fischerella* sp. in the rice field soil, the pH was changed and became acidic i.e. pH 5.0. It was also reported that acidic pH is mostly suitable for the rice crop which was possible due to the microbial activity in the soil. The soil EC was also significantly lowered with the inoculation of cyanobacterium in to the soil (Al-Sherif et al. 2015). But plants grew in garden soil and Cr^{6+} treated soil had shown unchanged in alkaline pH due to the lack of any microbial interaction. The nutrient (N, P, K and organic C) status of the soil get reduced in control and Cr^{6+} treated soil but get enhanced in the soil treated with Cr^{6+} with alga. Mohanty et al. 2011 reported that the initial nutrients were less in the soil because of the high contamination of Cr^{6+} which was increased later due to the interaction of rice plants with biofertilizer. A significant increase in the number of tillers and leaves were observed in soil treated with low amount of Cr^{6+} and the alga. Although the control plant had shown a good number of tillers and leaves but that was not more than the plant treated with chromium and alga. The alga removes excess toxic chromium present in soil, enhance soil health through nitrogen fixation and addition of dead organic remains (Sahu et al. 2015). Hence, the plant easily gets its micro and macro nutrients for its growth. High chromium concentration in the soil adversely affects shoot and root growth in comparison to control. At low concentration of Cr^{6+}, the algal dose was sufficient to reduce the toxic metal and to lessen its adverse effect on growth of the plant but with the increase amount of Cr^{6+} concentration, the same amount of algal dose might not be enough to remove the metal completely hence growth was slightly reduced. This might be due to the formation of large blocks of absorbent particles which can provide larger surface area for sorption of metal ions up to a certain limit (Al-Homaidan et al. 2018). However, growth was still higher in the plants treated with both

Cr^{6+} and the alga than that of both positive and negative controls. According to Sundaramoorthy et al. 2010, chromium toxicity inhibited root cell division and elongation hence, hampers the cell cycle extension. This inhibition in cell cycle extension leads to the reduction of shoot length. Reduced root growth hampers the transportation of nutrients and water to different plant parts and ultimately, lowers the weight of root and shoot (Ojha et al. 2018). Ahmad et al. 2011 reported that the shoot and root growth was reduced at higher chromium concentration (0 to 500mg/L) of chromium. Similarly, the leaf area is completely dependent on the number of leaves, their length and breadth. Leaf area decreased with increasing concentrations of Cr^{6+}.The reduction in cell size and damage to the ultra structure of leaves resulted in declining of cell numbers in leaves and their stunted growth (Sundaramoorthy et al. 2010; Tripathi et al. 2017). The number of grains per panicle and the weight of 1000 seeds were found to be high in plants grown in low chromium containing soil with cyanobacterium (T_4) while the same were found to be less in case of high chromium containing soil with cyanobacterium and negative control (T_5). It was least in plants grown in soil treated with high amount of chromium only (T_6) as the number of seeds were too less. Similarly, Sundaramoorthy et al. 2010 reported that the 1000 rice seed weight was 24.78gm in control while 11.0gm in treated plants having 200mg/L of Cr^{6+} and it is due to the application of cyanobacterial inoculums which reduced Cr^{6+} and enhanced plant growth and yield.

On the basis of biochemical analysis, the pigment content decreased with the increase in Cr^{6+} concentration. But the plant treated with high Cr^{6+} along with the alga (T_1) indicated higher chlorophyll content than the plant treated only with Cr^{6+}. This is because the alga was able to grow efficiently by utilising Cr^{6+} at high concentration of Cr^{6+} and hence, reduced the Cr^{6+} concentration of soil and improved the chlorophyll content of the plant. Available reports suggested that the activity of an enzyme δ- aminolevulinic acid dehydratase involved in chlorophyll biosynthesis is being inhibited due to the excessive amount of chromium in the soil (Hadif et al. 2015). Ghani, 2011 had also reported that high amount of chromium caused stomatal closure, reduction in intercellular spaces and alteration in chloroplast of plants. Chromium has also some deleterious effect on Calvin cycle, electron transport chain and thylakoid membranes by targeting their corresponding enzymes (Sharma et al. 2020). According to Gomes et al. 2017, there is deviation of electrons from the PSI electron donor side due to the presence of heavy metals. Hence, this leads to the inhibition of electron transport chain and disorganization in the ultra structure of chloroplasts which ultimately, leads to reduction in photosynthetic pigment content. However, they have also explained that some of those electrons might be used in the reduction of oxygen molecules which further creates oxidative stress in association with Cr^{6+}. The increase in Cr^{6+} concentration in soil raise the chromium toxicity level in plants, which is reflected with the loss of chlorophyll from leaves, depletion of crude protein, enzyme content and generation of oxidative stress. But the plants treated with alga were found to possess a good amount of pigment content, protein and antioxidative enzymes (SOD and catalase) activity. This is due to the availability of larger surface area of the alga for Cr^{6+} sorption and hence, converting the more toxic soil to less toxic. Panda and Choudhury, 2005 have stated that chromium can degrade the protein content in plants. The reduction in protein content may be due to the poor availability of nitrogen. It had also reported that chromium directly affects nitrogen bioavailability by reducing the activity of nitrate reductase involved in the conversion of nitrate to ammonia. Hasan et al. 2017 opined that proteins are the major target of heavy metals. They form complexes with functional groups of protein side chains or shift essential ions, form metallo-proteins, leading to impairment of physiological functions (Tamás et al. 2014). In addition to that, heavy metals also interfere with the native confirmations of proteins by inhibiting folding process of nascent or non-native proteins that manifest both in a quantitative deficiency of the affected proteins and in the formation of proteotoxic aggregates (Bierkens, 2000; Tamás et al. 2014). Moreover, chromium is also associated with the cellular redox activity of plants. A high amount of Cr^{6+} leads to the generation of more number of reactive oxygen species (ROS) which are virtually interacting with the cellular biomolecules including proteins. Ultimately, this activity encourages damage to proteins (Stambulska et al. 2018; Tripathi et al. 2012).

In conclusion, it is well known that rice plants are highly sensitive towards Cr^{6+} contamination, resulting in hindered growth and productivity. Physical and chemical methods used to reduce the toxicity of Cr^{6+} are expensive; hence, it is not advisable for their application in rice field. As an alternative approach bio-remediation using algae was proved to be beneficial and eco-friendly. Among three algal strains collected from chromite mining area for the bioremediation of rice field soil. It was observed that SM3 algal strain has a greater potential to reduce Cr^{6+} to Cr^{3+}. Microscopic study and molecular characterization indicated that SM3 algal strain is identified as *Fischerella* sp. Moreover, as it is a heterocystous cyanobacterium it can fix atmospheric nitrogen and also enrich in micro and macro nutrient content in soil. It is the first report that this alga strains are growing very well in the chromine mine spoil. Hence, it could be used for the detoxification of Cr^{6+} contaminated rice fields and to increase the soil fertility status.

Declarations

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Tables

Table 1 Physicochemical parameters of the soil samples collected from the opencast mining area and their adjoining rice field.

Name of the sample	pH	Temperature (℃)	Moisture content (%)	Electrical conductivity (µS/cm)	Total dissolve solid (ppm)	Cr^{6+} (ppm)
		Mean (Av.) ± SE*				
Soil from mining area	7.4	35.3±0.02	27.4±0.8	73.2±0.4	157.8± 1.3	47.5±0.8
Rice field soil	4.96	37.4±0.1	23.5±0.7	65.4±0.5	76.7± 0.91	40.4±0.6

* The experiment was repeated thrice with three replications

Table 2 Dry weight (gm/100ml) of algal isolates grown in the medium with different concentrations of hexavalent chromium (Cr^{6+}).

Different Concentrations of Cr^{6+} (ppm)	Different algal strains (Dry weight/ 100ml)(Mean average)		
	SM1	SM2	SM3
0	0.091±0.001	0.077±0.001	0.096±0.0023
20	0.132±0.001	0.085±0.003	0.114±0.002
40	0.156±0.001	0.088±0.002	0.185±0.002
60	0.174±0.001	0.105±0.003	0.181±0.004
80	0.168±0.002	0.125±0.002	0.156±0.002
100	0.154±0.002	0.185±0.002	0.133±0.003
120	0.107±0.002	0.168±0.002	0.106±0.003
140	0.085±0.003	0.136±0.002	0.096±0.002
160	0.062±0.002	0.085±0.001	0.075±0.005
180	0.032±0.001	0.041±0.001	0.043±0.002
200	0.002±0.00	0.026±0.002	0.008±0.001

* The experiment was repeated thrice with three replications/concentration.

Table 3 Percentage of Cr^{6+} available in the respective media after the culture of algal strains.

Different forms of Chromium	Algal strains used (Mean± S.E)*		
	SM1	SM2	SM3
Total Cr (ppm)	4.707±0.32	4.934±0.14	4.145±0.21
Cr^{6+}(ppm)	0.148±0.002	0.137±0.06	0.471±0.02
Cr available other than Cr^{6+} (ppm)	4.557±0.51	4.796±0.17	3.649±0.55
Percentage of total Cr removal (%)	88.239±1.34	87.76±1.68	89.63±1.52

* The experiment was repeated thrice with three replications

Table 4 Growth of rice (*Oryza sativa)* crop in different treatments during Rabi season, 2018.

Different treatment	Days after growth (d)	No. of tillers	No. of leaves/plant	Av. root length (cm)	Av. shoot length (cm)	Av. D.W. of root(mg)	Av. D.W. of shoot (mg)
		Mean ±SE*					
T_1	30- d	4.1±0.5	8.3±0.3	8.5±0.3	50.0±0.4	1.1±0.1	5.6±0.3
	60- d	6.3±0.3	19.3±0.4	9.2±0.2	72.3±0.5	7.4±0.3	8.8±0.4
	90- d	8.6±0.3	29.6±0.3	10.7±0.4	89.7±0.2	9.9±0.2	13.7±0.6
	120- d	10.3±0.5	30.2±0.5	12.7±0.8	102.9±8.2	11.9±0.7	17.4±0.2
T_2	30- d	4.3±0.3	11.3±0.3	9.6±0.6	53.1±0.9	1.6±0.1	5.8±0.2
	60- d	6.3±0.3	19.6±0.5	11.0±0.8	76.9±1.6	7.6±0.4	9.1±0.7
	90- d	9.2±0.5	28.6±0.4	12.6±0.2	90.9±1.7	10.9±0.8	14.2±0.4
	120- d	11.3±0.3	33.2±0.5	14.5±0.3	108.5±6.2	12.9±0.9	17.8±0.5
T_3	30- d	5.2±0.5	10.3±0.3	10.7±0.4	52.9±1.4	1.8±0.1	6.9±0.4
	60- d	10.3±0.3	28.6±0.4	12.4±0.5	84.6±1.6	10.2±0.4	10.4±0.3
	90- d	15.1±0.5	46.4±0.5	13.5±0.4	92.9±1.7	11.9±0.7	15.9±0.7
	120- d	14.6±0.3	48.3±0.4	15.7±0.3	110.9±8.3	12.4±0.4	19.1±0.2
T_4	30- d	4.2±0.5	10.3±0.3	10.2±0.4	57.8±1.4	2.2±0.2	6.9±0.2
	60- d	8.3±0.3	24.6±0.3	13.8±0.5	90.1±1.6	10.5±0.5	10.6±0.7
	90- d	14.4±0.5	38.2±0.5	16.8±0.1	94.6±0.2	12.6±0.8	16.7±0.6
	120- d	13.6±0.3	40.1±0.3	20.6±0.3	112.1±7.5	13.8±0.6	20.5±0.5
T_5	30- d	6.3±0.3	19.6±0.3	8.2±0.2	48.5±1.8	1.1±0.09	3.7±0.1
	60- d	8.6±0.3	23.6±0.3	10.7±0.5	71.2±1.2	6.7±0.3	9.3±0.4
	90- d	9.2±0.5	26.4±0.5	13.1±0.8	83.5±0.9	10.8±0.6	13.4±0.6
	120- d	8.6±0.3	28.2±0.5	21.1±0.4	94.6±1.1	12.2±0.3	15.5±0.4
T_6	30- d	2.6±0.3	4.6±0.3	6.2±0.3	43.1±0.8	1.2±0.03	3.2±0.1
	60- d	3.4±0.5	5.3±0.4	7.4±0.2	49.2±0.7	2.6±0.4	5.6±0.3
	90- d	3.6±0.3	7.6±0.5	9.2±0.1	60.5±1.6	4.8±0.6	7.8±0.4
	120- d	3.3±0.3	8.3±0.8	10.1±0.5	68.4±1.8	5.6±0.5	9.3±0.5

* The experiment was repeated thrice with fifteenreplications/treatment (p value at 0.5 level)

Table 5 Growth of rice (*Oryza sativa*) crop in different treatmentsduring Kharif season, 2019.

Different treatment	Days after growth (d)	No. of tillers	No. of leaves/plant	Av. root length (cm)	Av. shoot length (cm)	Av. D.W. of root(mg)	Av. D.W. of shoot (mg)
		Mean ±SE*					
T_1	30- d	4.3±0.3	11.3±0.5	8.3±0.1	48.7±0.6	1.1±0.1	6.8±0.3
	60- d	5.1±0.5	11.6±0.3	9.8±0.3	74.6±0.8	5.3±0.6	8.5±0.4
	90- d	11.3±0.6	27.6±0.4	11.2±0.2	88.6±1.1	11.9±0.9	13.8±0.6
	120- d	11.6±0.8	29.3±0.3	14.8±0.2	100.6±5.5	12.4±1.1	16.5±0.5
T_2	30- d	5.2±0.5	9.6±0.3	9.8±0.1	54.1±1.2	1.5±0.8	7.2±0.6
	60- d	6.6±0.3	15.6±0.3	10.4±0.2	79.1±1.3	8.6±0.7	10.1±0.7
	90- d	11.6±0.3	38.3±0.3	13.9±0.5	89.8±1.6	12.4±0.7	14.5±0.8
	120- d	13.6±0.3	39.5±0.8	16.5±0.4	103.8±6.2	13.6±0.8	17.4±0.6
T_3	30- d	8.3±0.3	21.6±0.3	11.5±0.2	55.2±1.3	1.8±0.4	6.5±0.5
	60- d	10.6±0.5	27.3±0.8	12.9±0.6	87.9±2.4	10.9±0.6	10.6±0.8
	90- d	12.6±0.3	38.3±0.3	14.9±0.4	92.8±1.3	12.4±0.8	15.9±0.7
	120- d	14.2±0.5	37.3±0.8	19.6±0.3	105.9±4.1	13.6±0.7	19.1±0.6
T_4	30- d	5.3±0.3	17.6±0.4	11.2±0.5	56.7±1.4	2.1±0.5	8.2±0.5
	60- d	7.6±0.3	22.3±0.6	15.2±0.4	90.6±1.7	11.4±0.8	11.5±0.4
	90- d	13.6±0.3	41.4±0.5	17.8±0.6	95.8±1.6	12.9±0.7	16.1±0.3
	120- d	13.8±0.5	39.3±0.5	21.9±0.3	106.7±7.2	14.1±0.9	20.5±0.6
T_5	30- d	6.6±0.6	16.2±0.5	9.9±0.3	51.7±1.2	1.03±0.2	4.8±0.4
	60- d	8.3±0.3	16.3±0.6	11.9±0.4	69.4±1.3	5.8±0.5	10.1±0.7
	90- d	11.5±0.5	24.3±0.6	12.5±0.6	87.2±1.4	10.2±0.8	13.4±0.8
	120- d	10.3±0.3	26.3±0.3	18.3±0.5	96.3±1.5	10.8±0.7	14.9±0.9
T_6	30- d	2.6±0.3	9.3±0.3	7.5±0.3	43.1±0.8	0.5±0.1	3.5±0.2
	60- d	4.3±0.6	8.6±0.3	8.6±0.5	49.6±0.9	2.2±0.4	5.7±0.5
	90- d	5.4±0.5	14.2±0.5	9.8±0.7	62.6±1.1	3.3±0.6	7.5±0.6
	120- d	5.3±0.3	15.6±0.3	10.5±0.8	66.9±1.5	4.4±0.5	9.4±0.8

* The experiment was repeated thrice with fifteen replications/treatment (p value at 0.5 level)

Table 6 Growth and yield performance of rice crop grown in different treatment during Rabi, 2018 and Kharif, 2019.

Different treatments	Rabi, 2018			Kharif, 2019		
	Leaf area (cm^2)	Grains/ panicle	1000 seed weight (gm)	Leaf area (cm^2)	Grains/ panicle	1000 seed weight (gm)
	Mean (Average) ±SE*					
T_1	33.4±1.2	89.3±1.2	29.5±0.4	32.5±0.7	90.4±1.3	29.6±0.3
T_2	37.1±1.1	91.4±0.9	37.2±0.7	37.8±0.8	92.6±1.1	42.6±0.7
T_3	36.9±0.8	91.6±1.1	43.3±0.8	36.2±0.6	92.8±0.8	48.8±0.8
T_4	40.2±0.7	94.2±0.6	45.9±0.9	40.3±0.8	95.3±0.6	48.3±0.6
T_5	33.9±0.4	79.4±0.7	28.3±0.6	33.7±0.6	81.5±0.9	29.0±0.7
T_6	25.8±0.6	60.6±0.8	12.9±0.8	25.2±0.6	62.3±0.8	13.1±0.2

* The experiment was repeated thrice with fifteen replications/treatment (p value at 0.5 level)

Table 7 Biochemical assay of the rice crop grown in different treatments during Rabi, 2018.

Different treatments	Pigment content (µg g^{-1})			Protein content (mg/g fresh wt. basis)	Carbohydrate content (mg/g fresh wt. basis)	Catalase activity (U/ mg of protein)	SOD activity (U/ mg of protein)
	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll (a+b)				
T_1	2.8±0.5	4.5±0.4	7.3±0.9	0.5±0.08	4.7±0.8	7.2±0.4	9.1±0.3
T_2	2.5±0.4	3.3±0.3	5.8±0.7	0.6±0.02	5.4±0.4	6.7±0.5	7.8±0.2
T_3	1.7±0.3	2.2±0.3	3.9±0.6	0.7±0.02	5.6±0.3	6.1±0.4	5.7±0.4
T_4	1.5±0.4	2.3±0.2	3.8±0.6	0.9±0.04	6.7±0.4	4.9±0.7	3.5±0.2
T_5	1.6±0.2	1.8±0.4	3.4±0.6	0.5±0.01	5.1±0.3	4.8±0.7	2.2±0.2
T_6	1.5±0.2	1.7±0.2	3.2±0.4	0.2±0.08	2.1±0.2	3.2±0.8	3.2±0.1

* The experiment was repeated thrice with three replications/treatment.

Table 8 Biochemical assay of the rice crop grown in different treatments during Kharif, 2019.

Different treatments	Pigment content (µg g^{-1})			Protein content (mg/g fresh wt. basis)	Carbohydrate content (mg/g fresh wt. basis)	Catalase activity (U/ mg of protein)	SOD activity (U/ mg of protein)
	Chlorophyll-a	Chlorophyll-b	Total Chlorophyll (a+b)				
T_1	2.5±0.2	4.4±0.3	6.9±0.5	0.7±0.02	5.6±0.2	8.8±0.4	9.9±0.8
T_2	1.3±0.1	2.5±0.2	3.8±0.3	0.8±0.05	5.9±0.6	7.8±0.5	8.7±0.4
T_3	1.1±0.2	1.9±0.3	3.0±0.5	0.8±0.01	6.5±0.3	6.7±0.2	6.7±0.4
T_4	1.1±0.1	1.7±0.2	2.8±0.3	1.05±0.3	7.6±0.4	5.7±0.6	5.4±0.3
T_5	0.5±0.05	1.1±0.2	1.6±0.25	0.4±0.08	5.3±0.3	4.4±0.7	3.5±0.2
T_6	0.4±0.08	0.7±0.1	1.1±0.2	0.2±0.03	1.3±0.4	3.4±0.8	3.0±0.6

* The experiment was repeated thrice with three replications/treatment.

Table 9 Elemental analysis of the identified cyanobacterium.

Name of the algal culture	Organic elements (%)				Inorganic elements (ppm)						
	C	H	N	S	P	Ca	Cu	K	Mg	Mn	Ni
Fischerella sp.	13.8±0.34	5.39±0.13	1.54±0.2	0.15±0.01	14.83±0.61	2.61±0.2	0.03±0.05	64.1±1.1	4.54±0.3	0.17±0.05	0.06±0.

* The experiment was repeated thrice with three replications

Figures

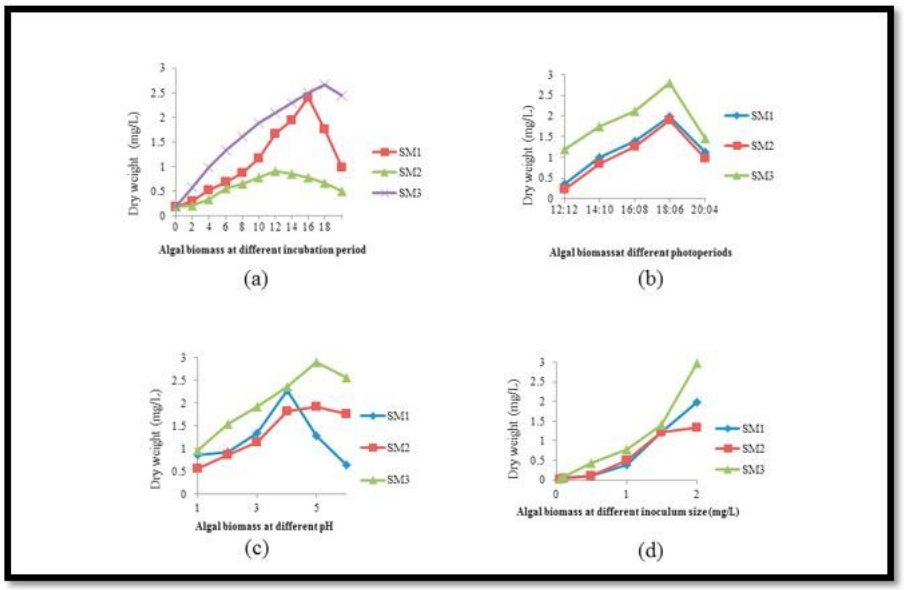

Figure 1

Optimization of growth of algal isolates in different growth medium: a. Incubation period, b. photoperiods, c. pH, d. inoculum size

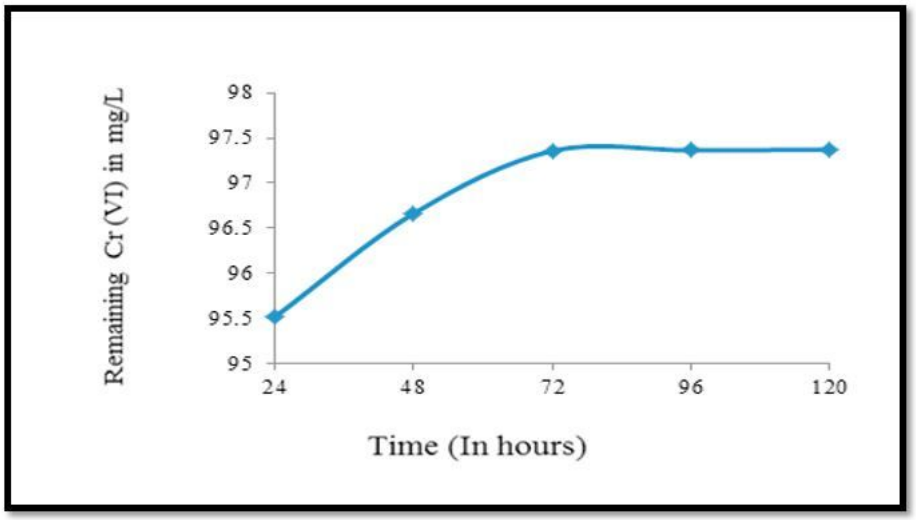

Figure 2

Effect of contact time on removal of Cr6+

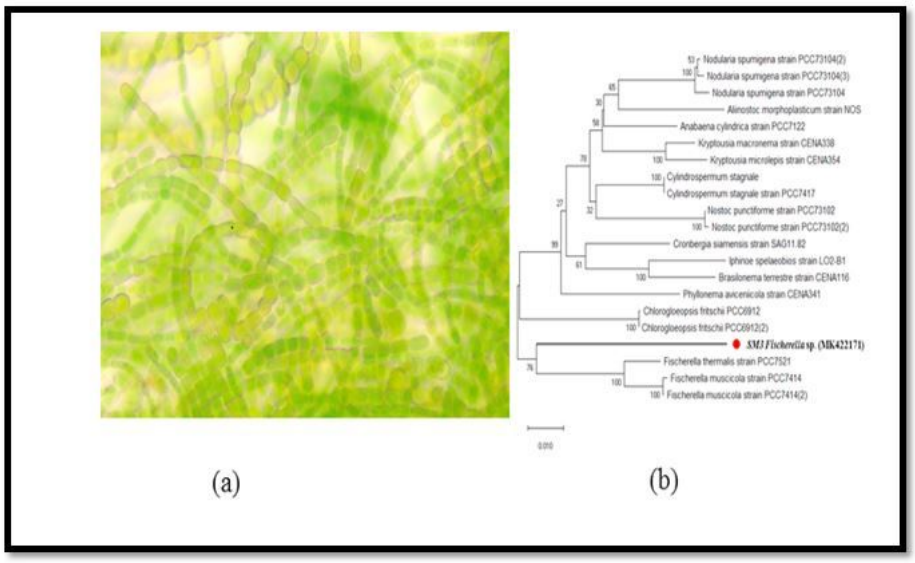

Figure 3

a. Microscopic photograph of the algal strain, b.Phylogenetic analysis of the selected alga

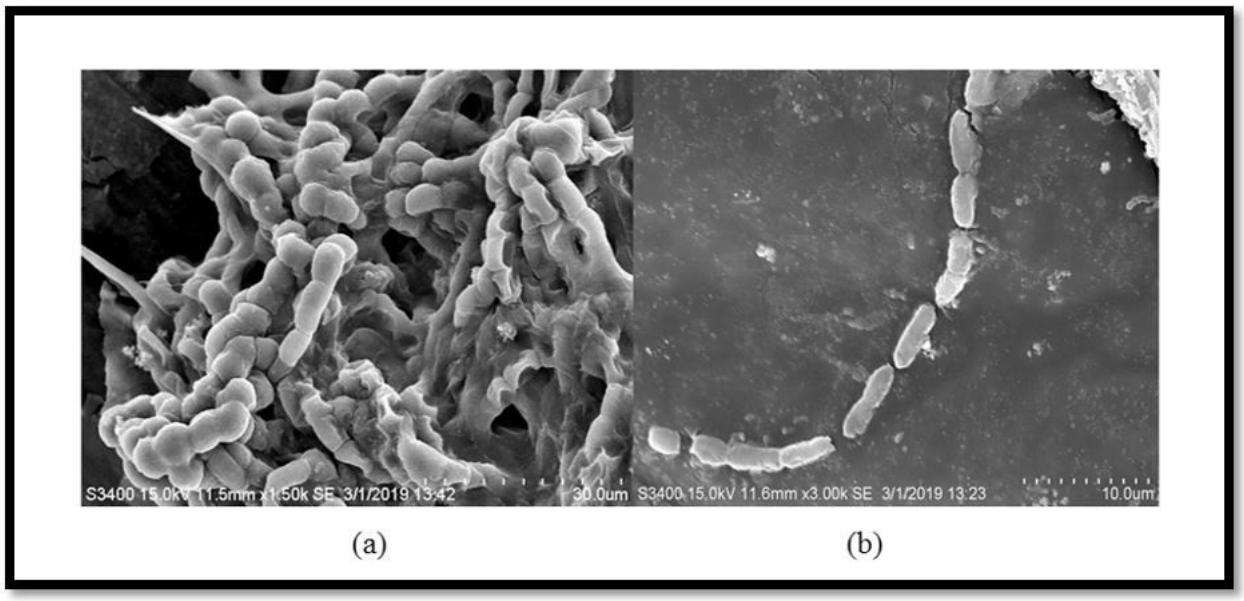

Figure 4

SEM micrograph of Cr6+ treated,b. Cr6+ untreated Fischerella sp. (MK422171)

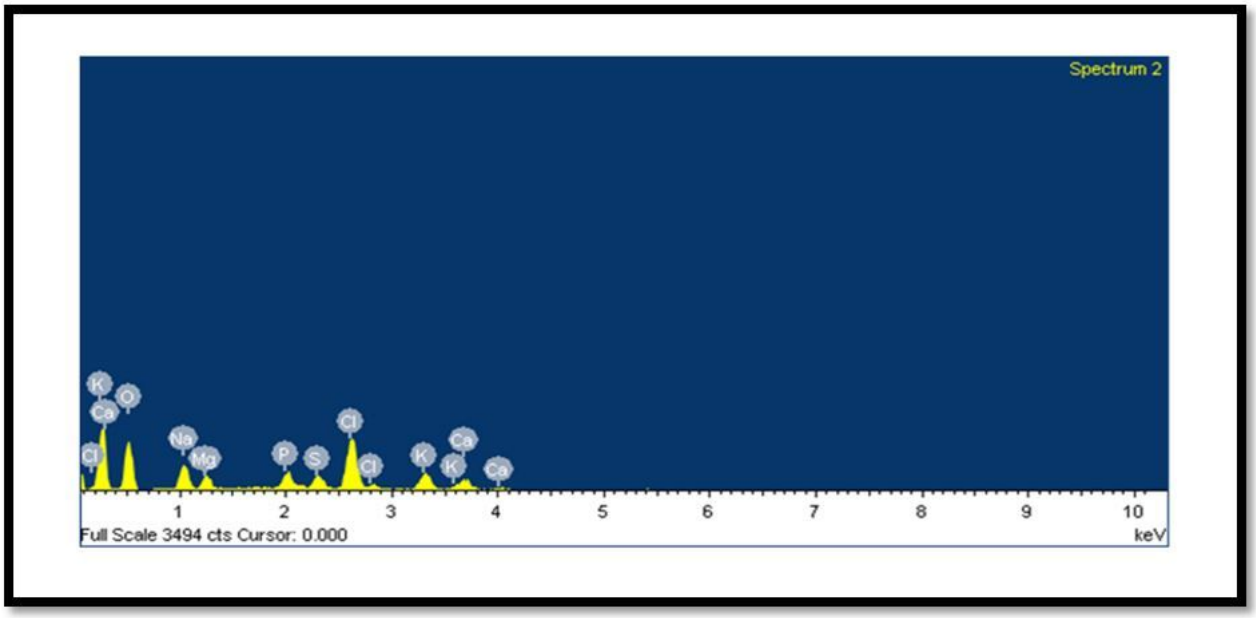

Figure 5

EDX report of Cr6+ treated Fischerella sp.(MK422171)

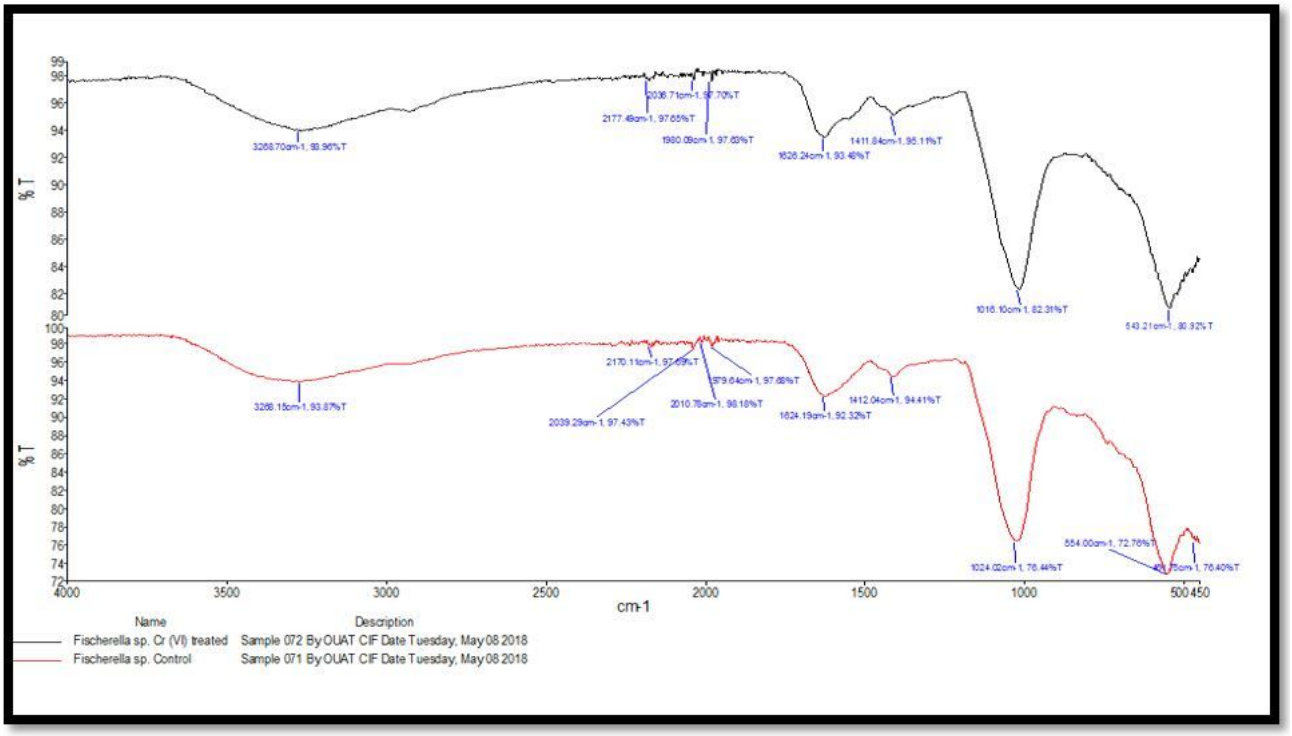

Figure 6

FTIR spectra of Cr6+ treated and untreated Fischerella sp. (MK422171)

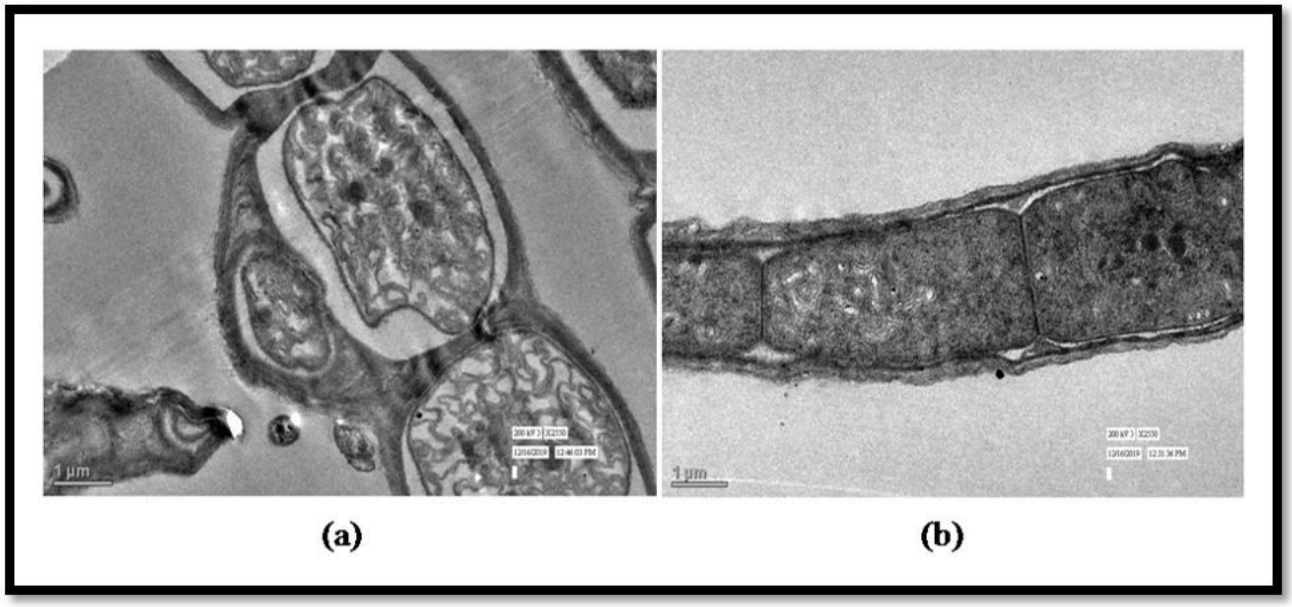

Figure 7

TEM micrograph of Cr6+ treated and 10.b. untreated Fischerella sp. (MK422171)

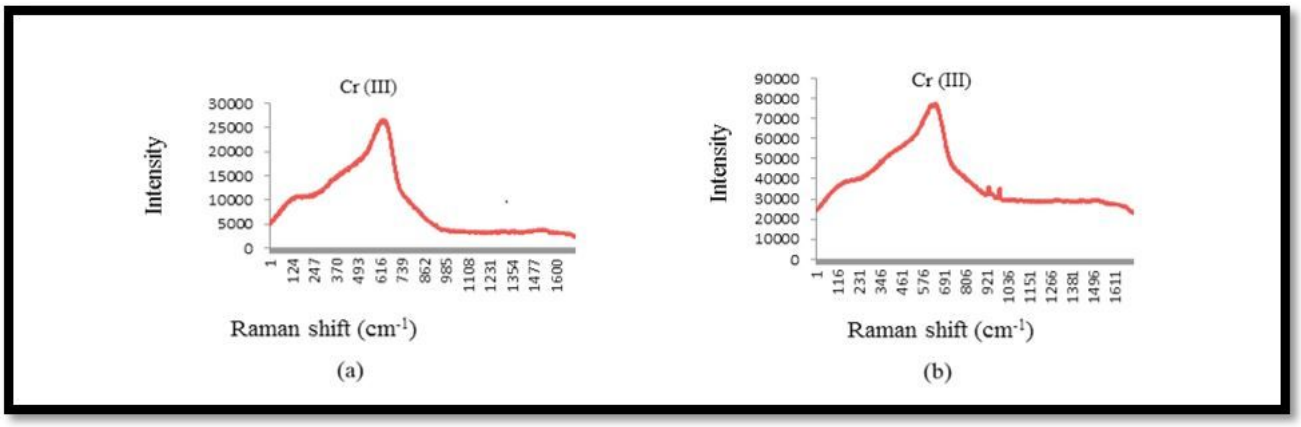

Figure 8

Raman spectra of a. Fischerella sp. cell free culture medium, b.Fischerella sp. (MK422171) cell itself treated with Cr6+