



Microalgal consortia differentially modulate progressive adsorption of hexavalent chromium

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Abstract A set of experiments was conducted to provide significant insights of micro-algal consortia regarding chromium adsorption. Four monocultures; *Scenedesmus dimorphus*, *Chlorella* sp., *Oscillatoria* sp., and *Lyngbya* sp., and their synthetic consortia were evaluated initially for chromium bio-adsorption at four different regimes of hexavalent chromium i.e. 0.5, 1.0, 3.0 and 5.0 ppm. Based on findings, only 1.0 and 5.0 ppm were considered for future experiments. Consequently, three different types of monoculture and consortia cells namely; live cells, heat-killed cells, and pre-treated cells were prepared to enhance their adsorption potential. Maximal adsorption of 112% was obtained at the dose of 1.0 ppm with 0.1% SDS pre-treated consortia cells over live consortia cells. In support, atomic absorption spectroscopy, laser induced breakdown spectroscopy, pulse amplitude modulated chlorophyll fluorescence, and scanning electron microscopy were performed to assess the structural and functional changes within consortia and their utilization in mitigation of elevated chromium levels.

Keywords Micro-algal consortia · Pulse amplitude modulated chlorophyll fluorescence (PAM) · Scanning electron microscopy (SEM) · Laser induced breakdown spectroscopy (LIBS) · Atomic absorption spectroscopy (AAS)

Introduction

Oxygenic photosynthesis and pollutant degradation by green algae and cyanobacteria have invigorated the dual concept of heavy metal-affected wastewater treatment and biomass production (Patel et al. 2014). These organisms have the good potential towards water purification by mitigation of the elevated levels of heavy metal toxicity (Khalida et al. 2012; Cardinale 2011; Weis et al. 2008; Cardinale et al. 2007). Individually, these organisms are not able to completely remove the heavy metals; therefore, considering the novelty of microalgae monocultures, efforts are ongoing to develop their suitable synthetic consortia that can be able to alleviate the enhanced levels of heavy metal pollution in different water reservoirs. In addition, regular practices to produce the high-quality microalgae biomass from such wastewater treatment plants is also in progress (Cardinale et al. 2006). Utilization of these biological agents as designer consortia for nutrient removal, pollutant degradation and biomass production from wastewater may enhance the chances of their acceptance by public (Power and Cardinale 2009). Different microalgae have varying inherent traits that define their morphological appearance and functional roles in nature as well as in the heavy metal-affected environment. High concentrations of organic pollutants like styrene and phenolic compounds inhibit the growth of microbial community due to their low aqueous solubility. Consequently, heavy metals such as chromium, mercury, copper and lead are soluble in water up to a high extent. Therefore, utilization of such heavy metal polluted water in plant irrigation or direct intake by population is the major cause behind a number of serious metabolic disorders or diseases (He et al. 2012). Amongst the monocultures, some species have very strong potential while others have limited or

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negligible efficiency towards the heavy metal detoxification.

Furthermore, their ability towards the detoxification may vary alone or within a group of similar or related taxa (Bose et al. 2011). These variations are due to the alterations in the structure and functioning of entire consortia. Sometimes, combinatorial activities of these monocultures either in fresh or in contaminated water may lead to enhance rates of CO₂ mitigation, heavy metal adsorption and biomass production. Therefore, selection of novel species that can have the higher potential for heavy metal degradation is the target of present day biotechnologists. In this aspect, a little success has been achieved (Ruffing and Trahan 2014; Stevenson 2014; Singhvi and Chhabra 2013; Spolaore et al. 2006; Tilman et al. 1996). However, utilizing a single species for microbial degradation of the pollutants has some bottlenecks; therefore, it requires a coordinated approach involving both biochemical and molecular tools to investigate the novel insights into the microalgal consortia for heavy metal polluted water restoration. In comparison to the introduction of genes or enzymes in a single organism which requires their integration within the regulatory and metabolic network for the proper expression (Silva-Rocha and de Lorenzo 2010), engineering of the microbial consortium is comparatively easier, achievable and acceptable. Developing consortia on the basis of the division of labour by a combination of tasks of constituent members could provide a better understanding of the natural assemblages of microbial communities (Patel et al. 2017). It could be a possible way to get the microalgal consortia with enhanced abilities towards the trial missions of pollutant degradation, mitigation of CO₂ by its dark photosynthetic fixation reactions, and to get increased biomass yields for commercial production of metabolites of different biotechnological importance (Ortiz-Marquez et al. 2013).

Chromium, a highly poisonous chemical that is responsible for a number of metabolic disorders and incurable serious ailments, being deposited excessively in most of the water reservoirs from industrial sectors such as leather tanning, textile industries, battery, and electroplating in relatively larger amounts. Leaching of rocks and topsoil are natural sources of chromium entry in different water bodies. Most common sources of groundwater chromium contamination are improper disposal of wastes from chromate processing units. According to World Health Organization (WHO), Geneva (1988) Environmental Health Criteria No. 61, levels of chromium in drinking water should not be more than 0.05 ppm. On the other hand, its level in rainwater, seawater, surface water, groundwater (irrigation water) should be between 0.2–1.0, 0.04–0.5, 0.5–2.0 and 1.0–10 µg L⁻¹, respectively. Water purification tanks are being planted to purify chromium

contaminated water reservoirs; however, a little success has been achieved (Berg et al. 2009). A number of physical and chemical methods such as coagulation followed by filtration, membrane filtration, adsorption, and ion-exchange are being used to remove the increased levels of chromium from drinking water and non-drinking water. Yet, these methods have a number of limitations which does not allow them to meet the standards with their application. However, coagulation–filtration is still being used as a common method for chromium removal. Membrane technology has been found efficient in removing both Cr⁶⁺ and Cr³⁺. In the absence of proper technology, still a number of efforts are ongoing, and biotechnologists are searching the potential microalgae species that could be actively engaged in chromium-contaminated water purification processes. Moreover, a number of studies have been done with monocultures, especially with green algae *Chlorella*, and cyanobacteria *Oscillatoria* and *Lyngbya*; globally, the role of microalgae consortia in chromium adsorption is underway globally. According to the Safe Drinking Water Act (SDWA) (1991), maximum contaminated level of water with hexavalent chromium should not be more than 100 ppb i.e. 0.1 ppm; Consequently, its toxicity level has increased beyond this limit in industrial sectors, and some of which is contaminating the rivers that are causing the serious water issues in human beings and other animals. Therefore, several water purification programs are ongoing, which majorly involve cyanobacteria and green algae cultures. Considering all these facts that may provide improved water quality and higher biomass yields, this study was proposed, which processes of microalgal consortia were assessed in mitigation of chromium toxicity under different regimes of hexavalent chromium to see that how the changes in microalgal community structure could affect its processes (Volland et al. 2013). Green algae *Chlorella* sp., *Scenedesmus dimorphus*, cyanobacteria *Oscillatoria* sp., and *Lyngbya* sp. were in vitro evaluated to alleviate the levels of chromium in BG11⁺ broth. Monocultures as well as consortia of these organisms were investigated in their natural habitat and chromium enriched environment to develop a successful micro-algal technology which could reduce the elevated chromium levels in heavy metal enriched fresh water systems, and can produce biomass (Berg et al. 2009; Renuka et al. 2013). Consortia of these photosynthetic organisms could provide not only less polluted water but also sustainable biomass which can be utilize for other purposes after complete recycling of its adsorbed chromium (Khalida et al. 2012); otherwise, it may further create the problem of bio-accumulation or toxicity. Four organisms were selected; *Chlorella*, *S. dimorphus*, *Oscillatoria* and *Lyngbya* for this study, since these were previously tested by a number of research groups and identified as potential agents in chromium

removal. Therefore, evaluating them in consortia could provide their better exploitation in chromium removal (Renuka et al. 2013).

Materials and methods

Microalgae cultures

The members of family Chlorophyceae and Cyanophyceae; *Chlorella* sp., *S. dimorphus*, *Oscillatoria* sp., *Lyngbya* sp. and their consortia were selected for the study. These cultures were available at the Phycology laboratory, Centre of Biotechnology, University of Allahabad, India. Axenic cultures were maintained on BG11⁺ agar media (pH 7.8) supplemented with 10 mM sodium thiosulphate (Behera et al. 2015; Gross et al. 2014; Cardinale et al. 2007).

Culture conditions

Microalgae cultures were incubated at 27 ± 1 °C and day-light fluorescent lamps were used for providing illumination at the irradiance of $92.5 \mu\text{mol photons m}^{-2} \text{S}^{-1}$ in 14:10 light–dark diurnal cycle. Furthermore, at the start of experiments and for various physico-chemical studies, equal number of cells of above mentioned cultures of Chlorophyceae and Cyanophyceae (optimized through corresponding O.D. and cell counts) were inoculated from the late log phase in equal volume of BG11⁺ culture broth under two different experimental setups; first set involved cultivation with 0.5, 1.0, 3.0 and 5.0 ppm doses of chromium while second set was deficient in chromium (Shukla et al. 2012).

Experimental designs

Experiments were performed in three replicates, a Complete Randomized Design (CRD) was used to elucidate the effects of Cr⁶⁺ on the microalgae consortia consisting *Chlorella* sp., *S. dimorphus*, *Oscillatoria* sp. and *Lyngbya* sp. Hexavalent chromium stock of 100 ppm was prepared by dissolving 2.830 g potassium dichromate (K₂Cr₂O₇) in 1000 mL sterile distilled water. Consequently, the stock was used to make the further dilutions of 0.5, 1.0, 3.0 and 5.0 ppm. Before the start of experiment, inoculums were grown up to the late log phase. An equal number of cells of green algae and cyanobacteria were mixed to get the microalgae consortia. Based on our previous standardizations (O.D. vs. cell counts), the cells and filaments were mixed. In each treatment, equal numbers of cells of monocultures or consortia having equal cells of monocultures were inoculated in separate flasks. Thus, initially, each flask contained 1×10^7 cells mL⁻¹ of culture broth (BG11⁺ media supplemented with or without different

doses of Cr⁶⁺. The flasks without chromium treatments were considered as experimental controls. To nullify the experimental error, the optical density of un-inoculated BG11⁺ supplemented with chromium was normalized with O.D₇₃₀ of natural BG11⁺. The chromium-induced stress effects and changes in physico-chemical parameters of monocultures as well as of consortia were evaluated under four different regimes including; 0.5, 1.0, 3.0 and 5.0 ppm for *S. dimorphus*, *Chlorella* sp., *Oscillatoria* sp. and *Lyngbya* sp. in consortia of BG11⁺ media (Fox, 2004). Chromium treated cultures along with respective experimental controls (BG11⁺) were incubated up to 25 days under above described culture conditions. Consequently, different controls (different microalgae cultivated in BG11⁺ individually as well as in consortia) were compared with treated monocultures and consortia to record changes in the original physiological consequences and role of different regimes of chromium stress on their biomass production ability.

Analytical methods

Bio-sorption of chromium by different species and consortia

Bio-sorption of chromium by different microalga sp. and their consortia was studied by three different treatment methods; in first the one, living mono culture cells and consortia were used to monitor the rates of uptake of chromium heavy metal by supplementing the basal media with different concentrations (1.0 and 5.0 ppm) of chromium stock solutions. In the second treatment, heat-killed cells were prepared by taking fresh biomass and incubating it in water at 100 °C for 5 min. In third treatment, pre-treated cells were made by suspending the fresh biomass in 0.1 N NaOH and 0.01% SDS separately followed by incubating these cells at room temperature (R.T.) for 20 min. The biomass obtained from each treatment was later suspended in solutions containing different concentrations of chromium. The biomass was left for 1 h in the respective chromium containing solutions of 1.0 and 5.0 ppm; cell-free supernatants were collected from each, and analyzed for chromium bio-sorption by measuring the optical density at 540 nm wavelength (Jayashree et al. 2012, Micheli et al. 2014).

Calculation of LD₅₀

The LD₅₀ of Cr⁶⁺ was determined calorimetrically to decide the optimal dose at which various monocultures and consortia could perform effectively. Simultaneously, the maximal dose at which major processes of these cells get inhibited was also selected for comparative analyses (Stohs et al. 2001).

Chlorophyll-a (chl-a) fluorescence

The *chl-a* fluorescence of various microalgae and their consortia under different experimental conditions was monitored by a dual modulation kinetic fluorometer FL3500/F (PSI, Brno, Czech Republic, version 3.7.0.1). The monocultures and their consortia were pre-adapted for 15 min in the dark before examining their fluorescence rates (Maxwell and Johnson 2000; Patel et al. 2016).

Microscopy

The microscopic observations were made at the end of cultivation to study the structural changes within control and treated cells of monocultures and consortia. The cells were harvested and washed with sterile distilled water two times, and suspended to the original volume by adding the fresh BG11⁺ media. Two drops of the cultures were placed on the neat and clean slides and observed under 40× objective of the compound microscope.

Scanning electron microscopy (SEM)

The high resolution micrographs of control and adsorbed samples of micro-algal consortia were obtained under the Scanning Electron Microscope (SEM) through FEI Quanta 200. Consortia grown in original BG11⁺ media, 1.0 and 5.0 ppm were harvested by centrifugation at 2000 rpm for 5 min, washed three times with Sorenson's buffer. Subsequently, each micro-algal consortia sample was fixed with a fixing solution containing 4.0% v/v formaldehyde and 2.5% glutaraldehyde for 4 h at R.T. Fixing solution was prepared in 0.2 M Sorenson's phosphate buffer. Fixed samples were washed with 0.2 M Sorenson's phosphate buffer three times with 15 min for each wash. Consequently, each sample was dehydrated in a series of sequential series of 20, 40, 60, 80 and 100% absolute ethanol (Bharti et al. 2016). Finally, each sample was dried in a critical point dryer under CO₂ enriched environment and examined by using the FEI Quanta 200.

Laser induced breakdown spectroscopy (LIBS)

To perform the LIBS, control consortia and consortia treated with 1.0, and 5.0 ppm Cr⁶⁺ were grown in 2000 mL of BG11⁺ media in 5 L Conical flasks up to 25 days. Proper light and dark treatments was maintained and hand shaking was provided timely to mix the content throughout cultivation. Each sample was centrifuged and filtered with Whatman filter paper (2.0 μm pore size), and oven-dried to remove moisture completely. Equal biomass of 1.0 g from each sample was further considered for the preparation biomass pellets by the help of KBR-press device.

Furthermore, pellets were used for LIBS analysis. Considering the sensitive nature of microalgae biomass pellets and destruction by Laser-pulse irradiation induced thermal shock, shot to shot protocol was used to minimize any destruction and to get the high degree of reproducibility. The peaks obtained after the LIBS, were compared with the CN bands available at NIST (National Institute of Standards and Technology, Gaithersburg, Maryland) to get the presence or absence of chromium (Kumar et al. 2014).

Atomic absorption spectroscopy (AAS)

Atomic Absorption Spectrophotometer was used to perform the analysis by following the method suggested by Singh et al. 1989. To perform the AAS, equal dried biomass of each consortia control, consortia 1.0 and 5.0 ppm Cr⁶⁺ treated samples were washed by double distilled water two times and oven-dried, and equal dried biomass of 1.0 g was considered for analysis. Single acid digestion was done with HNO₃, and samples were kept in hot air dry-oven for complete drying. Acid digested samples were then mixed in 0.5% HNO₃, and final volume was made to 100 mL. Quantitative determination of Cr⁶⁺ was done by AAS of Perkin Elmer at scientific and Industrial research organization, Centre of Food technology, University of Allahabad, Allahabad. The experimental standards were prepared by dissolving the Cr⁶⁺ in similar way as done for various algal samples in 0.5% HNO₃. The results were compared to standards to get the concentrations of Cr⁶⁺ adsorbed in consortia biomass by available list of metal spectra in NIST database.

Statistics

Culture related experiments were performed in three replications while analytical evaluation through biochemical and spectrophotometric methods were done in six replications. Various experimental data were analyzed by either one way or two way ANOVA or mixed ANOVA as where needed by using either Graph Pad Prism 5.0 or Origin 8.5 statistical software (Loreau et al. 2001). The Null hypothesis was rejected or accepted on the basis of Fisher ratios (*F* value) and probability ($p \leq 0.05$) at 95% confidence levels.

Results and discussion

Chromium induced changes in microalgae community

Exogenous supplementation of different doses of chromium significantly affected the structure and functioning of various monocultures and consortia. In order to find out the tolerance limits of these cyanobacteria, green algae and

their consortia against chromium, their growth media was supplemented with four concentrations of Cr^{6+} in different experimental flasks up to 25 days. In natural environment, these photosynthetic microbes had different behavior towards chromium when compared to other treatments. Highly drastic morphological distortions were seen under microscope at higher doses of Cr^{6+} . Almost all the cells of

Oscillatoria were lost at the 5.0 ppm of Cr^{6+} whilst *Chlorella*, *Lyngbya* sp. and *Scenedesmus* showed comparatively better stress tolerance in consortia. Microscopic observations showed that at the 0.5 ppm Cr^{6+} , the cell morphology of monocultures and consortia cells was not affected significantly (Adinath et al. 2015; Pereira et al. 2013; Tchounwou et al. 2012). On the other hand;

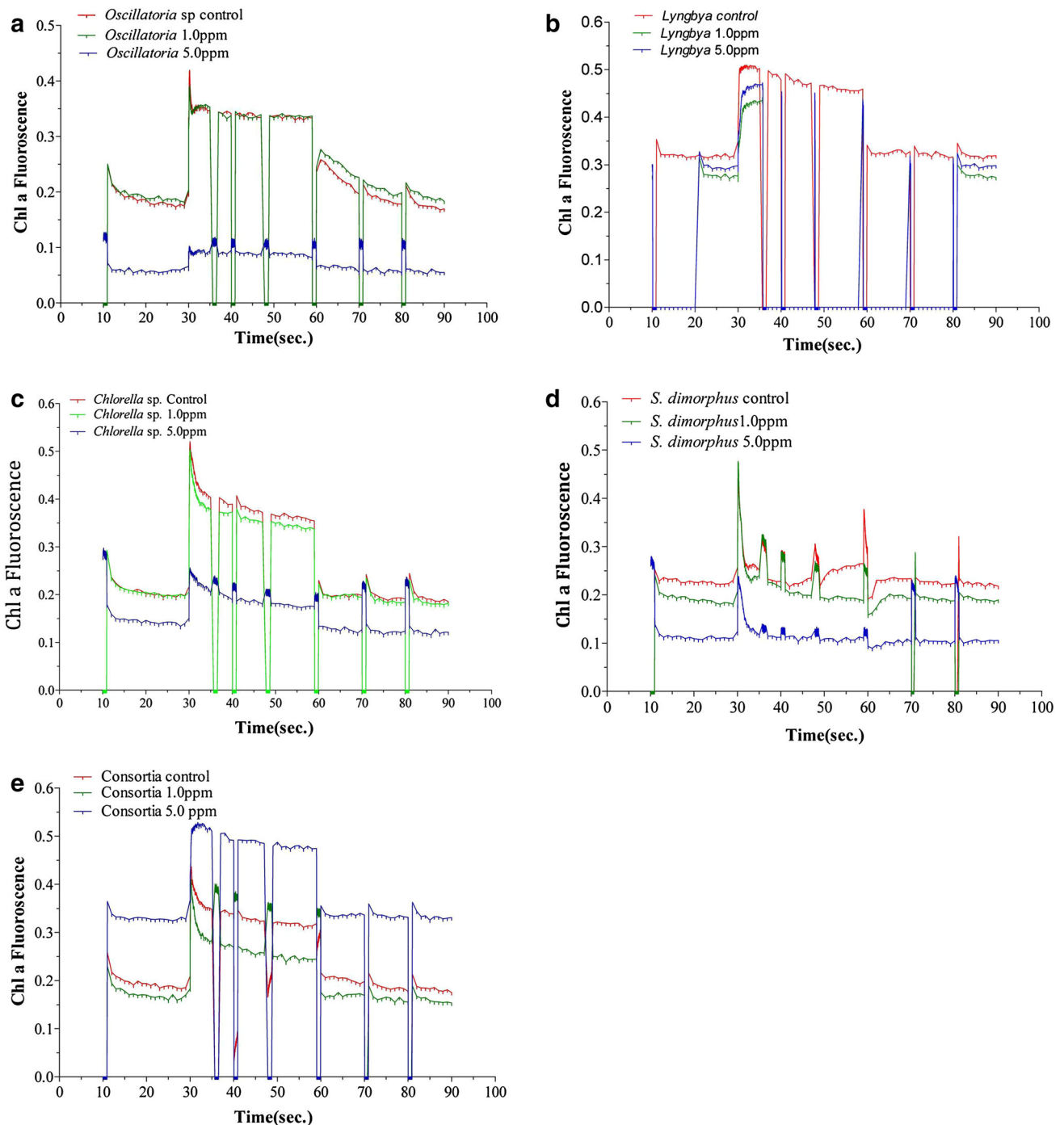


Fig. 1 OJIP curves showing the effects of Cr^{6+} on photosynthetic rates of different strains of cyanobacteria, green algae and their consortia. **a** *Chlorella*, **b** *Oscillatoria*, **c** *S. dimorphus*, **d** *Lyngbya*, **e** Consortia

appearance of these photosynthetic organisms was altered at higher doses of Cr^{6+} . However, at the lower doses of 0.5 and 1.0 ppm, most of the cells were slightly swollen. Since, swollen cells have smaller surface to volume ratios, therefore, it may be a reason for slightly increased physiological performance at lowest dose (0.5 ppm) of Cr^{6+} . In addition, cell surfaces of different algae and cyanobacteria get blocked by adsorption of Cr^{6+} which alternatively affected the original rate of exo/endo-osmosis of important metabolites. Therefore, natural physiology of chromium treated cells gets altered that highly affected the growth rates and biomass yields (Fig. 1a–e).

Similar trends were recorded for the consortia at different doses of Cr^{6+} (Fig. 2). The monocultures in control

consortia showed the following order of tolerance as observed by cell counts at the time of harvest; *Oscillatoria* sp. < *S. dimorphus* < *Chlorella* < *Lyngbya* sp. respectively. Therefore, within consortia *Lyngbya* sp. was found to be dominant sp. Based on the structural changes and calculated LD_{50} values of various monocultures and consortia under stress, we selected only 1.0 and 5.0 ppm doses for future studies. At 1.0 ppm Cr^{6+} , cells performed optimally whilst at highest dose of 5.0 ppm highly diminished physiology was obtained for each culture (Fig. 3). Under stressed environment, assemblages of cyanobacteria and green algae produce unusual compounds to protect them from adverse environmental effects. In another study by Dixit et al. (2017), Jaishankar et al. (2014), they isolated

Fig. 2 Bio-sorption estimation of various microalgae and their consortia

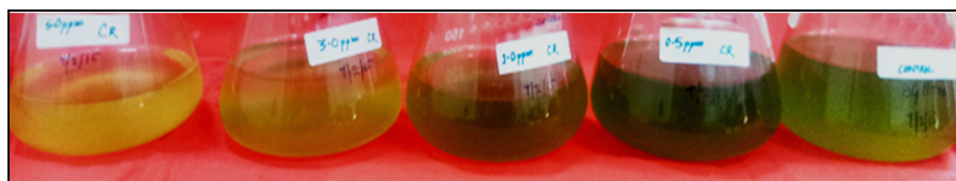
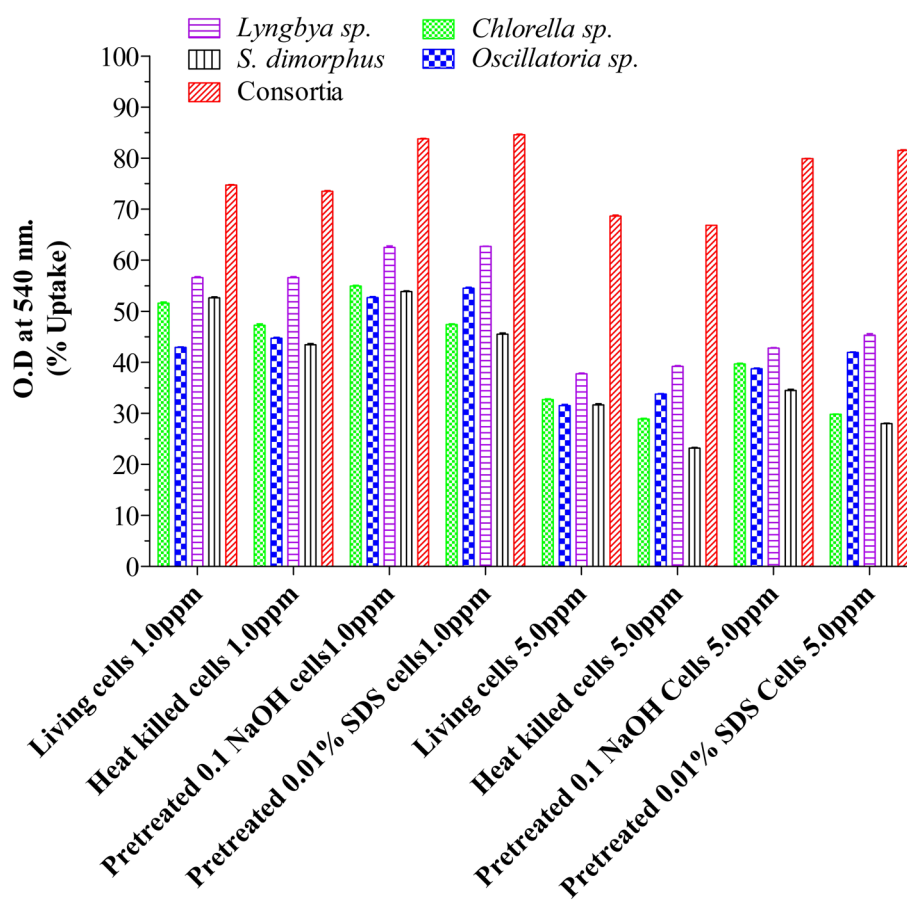


Fig. 3 Volumetric appearance of synthetic microalgae consortia and consortia treated with different doses of chromium; from right first flask have natural community whilst second, third, fourth and fifth

flasks communities are treated with 0.5, 1.0, 3.0 and 5.0 ppm of hexavalent chromium

Table 1 Elemental characterization by laser induced breakdown spectroscopy

Elements	Wavelength/s (nm)
Carbon (C)	247.8
Iron (Fe)	238.2, 275.5, 294.7
Magnesium (Mg)	277.6, 279.0, 279.5, 280.2, 285.2, 382.9, 383.2, 383.7
Sodium (Na)	293.6, 330.2, 589.0, 589.6
Oxygen (O)	777.3, 844.8
Nitrogen (N)	746.8, 822.2, 824.2
Calcium (Ca)	315.9, 317.7, 370.6, 373.7, 393.3, 396.8, 422.6, 430.2, 585.7, 610.2, 612.2, 616.1, 643.8, 644.9, 646.2, 647.1, 649.3, 649.9, 714.7, 720.2, 732.6, 849.7, 854.2
Potassium (K)	404.4, 404.7, 460.7, 766.4, 769.9
Hydrogen (H)	656.2
Chromium (Cr)	425.4, 427.4, 428.9

two strains of *Oscillatoria* sp. RBD01 and *Leptolyngbya* sp. RBD05 were isolated from nutrient and heavy metal polluted stretch of Ganga River, and characterized them as producer of microcystin. These compounds highly affect the growth rates of other individuals of consortia (Table 1).

Fluorescence response of chlorophyll

Cumulative interactions of these photosynthetic monoculture cyanobacteria and green algae in consortia towards the chromium tolerance were highly reduced at the 5.0 ppm (Govindjee 2005; Holt et al. 2004). However, *Chl. a* fluorescence of consortia was less affected than monocultures. Among the monocultures, maximum Fv/Fm ratio was obtained for the *Lyngbya* sp. followed by *Chlorella* sp., *S. dimorphus* and *Oscillatoria* sp. respectively. Moreover, with respect to various monoculture controls 2.802, 0.813, 10.886, 4.583, 42.51, 70.49, 15.44, 44.87% inhibition in the rate of *Chl-a* fluorescence was obtained at the 1.0 and 5.0 ppm Cr⁶⁺ respectively for *Chlorella* sp., *Oscillatoria* sp., *Lyngbya* sp., and *S. dimorphus*. In contrary, only 14.57 and 7.32% inhibition was recorded for consortia when compared to the consortia control (Table 2). Therefore, enriching the consortia of these organisms with 1.0 ppm Cr⁶⁺ enriched water reservoirs could be a possible way to get the more photosynthetic yields. Beyond that, these

organisms would have very negligible contribution in reducing the Cr⁶⁺ toxicity. At the higher concentrations, all the cultures had lower cell counts and decreased *chl-a* fluorescence. It might be a reason for the increased perennation on the surface of culture broth in stressed environments. Rate of perennation was increased with increasing in the level of Cr⁶⁺ stress. It indicates that they were reached their succession stages much earlier than the respective controls. Micrographs and Fv/Fm ratios of various cultures collectively showed that *Oscillatoria* sp. was highly affected whilst *Lyngbya* was dominating species within consortia. Since, both at the 1.0 and 5.0 ppm Cr⁶⁺, the percentages inhibition in *chl-fluorescence* was found to be lowest in the case of *Lyngbya* followed by *Chlorella*, *Scenedesmus* and *Oscillatoria* respectively. Therefore, *Lyngbya* was photo-synthetically most active in Cr⁶⁺ rich environment. For the natural habitats affected with chromium, similar trends can be expected with some deviations, since in natural water reservoirs there are several other independent factors or variables which affect the structure and function of natural assemblages of green algae and cyanobacteria. These variables may be of biological, physical or chemical nature. Therefore, above 1.0 ppm Cr⁶⁺, these microalgae lose their original features. The measurement of fast kinetics and multiphase (OJIP) transitions showed that after an adaptation of algae in dark, the fluorescence yield from the transition 'O' (F₀) is the emission of light energy and excitement before going to the reaction center by chlorophyll antenna of PSII (Iara and Hendrik 2009). At this level, the reaction centers are opened and the QA is completely oxidized, because the energy is not sufficient to induce the separation of charge. Normally, the yield of F₀ is constant; however, the performance change of F₀ is an alteration of pigment-protein complexes associated with PSII. Decreased surfaces to volume ratios of stressed cells are directly related to the size of intracellular contents. At lower doses of 0.5 and

Table 2 Percentages inhibition of the maximum photosynthetic yields (Fv/Fm ratios) of four organisms and consortia at 1.0 and 5.0 ppm Cr⁶⁺ with respect to controls

Organism	Control (%)	1.0 ppm (%)	5.0 ppm (%)
<i>S. dimorphus</i>	1.0	4.583	44.87
<i>Chlorella</i> sp.	1.0	2.802	42.51
<i>Lyngbya</i> sp.	1.0	10.88	15.44
<i>Oscillatoria</i> sp.	1.0	0.813	70.49
Consortia	1.0	7.32	14.57

1.0 ppm Cr^{6+} , due to increased cell surfaces the antenna complex surface may increased that can be a major cause for slightly increased or unaltered rate of transitions of electrons.

Scanning electron microscopy (SEM)

SEM generated micrographs of the control, 1.0 and 5.0 ppm treated consortia are shown in Fig. 4a–c respectively. From micrographs, it is evident that with increased doses of Cr^{6+} up to 1.0 and 5.0 ppm, morphology of constituent cells of consortia gets highly distorted. It clarifies that how the increment in the concentrations of Cr^{6+} can create morphological changes. It was reported that in the presence of excess Cr^{6+} , due to hydrolysis of polysaccharides more number of hydroxyl ions get accumulated on the surface of consortia and decreases overall effective sizes of cells (Shukla et al. 2012; Zhang et al. 2016). Possibly, negative charges were distributed throughout the cell surface of cyanobacteria and algae which provided an enormous surface area for binding of the positively charged chromium ions.

Bio-sorption of chromium by various monocultures and their consortia

The bio-sorption of Cr^{6+} was performed by preparing three types of cells; live cells, heat killed cells, and pre-treated cells. 0.1% SDS pre-treated consortia cells showed maximal metal uptake of 84.5% at 1.0 ppm. In contrary, 0.1 N NaOH pre-treated consortia cells could uptake 83.6% (Fig. 2). In general, metal uptake by pre-treated cells was found to be more than live cells or heat-killed cells. Sodium hydroxide and sodium dodecyl sulphate are anionic detergents having negatively charged $-\text{OH}$ groups. Therefore, when these detergents come in contact with positively charged cations such as Cr^{6+} , Cd, Pb etc., they

get bind to them. So, it may be a cause behind the optimum adsorption of positively charged Cr^{6+} cations to NaOH and SDS pre-treated cyanobacterial and green algae cells (Fisher et al. 1984). In a study by Rabsch and Elbracher (1980), they observed that heat-killed cells of *Coscinodiscus granii* could accumulate three times more zinc than its live cells. The uptake of zinc metal by microalgal consortia is comparatively higher (Ahuja et al. 1999). Some studies showed that in immobilized monocultures or consortia on alginate have greater metal uptake potential (Brun et al. 1998; Gloaguen et al. 1996; Ye et al. 1997).

Laser induced breakdown spectroscopy (LIBS)

To prove the presence of Cr^{6+} in microalgal consortia, different treated samples were subjected for LIBS analyses. LIBS is an emerging and most suitable laboratory analytical technique, being routinely used for wide range elemental analysis of solid, liquid, gaseous samples. It is a rapid and non-distractive technique which requires no to minimal sample preparation. With their qualitative and quantitative analysis, LIBS can simultaneously analyze the presence of all the elements which are present in the sample. Analytical results of LIBS of algal consortia favor for the presence of elements C, Mg, Ca, Na, O, N, H, K and Cr (Fig. 5a–d) respectively for control and with Cr^{6+} treated consortia. Peaks obtained during studies showed the spectral signature for the elements C, Mg, Ca, Na, O, N, H, K and Cr (Kumar et al. 2014) as compared with available NIST database list (Table 1). Published literature showed that CO_2 assimilation and photosynthesis of algal consortia is highly decreases due to the toxic effects of Cr^{6+} . In addition, PSII reaction center is highly sensitive to any damage caused by heavy metal stress which alternatively affects the photosynthetic carboxylation reactions, photosystem II (PS II) electron transport system (ETS) and oxygen-evolving complex. Therefore, experimental results

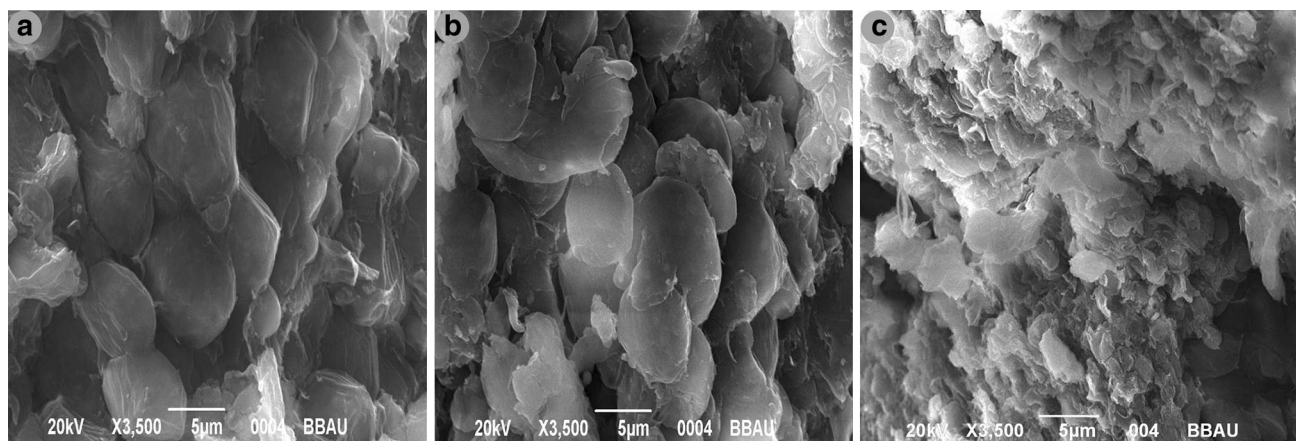


Fig. 4 Scanning electron microscopy of **a** consortia control, **b** consortia 1.0 ppm, **c** consortia 5.0 ppm respectively

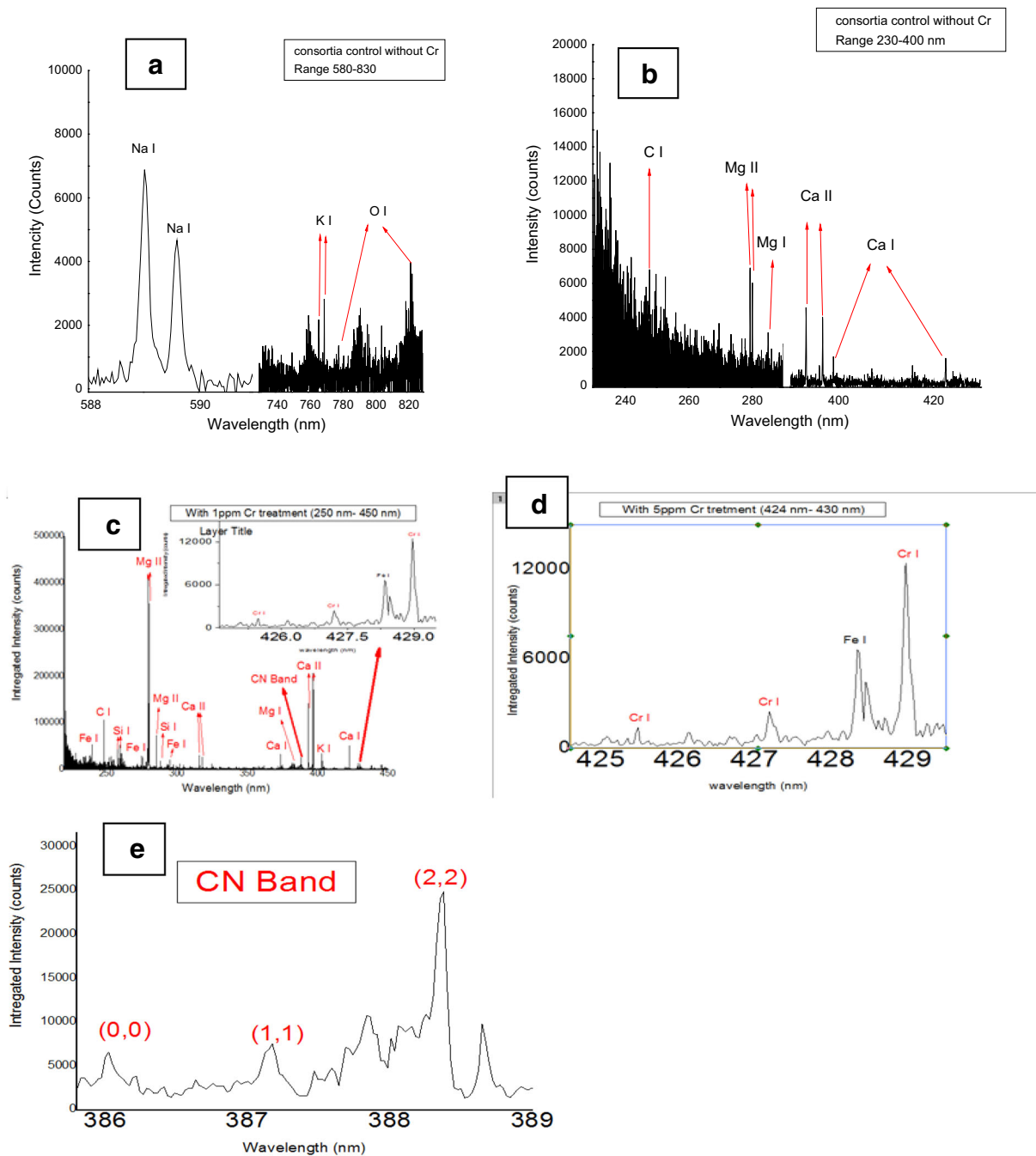


Fig. 5 Analysis of hexavalent chromium in consortia by laser induced breakdown spectroscopy (LIBS)

of the present study clearly demonstrate that Cr^{6+} affected the cell morphology and physiological parameters. Presence of spectral peak of Cr^{6+} was obtained at 357.8, 359.5, and 360.4 nm in the LIBS spectra for the stressed cells of consortia. LIBS spectra are shown in Fig. 5a–e for control and treated consortia. Presence of peak in treated cells clearly indicates that consortia cells had adsorbed the Cr^{6+} . In contrary, complete absence of spectral peak in control consortia clarify the absence of chromium in the sample.

Atomic absorption spectroscopy (AAS)

Micro-algal consortia can perform functions which are difficult or even impossible for the individual species (Brenner et al. 2008; Perales-Vela et al. 2006). Living together may provide robustness to the environmental fluctuations, ability of cumulative metabolite production, and resistance to invasion by other species. A number of proof-of-principle studies on the consortia of cyanobacteria/microalgae–bacteria for pollutant can be retrieved from

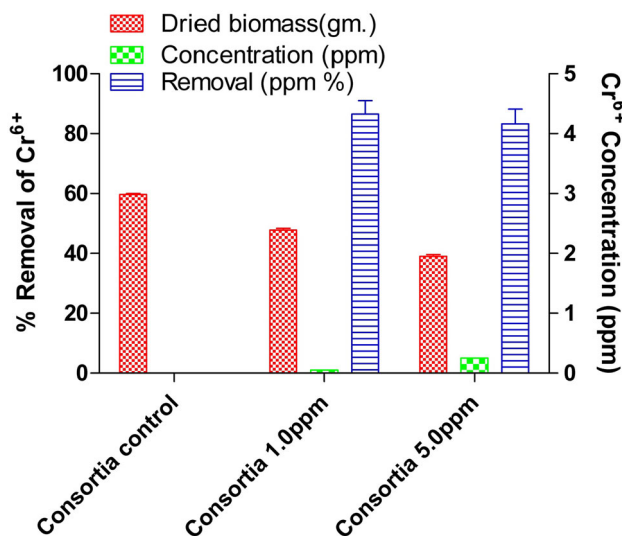


Fig. 6 Quantification of hexavalent Cr⁶⁺ by AAS

the published literature. The results of this study indicated that the biomass of consortia could be developed as a suitable technology for the efficient removal of chromium from the waste water. Present results showed that highest amounts of 92.0 and 84.0% of Cr⁶⁺ metal could be adsorbed at 1.0 and 5.0 ppm respectively by the consortia of cyanobacteria and green algae (Fig. 6).

Conclusions

Increasing concentration of heavy metals in different water reservoirs, especially drinking water has generated the serious issues of bio-accumulation at higher tropic levels. Chromium in excess creates a number of serious health issues; therefore, regular efforts to remove it from water are underway. Different approaches of microbial environmental biology are being tried to reach the target. In this aspect cyanobacteria and green algae are identified as potential agents. In natural habitats these organisms are in different assemblages. Consortia based mitigation with increased diversity may lead to enhanced water purification (Zimmerman and Cardinale 2014; Chekroun and Baghour 2013; Chakraborty et al. 2011; Dwivedi et al. 2010; Cervantes et al. 2001) through mitigating the increased chromium levels.

Biomass yields in control community was highest than all the monocultures, however, in the presence of Cr⁶⁺ perturbation at different regimes, ranging from 1.0 to 5.0 ppm, overall growth was inhibited. As Cr⁶⁺ affects the photosynthetic apparatus of algae, at its lowest dose of 5.0 ppm there was an increase in the fluorescence 'F₀'. Consequently, at slightly higher dose of 1.0 ppm Cr⁶⁺ due to altered light harvesting antennae complex of PSII, we

noticed a decrease in the fluorescence. It shows the degradation of electron transfer system at higher doses. Therefore, it could be said that as a consequence of damage to PSII the Fv/Fm ratio was decreased with increased concentration of Cr⁶⁺. Conclusively the use of chlorophyll fluorescence as an indicator of toxicity of chromium is a simple and fast method, it gives us an indication of the photosynthetic apparatus of the algae, therefore, we could use this as bioassays to detect environmental stress. Simultaneously, SEM proved the presence of Cr⁶⁺ due to altered morphological appearances. In the same direction, LIBS analysis of control consortia revealed the presence of elements C, Mg, Ca, Na, O, N, H and K, however, consortia with Cr⁶⁺ contained the spectral signature of elements C, Mg, Ca, Na, O, N, H, K and Cr. In fact, the pre-treated cells have shown best results to the remove chromium at various concentrations. Therefore, these studies could be a possible tool to reach the physiological consequences in consortia under Cr⁶⁺ stress.

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Author's contributions Shanthi Sundaram and Adinath designed the experiments, Adinath performed all the experiments. P K Tiwari and AK Rai helped Adinath in elemental analysis of chromium. Adi Nath and Shanthi Sundaram drafted the manuscript, Shanthi Sundaram reviewed the manuscript.

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