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Characterization of native microalgal strains for their chromium bioaccumulation potential: Phytoplankton response in polluted habitats

S. Dwivedi, S. Srivastava [1], S. Mishra, A. Kumar, R.D. Tripathi *, U.N. Rai, R. Dave, P. Tripathi, D. Charkrabarty, P.K. Trivedi

Ecotoxicology and Bioremediation Group, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, U.P., India

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ABSTRACT

Due to its various uses, Cr contamination has become widespread in a diverse array of environments. The present study was carried out during 2007–2008 to investigate the accumulation potential of metals (Cr, Cu, Fe, Mn, Ni and Zn) and metalloid (As) by green (GA) and blue green (BGA) microalgae growing naturally in selected Cr-contaminated sites in districts Unnao and Kanpur (Uttar Pradesh, India). This investigation is a preliminary work to identify suitable native microalgae for biomonitoring and phytoremediation purposes. A total of 22 GA and 11 BGA were encountered in three seasons (summer, rainy and winter). Among these, the accumulation potential was evaluated in high biomass producing strains of BGA (three) and GA (nine). The maximum accumulation of Cr was shown by *Phormedium bohneri* (8550 $\mu g\,g^{-1}$ dw) followed by *Oscillatoria tenuis* (7354 $\mu g\,g^{-1}$ dw), *Chlamydomonas angulosa* (5325 $\mu g\,g^{-1}$ dw), *Ulothrix tenuissima* (4564 $\mu g\,g^{-1}$ dw), and *Oscillatoria nigra* (1862 $\mu g\,g^{-1}$ dw); all of which demonstrated a transfer factor of >10% for Cr. The results also indicate that the phytoplankton diversity was modified by Cr pollution. BGA represented the dominant community where Cr concentration was higher (11.84 and 2.27 $mg\,L^{-1}$) ($r = 0.695$), whereas GA showed negative correlation with respect to Cr concentration ($r = -0.567$). In conclusion, different algal species were able to grow in Cr-contaminated sites and to accumulate significant amounts of Cr with a high transfer factor.

1. Introduction

The presence of various metals in aqueous streams, arising from the discharge of untreated metal containing effluents into water bodies, is one of the most important environmental issues [1] as it poses human health risks and causes harmful effect to living organisms [2]. Chromium (Cr) is the seventh most abundant element on Earth [3]. In last few decades, the amount of Cr in aquatic and terrestrial ecosystems has increased as a result of different human activities such as mining, chrome plating, leather tanning, wood preservation and nuclear power plants [4,5]. Effluents from industries, like paper mill and leather, contain Cr at concentrations ranging from 10 to 80 $mg\,L^{-1}$ [6].

Chromium exists in several oxidation states (−2 to +6), however the most stable and common forms are hexavalent Cr(VI) and trivalent Cr(III) [7]. Both Cr species produce serious damages to plant tissues and organs, albeit at different concentrations [8]. The toxic action of Cr(VI) is due to the negatively charged hexavalent chromate ions, which easily cross cellular membranes by means of sulfate ion channels, and then undergo immediate reduction reactions leading to the formation of various reactive intermediates [9,10]. These intermediates are themselves harmful to cellular organelles, proteins and nucleic acids [6]. Cr interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced root growth and phytomass, chlorosis, photosynthetic impairment, stunting and finally plant death [11–13]. Due to its high oxidation power, Cr(VI) can inhibit uncoupled electron transport in plant and animal mitochondria and induce generation of superoxide radicals [14]. In plants, Cr(III) has been reported to affect the net photosynthesis [15], lower the biomass and decrease the concentrations of most of the nutrients, such as Cu, Fe, K, Mg, Mn, and P.

The use of plants, for either the removal of organic or inorganic pollutants, such as hydrocarbons of petroleum [16], toxic by-products of the industry [17] and heavy metals [18–20] from the environment or for rendering them to harmless species, is defined as 'phytoremediation' [21]. This solar driven technology is low-cost when compared to other available physical and chemical remediation methods [22]. Phytoremediation, therefore, is a possible strategy that may be used for decontamination of Cr-contaminated

* Corresponding author. Tel.: +91 522 2205831 35x222; fax: +91 522 2205836 39.
E-mail addresses: tripathi_rd@rediffmail.com, tripathird@gmail.com (R.D. Tripathi).
[1] Present address: Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400085, India.

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sites. Phytoplankton are able to accumulate significant amount of metals from the contaminated surface water [23], have rapid growth, wide habit and habitat range, and thus may be used as bioindicator [24,25] and phytoremediator of heavy metals in aquatic bodies [26]. A number of microalgae viz. *Chlorella* sp., *Ankistrodesmus* sp. and *Eumosphaera* sp. have been used for heavy metal removal from the aqueous medium [27]. Recent reports demonstrate that some microalgal species, such as *Oscillatoria* spp., *Phormedium* spp., *Spirogyra* spp. etc. are able to accumulate significant amount of metals from the contaminated environment [28,29].

According to Yoon et al. [30], species growing naturally on a contaminated site respond better under stress conditions than plants introduced from other areas in terms of survival, growth and reproduction. Besides, the species obtained from such a contaminated site might possess a greater potential for accumulating high concentration of metals and metalloid [31]. Therefore, it is worthwhile to evaluate the metals and metalloid accumulation potential of native phytoplankton growing in contaminated sites to use them for phytoremediation purposes in future. The present study was, thus, undertaken to screen blue green and green microalgae growing naturally in different seasons in Cr-contaminated areas of districts Unnao and Kanpur (Uttar Pradesh, India). The phytoplankton samples were collected from the polluted areas so as to find Cr tolerant and accumulating species. The study included the analysis of physico-chemical properties of contaminated water and its co-relation with phytoplankton density and metals and metalloid level in biomass of various microalgal species collected from the selected sites. The accumulation of some other metals viz., Cu, Fe, Mn, Ni and Zn and metalloid i.e. As, by the microalgal biomass was also investigated.

2. Material and methods

2.1. Sampling sites

The study was conducted for one year during 2007–2008. A total of five (A–E) sites i.e. combined effluent treatment plant (CETP) (A), pond near police station (B), pond near river Ganga (C), Gagan Khera (D) and pond at Shuklaganj (E), were selected in the districts Unnao (26°33′00″N 80°29′00″E) and Kanpur (26°27′39″N 80°20′00″E) (Uttar Pradesh, India). The site selection was based on the level of Cr pollution in the water bodies as well as on our previous study on monitoring and remedial potential of aquatic plants [32,33] carried out in this area. All water bodies were within the 10 km range of tannery complex and were receiving effluent from various point and non-point sources.

2.2. Collection of microalgae and characterization of microalgal community

The phytoplankton (10–105 μm) were collected from the above sites periodically (on four month intervals) using a phytoplankton net (5–28 μm mesh size; Aquatic Research Instruments, Wellington place) and were kept in 250 mL plastic bottles. The samples were examined for the identification of microalgal forms after bringing them to laboratory in living condition. For blue green algal (BGA) strains, staining was done with 1% aqueous methylene blue (E – Merck) solution to bring out mucilaginous envelope, while green algae (GA) were stained with iodine solution (E – Merck). Microalgal samples were also fixed in 4% formaldehyde (E – Merck) solution for proper identification in the laboratory. Identification of various BGA strains collected from Cr-contaminated environment was done by using the taxonomic keys given in Desikachary [34], while different forms of GA were identified with the help of Prescott [35], Philipose [36] and Prasad and Mishra [37]. The frequency of microalgal species was determined by the heamocytometer (Micrometer scale, Neudauer Feinoptik Bad Bankendurd, Japan) based on the percent occurrence of an individual species. It was calculated by counting total number of all species and that of each individual species by following formula:

$$\text{Frequency} = \frac{\text{Total number of individual species}}{\text{Total number of all species}} \times 100$$

After counting, they were classified as dominant (>50%), common (20–50%) and rare (<20%) based on their frequency of occurrence.

2.3. Characterization of physico-chemical parameters of the selected sites

The tannery effluent and contaminated surface water from selected ponds were collected in 5 L acid washed plastic containers for estimation of various physico-chemical parameters. A few physico-chemical parameters like colour, odour, pH, temperature, electrical conductivity (EC) and total dissolved solid (TDS) of the samples were recorded on the spot by using portable water analysis kit (Orion 5 star, Thermo, Electron Corporation). Other physico-chemical parameters like biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were estimated in laboratory by following the procedures given in APHA [38].

2.4. Metal and metalloid concentration of the water and phytoplankton

The metals and metalloid accumulation potential was analyzed in a limited number of phytoplankton strains. Selection was based on their ability to produce high biomass and to be abundantly growing. The species, considered as high biomass producing strains were selected on the basis of bloom forming [39] characteristics. They included mostly the filamentous forms, which could be easily harvested. The two unicellular forms e.g. *Chlamydomonas angulosa* and *Chlorococcum vitiosum* were also forming bloom dominantly at sites A and D, respectively, and so could be easily screened and harvested. Microalgal species were collected in bulk through mesh (5–28 μm mesh size) and kept in polythene carry bags (2 kg capacity). After bringing to laboratory, all samples were subjected to repeated washing under running tap water followed by double-distilled water and Milli Q water (three times at each step) so as to remove any non-algal particulates and adsorbed metals and metalloid ions. Further, before drying, the samples were observed under microscope for removing any contamination of non-algal particulates or mixture of other microalgae. Then, samples (1 g) were oven dried at 80 °C for 2 d to constant weight. The dried samples were digested with HNO_3:$HClO_4$ (3:1, v/v) at 80 °C and then diluted with Milli Q water. The digested samples were analyzed for different metals viz. Cr, Cu, Fe, Mn, Ni, and Zn by measuring the absorbance of samples on an atomic absorption spectrophotometer (GBC Avanta Σ). For quantification of total As, microalgal tissue was digested by following the method of Bleeker et al. [40] and after dilution in Milli Q water, the As concentrations were determined on an atomic absorption spectrophotometer (GBC Avanta Σ, Australia) coupled with a GBC hydride generation system (HG 900). The total metals and metalloid concentrations in effluent and surface water were detected after filtration (using Whatman filter paper no. 41, 20–25 μm) for removal of phytoplankton and other organisms. The digestion and analysis of the samples were done as described above for microalgal samples. Metals and metalloid transfer factor in algal species was evaluated following Huang et al. [41]. It was computed by the formula: transfer factor (TF_{total}) = metal content in microal-

Table 1
Physico-chemical characteristics and, metal and metalloid contents of the selected water bodies and effluent collected from industrial complex at Unnao. Values are means of triplicates ± SD.

Parameters	CETP, Unnao (A)	Pond near police station (B)	Pond near River Ganga (C)	Gagan Khera (D)	Shuklaganj (E)
Colour	Blackish brown	Light brown	Light brown	Muddy colour	Muddy colour
Odour	Foul smell	Smell less	Smell less	Smell less	Smell less
Temperature (°C)	24.66 ± 0.67	26.82 ± 8.71	23.14 ± 0.42	23.68 ± 0.32	26.44 ± 0.52
pH	8.20 ± 0.18	7.90 ± 0.16	7.90 ± 0.18	7.50 ± 0.15	7.8 ± 0.18
EC (μS/cm)	30.68 ± 1.82	23.66 ± 2.08	18.42 ± 1.60	12.22 ± 0.69	24.02 ± 0.22
TDS	4560 ± 79.61	1886 ± 94.66	2241 ± 76.08	1282 ± 107.02	788 ± 44.21
BOD	1188 ± 64.12	326 ± 27.22	422 ± 18.62	602 ± 17.41	521 ± 18.09
COD	15712 ± 112.61	1276 ± 48.62	872 ± 27.49	721 ± 26.22	842 ± 21.62
Cr	11.84 ± 1.26	0.19 ± 0.02	0.74 ± 0.07	0.86 ± 0.008	2.27 ± 0.09
Cu	0.25 ± 0.03	3.49 ± 0.92	9.00 ± 0.88	3.39 ± 0.71	14.77 ± 1.87
Fe	141 ± 8.42	500 ± 23.69	79.00 ± 7.62	104 ± 7.45	487 ± 20.48
Mn	2.90 ± 0.06	101 ± 6.08	199 ± 16.48	207 ± 16.99	277 ± 11.17
Ni	0.92 ± 0.03	4.84 ± 0.89	3.95 ± 0.73	7.70 ± 1.07	7.10 ± 0.82
Zn	5.80 ± 0.77	39.30 ± 1.07	56.10 ± 4.33	54.42 ± 4.21	23.41 ± 2.42
As	0.09 ± 0.002	0.06 ± 0.003	0.02 ± 0.001	0.04 ± 0.008	0.02 ± 0.004

CETP: combined effluent treatment plant; all values are in mg L^{-1} except for those otherwise mentioned.

gal strains (μg g^{-1} dw)/ambient metal concentration in pond water (μg L^{-1}).

2.5. Analytical quality control

The standard reference materials of Cr, Cu (EPA quality control samples; Lot TMA 989), Fe, Zn (BND 1101.02; provided by the National Physical Laboratory, New Delhi, India), Mn, Ni and As (E – Merck, Germany) were used for the calibration and quality assurance. Analytical data quality of the metals was ensured through repeated analysis (n = 6) of standard reference samples and the results were found to be within ±2.11% to ±2.98% of certified values. The mean recovery was about 96–99% for different metals. The blanks were run in triplicate to check the precision of the method with each set of samples. The detection limits for the metals, Cr, Cu, Fe, Mn, Ni and Zn were 0.05, 0.001, 0.02, 0.02, 0.02 and 0.005 mg L^{-1}, respectively, whereas for the metalloid As it was 0.001 mg L^{-1}.

2.6. Statistical analysis

The relationship among various physico-chemical properties of the water and the presence of specific algal strains in different seasons was tested using a linear correlation coefficient. All determinations were carried out in three replicates in each case. To confirm the validity of the data an analysis of variance (ANOVA < 0.01) was performed and significant differences in metal accumulation in various algal species were tested by Duncan's multiple range test (DMRT < 0.05) following Gomez and Gomez [42].

3. Results and discussion

3.1. Water quality

The physico-chemical properties of effluent and surface water varied considerably from one water body to the other. The effluent collected from CETP, Unnao was significantly contaminated as it was highly alkaline (pH 8.2) and had high values of total dissolved solids (TDS; 4560 mg L^{-1}), biological oxygen demand (BOD; 1188 mg L^{-1}) and chemical oxygen demand (COD; 15712 mg L^{-1}) as compared to the effluents collected from other sites (Table 1).

In addition, it also contained high concentration of metals, such as up to 11.84 mg L^{-1} Cr (Table 1). Cr concentration in the water samples collected from the selected sites was also higher (Table 1) than the maximum permissible limit of Cr (0.01 mg L^{-1}) for effluent discharges [43]. The Fe concentration in all samples was high; with the maximum being in the pond near police station (500 mg L^{-1}) followed by Shuklaganj (487 mg L^{-1}). In addition, the concentration of Mn and Zn were considerably high in all water bodies, except the effluent of CETP, Unnao, where their concentration was 2.90 and 5.80 mg L^{-1}, respectively.

3.2. Phytoplankton community structure

A total of thirty three microalgal species belonging to 24 genera were found growing in Cr-contaminated water bodies; out of which 11 species belonged to BGA and 22 to GA (Tables 2 and 3). A contrasting algal diversity was observed among BGA and GA with respect to their habitat, occurrence, seasonal variation and metal tolerance (Tables 2 and 3). During the study, it was observed that the maximum diversity of GA occurred where Cr concentration was low (sites: B and C), though, the frequency of GA in these sites was rare (<20%) except *Oedogonium capilliforme* and *Ulothrix tenuissima* (20-50%). It was interesting to note that out of 22 GA species, only *C. angulosa* was growing dominantly (>50%) at the highly Cr-contaminated site (A) during rainy season. Among other GA members, *Chlorococcum humicolo* was growing rarely (<20%) at site B while *C. vitiosum* was growing dominantly (>50%) at site D during summer season. The species diversity of GA at sites B and C (Cr 0.185 and 0.735 mg L^{-1}, respectively) and their absence at other sites especially A and E (Cr level 11.84 and 2.27 mg L^{-1}, respectively) showed that these taxa were sensitive to high Cr concentration. Earlier, Dwivedi et al. [28] found a direct correlation between dominance of phytoplankton and metal contamination

Table 2
Distribution and dominance of BGA in chromium contaminated environment at Unnao.

S. no.	Genus/species	Site	Frequency	Seasons
1	*Aphanocapsa grevilli*	A	R	S
2	*Oscillatoria angustissima*	A, E	R	R
3	*O. nigra*	C	D	R, W
4	*O. tenuis*	A	D	S, W
5	*O. terebriformis*	B	R	S, R
6	*O. curviceps* var. *anjusta*	B, E	C	S
7	*O. laete-virens*	E	R	W
8	*Phormedium bohneri*	A	C	S, R
9	*Spirulina subsalsa*	B	R	W
10	*Arthrospira tenuis*	A	R	R, W
11	*Anabaena ambigua*	E	R	S

BGA: blue green algae; D: dominant (>50%); C: common (>20–50%); R: rare (<20%); S: summer; R: rainy; W: winter. A: combined effluent treatment plant (CETP), Unnao; B: pond near police station; C: pond near River Ganga; D: Gagan Khera, Unnao; E: pond at Shuklaganj, Kanpur.

Table 3
Distribution and dominance of GA in chromium contaminated environment at Unnao.

S. no.	Genus/Species	Site	Frequency	Season
1	*Gonion compactum*	B	R	R
2	*Chlamydomonas angulosa*	A	D	R
3	*Chlorococcum humicolo*	B	R	S
4	*C. vitiosum*	D	D	S
5	*Hydrodictyon reticulatum*	C	D	S
6	*Scenedesmus dimorphus*	B, C	R	W
7	*Scenedesmus* sp.	B, C	R	W
8	*Coelostrum cambricum* var. *intermedium*	B	R	S
9	*Oocystis naegeli*	B	R	W, S
10	*Rhizoclonium hieroglaphicum*	C	C	S, W
11	*Oedogonium* sp. I	B	C	S
12	*Ulothrix tenuissima*	B	C	W
13	*Spirogyra adenata*	B, E	D	S, W, R
14	*Spirogyra* sp. I	E, C	D	S
15	*Closterium acerosum* var. *elongatum*	B, E	R	R, S
16	*Oedogonium* sp. II	E	C	S
17	*Pedistrum tetras var. excisum*	B	C	S
18	*Tetraedron muticum*	B	R	W
19	*Nephro cytium agardhium*	D	R	S
20	*Ankistrodesmus spiralis*	E	C	S
21	*Closterium rectimargenatum*	E	R	W
22	*Cosmarium circulari*	B	R	S, W

BGA: blue green algae; D: dominant (>50%); C: common (>20–50%); R: rare (<20%); S: summer; R: rainy; W: winter. A: combined effluent treatment plant (CETP), Unnao; B: pond near police station; C: pond near River Ganga; D: Gagan Khera, Unnao; E: pond at Shuklaganj, Kanpur.

in river Ganga surface water polluted through fly-ash leaching. But, high abundance of *C. angulosa* at site A showed that it has a remarkable tolerance to Cr present in the effluent. Similarly, Kalin et al. [44] have reported that *Chlamydomonas* spp. was apparently tolerant to a wide range of physical and chemical conditions. The frequency of various BGA species with seasonal variation is shown in Table 2. The strain *Oscillatoria tenuis* was growing dominantly (>50%) at site A, while *Anabaena ambigua* was growing rarely (<20%) at site E. *Oscillatoria laetevirens* and *Spirulina subsalsa*, other BGA species, occurred rarely (<20%) in winter season, whereas, *Oscillatoria angustissima* occurred rarely in rainy season at sites A and E. Out of 11 BGA species, only *O. tenuis* occurred in both summer and winter seasons, while *Phormedium bohneri* and *Arthrospira tenuis* were encountered during rainy and winter seasons. Interestingly, out of 11 cyanophycean species, 10 belonged to family Oscillatoriaceae and most of them were encountered at sites A and E, except *Oscillatoria terebriformis* and *Oscillatoria nigra*, which occurred at sites B and C, respectively. Similarly, in an earlier study, Dwivedi et al. [28] observed the dominance of non-heterocystous species of BGA at highly contaminated site of river Ganga, polluted by metals through fly-ash leaching. In addition, waste of various industries causing eutrophication in flowing waters has been found to result in an increased population of *Oscillatoria* sp. and *Phormedium* sp. [45].

The percent seasonal distribution of phytoplankton community in different seasons and sites is presented in Fig. 1. The most diversified community of phytoplankton was found in the pond near police station, Unnao (B) during summer season, dominated by *Spirogyra adenata*. In general, the data collected from different sites showed that member of Chlorophyceae dominated over cyanophycean forms where Cr concentration was low. By contrast, sites A and E, having high Cr concentrations, showed the maximum percentage of cyanophycean forms during summer and winter seasons. The phytoplankton diversity and its correlation with water quality in different water bodies has been reported earlier by Dwivedi et al. [46] and Rai et al. [47]. Similarly, heavy metal pollution and phytoplankton community structure and their periodicity in the River Moosi has been reported by Kumari et al. [24] who also emphasized the use of certain plankton species as an indicator of metal pollution. Algal communities have also been found as an indicator of trophic status of water bodies [25].

Correlation analysis showed interesting differences among BGA and GA forms with respect to their preference for colonization of an area (Table 4). Both temperature and Fe content showed positive correlation with BGA and GA diversity. BGA showed good positive correlation with pH, EC, TDS, BOD, COD, Cr, and As while GA showed either negative or non-significant positive correlation with these parameters. This indicates that BGA forms were more tolerant to contaminated habitat with high pH, EC, TDS, BOD and COD values as compared to GA forms, which were colonized mainly in relatively non-contaminated habitats. The most important was to find good positive correlation of BGA diversity with both Cr and As indicating the Cr and As tolerance abilities of BGA forms. However, they showed a negative correlation with Cu, Zn, Mn and Ni while GA forms showed a non-significant positive correlation with these metals.

3.3. Metal and metalloid accumulation

Various studies on living and non-living biomass have been conducted for their heavy metal uptake/adsorption capacities with the view of utilization for remediation purposes [31]. In the present study, among the collected algal strains, a total of 12 were bloom (high biomass) forming strains [39] and were also abundantly growing at the contaminated sites. Out of 12, three belonged to BGA viz. *O. tenuis*, *O. nigra* and *P. bohneri*, while remaining nine represented the GA, namely *C. angulosa*, *C. vitiosum*, *Hydrodictyon reticulatum*, *Rhizoclonium hieroglaphicum*, *U. tenuissima*, *O. capilliforme*, *O. epiphyticum*, *S. adenata* and *Spirogyra crassa*. The metals (Cr, Cu, Fe, Mn, Ni) and metalloid (As) accumulation potential of various BGA and GA strains was found to vary from species to species and was also dependent on the season; the accumulation followed the pattern: summer > winter > rainy. The data presented in Tables 5 and 6 show the mean values of accumulation of different metals by various strains in various seasons. The maximum accumulation of Cr was shown by *P. bohneri* (8550 $\mu g\, g^{-1}$ dw) followed by *O. tenuis* (7354 $\mu g\, g^{-1}$ dw), *C. angulosa* (5325 $\mu g\, g^{-1}$ dw), *U. tenuissima* (4564 $\mu g\, g^{-1}$ dw) and *O. nigra* (1862 $\mu g\, g^{-1}$ dw); all of which demonstrated a transfer factor of >10% for Cr. The least Cr accumulation was shown by *R. hieroglaphicum* (10.61 $\mu g\, g^{-1}$). The values given in the brackets in Tables 5 and 6 denote the transfer factor values of individual algal species. In general, the transfer factor values of Cr were found to be higher in BGA than in GA forms. The *P. bohneri* dominated at site A and showed the maximum transfer factor values (72%) for Cr. Among GA, *C. angulosa* from the same pond dominated during rainy season and showed the maximum transfer factor of 44%. The Fe was also preferentially accumulated by all microalgal species. The maximum amount of Fe was found to be accumulated by *R. hieroglaphicum* (3583 $\mu g\, g^{-1}$ dw) followed by *H. reticulatum* (2707 $\mu g\, g^{-1}$ dw), *S. adenata* (2708 $\mu g\, g^{-1}$ dw) and *U. tenuissima* (2080 $\mu g\, g^{-1}$ dw); all of them demonstrating a transfer factor of >1% for Fe. Arsenic accumulation in all microalgal species were negligible, with the maximum being 3.71 $\mu g\, g^{-1}$ dw by *H. reticulatum* among GA and 3.44 $\mu g\, g^{-1}$ dw among BGA. This seems to be primarily related to low concentrations of As in various effluents as compared to the concentration of all other metals. Further, As speciation plays an important role in its uptake by plants [48] and at such low concentrations, its uptake may be competitively inhibited by ions like phosphate [49] and borate [50]. Higher Cr accumulation and transfer factors demonstrated by BGA forms may be due to the presence of thin mucilage sheath over trichome and other multiple binding sites comprising of polysaccharides, proteins and lipids, which are

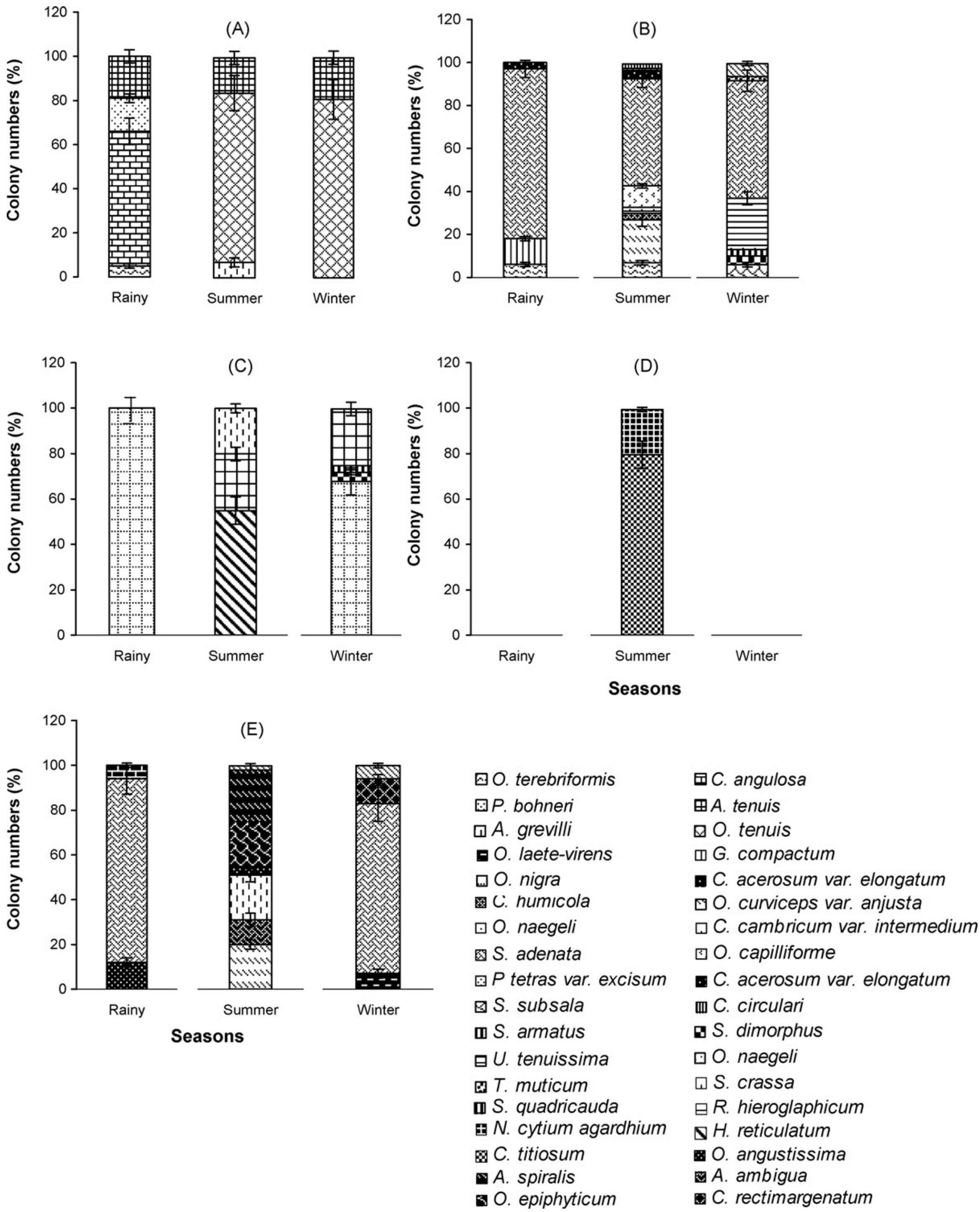

Fig. 1. Frequency of various algal forms collected from different chromium contaminated water bodies during different seasons of the year: (A) CETP, Unnao; (B) pond near police station; (C) pond near River Ganga; (D) Gagan Khera; (E) Shuklaganj.

Table 4
Correlation coefficient values between physico-chemical parameters, metals and metalloid concentration and microalgal forms from selected sites.

	Physico-chemical parameters/metals and metalloid												
	Temperature	pH	EC	TDS	BOD	COD	Cr	As	Cu	Zn	Mn	Fe	Ni
BGA	0.615	0.778*	0.969**	0.487	0.524	0.658	0.695	0.498	−0.011	−0.953**	−0.477	0.467	−0.545
GA	0.694	−0.004	0.079	−0.382	−0.728	−0.494	−0.567	−0.134	0.176	0.197	0.066	0.770	0.174

Tabular $r = 0.917$, $p < 0.01\%$ (denoted as **), $r = 0.729$, $p < 0.1\%$ (denoted as *).

Table 5
Metals and metalloid concentration in bloom (high biomass) producing green algae collected from Cr-contaminated environment. Values are means of triplicates ± SD. Different superscript letters denote significant difference ($p < 0.05$) between the algal species according to DMRT. Values in brackets denote transfer factor of the metals and metalloid.

S. no.	Genus/species	Site	Metals and metalloid accumulation ($\mu g\,g^{-1}$ dw) in GA species						
			Cr	Cu	Fe	Mn	Ni	Zn	As
1	*Chlamydomonas angulosa*	A	$5325^{c} \pm 146$ (44.0%)	$18.65^{de} \pm 1.81$ (7.6%)	$557^{fg} \pm 12.26$ (0.4%)	$79.38^{de} \pm 4.81$ (2.7%)	$18.44^{cde} \pm 2.07$ (2.0%)	$362^{b} \pm 6.18$ (6.2%)	$2.07^{a} \pm 0.42$ (2.3%)
2	*Chlorococcum vitiosum*	D	$210^{e} \pm 3.42$ (24.3%)	$16.31^{de} \pm 1.32$ (0.5%)	$2245^{c} \pm 46.80$ (2.2%)	$361^{ab} \pm 17.12$ (0.2%)	$19.07^{cde} \pm 2.20$ (0.2%)	$60.72^{ef} \pm 4.08$ (0.1%)	$2.28^{a} \pm 0.76$ (5.6%)
3	*Hydrodictyon reticulatum*	C	$10.97^{e} \pm 0.89$ (1.5%)	$18.76^{de} \pm 0.89$ (0.2%)	$2707^{b} \pm 41.02$ (3.4%)	$66.44^{de} \pm 7.31$ (0.3%)	$63.35^{a} \pm 3.42$ (1.6%)	$390^{b} \pm 18.61$ (0.7%)	$3.71^{a} \pm 0.77$ (18.5%)
4	*Rhizoclonium hieroglaphicum*	C	$10.61^{e} \pm 0.86$ (1.4%)	$18.44^{de} \pm 1.22$ (0.2%)	$3583^{a} \pm 40.89$ (4.5%)	$321^{ab} \pm 11.25$ (0.2%)	$12.08^{def} \pm 1.07$ (0.3%)	$77.29^{ef} \pm 6.68$ (0.1%)	BDL (-)
5	*Ulothrix tenuissima*	A	$4564^{c} \pm 217$ (38.5%)	$86.67^{b} \pm 2.68$ (35.3%)	$2080^{cd} \pm 154$ (1.5%)	$117^{cd} \pm 3.16$ (4.0%)	$23.08^{bcd} \pm 2.89$ (2.5%)	$319^{bc} \pm 14.79$ (5.5%)	$1.44^{a} \pm 0.23$ (1.6%)
6	*Oedogonium* sp. I	B	$161^{e} \pm 12.20$ (87.0%)	$182^{a} \pm 11.87$ (0.05%)	$1210^{e} \pm 55.41$ (0.2%)	$372^{a} \pm 24.42$ (0.4%)	$28.16^{bc} \pm 4.99$ (0.6%)	$781^{a} \pm 46.02$ (1.9%)	BDL (-)
7	*Oedogonium* sp. II	E	$229^{e} \pm 17.47$ (10.0%)	$55.48^{bc} \pm 6.86$ (0.1%)	$1861^{d} \pm 62.11$ (0.4%)	$167^{c} \pm 8.91$ (0.06%)	$10.07^{ef} \pm 1.98$ (0.1%)	$215^{cd} \pm 13.03$ (0.9%)	$1.02^{a} \pm 0.08$ (6.0%)
8	*Spirogyra adenata*	C	$44.82^{e} \pm 3.67$ (5.9%)	$18.19d^{e} \pm 1.05$ (0.2%)	$2708^{b} \pm 78.07$ (3.4%)	$309^{b} \pm 16.08$ (0.2%)	$32.82^{b} \pm 2.69$ (0.8%)	$28.44^{f} \pm 1.92$ (0.05%)	BDL (-)
9	*Spirogyra* sp. I	B	$12.44^{e} \pm 6.17$ (6.7%)	$5.74^{e} \pm 0.46$ (0.2%)	$779^{fg} \pm 22.21$ (0.9%)	$140^{c} \pm 9.06$ (0.1%)	$1.35^{f} \pm 0.08$ (0.02%)	$164^{de} \pm 6.07$ (4.1%)	$2.97^{a} \pm 0.06$ (4.9%)

A: combined effluent treatment plant (CETP), Unnao; B: pond near CETP; C: pond water near River Ganga; D: Gagan Khera, Unnao; E: pond at Shuklaganj, Unnao; BDL: below detection limit; GA: green algae.

Table 6
Metals and metalloid concentration in bloom (high biomass) producing blue green algae collected from Cr polluted environment. Values are means of triplicates ± SD. Different superscript letters denote significant difference ($p < 0.05$) between the algal species according to DMRT. Values in brackets denote transfer factor of the metals and metalloid.

S. no.	Genus/species	Site	Metals and metalloid accumulation ($\mu g\,g^{-1}$ dw) in BGA species						
			Cr	Cu	Fe	Mn	Ni	Zn	As
1	*Oscillatoria tenuis*	A	$7354^{b} \pm 268$ (62.1%)	$18.44^{de} \pm 0.58$ (7.5%)	$1192^{e} \pm 8.66$ (0.8%)	$21.73^{e} \pm 0.67$ (0.7%)	$7.22^{ef} \pm 0.84$ (0.8%)	$140^{de} \pm 4.22$ (2.4%)	$3.44^{a} \pm 0.86$ (3.8%)
2	*Oscillatoria nigra*	A	$1863^{d} \pm 107$ (15.7%)	$23.42^{de} \pm 3.61$ (9.5%)	$480^{g} \pm 18.61$ (0.3%)	$51.73^{e} \pm 5.66$ (1.8%)	$28.61^{bc} \pm 2.42$ (3.1%)	$59.91^{ef} \pm 5.66$ (1.0%)	$0.86^{a} \pm 0.02$ (0.9%)
3	*Phormedium bohneri*	A	$8550^{a} \pm 294$ (72.2%)	$44.64^{cd} \pm 6.31$ (18.2%)	$841^{f} \pm 18.22$ (0.6%)	$66.08^{de} \pm 4.80$ (2.2%)	$17.82^{cde} \pm 1.61$ (1.9%)	$227^{cd} \pm 11.78$ (3.9%)	$2.66^{a} \pm 0.77$ (2.9%)

A: combined effluent treatment plant (CETP), Unnao; BGA: blue green algae.

able to bind large amount of heavy metals [31]. The accumulation potential of *Oscillatoria* and *Phormedium* for various metals (Cu, Fe, Mn, Ni, Pb and Zn) has been demonstrated previously during analysis of algal samples collected from polluted regions of the River Ganga (Raebareli, India) [28] and common effluent treatment plant (Unnao, India) [29]. Green algae were also found to accumulate significant amount of metals. *U. tenuissima* accumulated appreciable amount of Cr (4564 $\mu g\,g^{-1}$ dw) followed by Fe (2080 $\mu g\,g^{-1}$ dw), Zn (318 $\mu g\,g^{-1}$ dw), Mn (117 $\mu g\,g^{-1}$ dw), Cu (87 $\mu g\,g^{-1}$ dw), Ni (23 $\mu g\,g^{-1}$ dw) and As (1.44 $\mu g\,g^{-1}$ dw). Similar metals and metalloid accumulation order (Cr > Fe > Zn > Mn > Cu > Ni > As) was shown by *C. angulosa*. Radwan et al. [51] concluded that the bioaccumulation of heavy metals in planktons depends on many factors, such as absorptive ability of individual species and season. Similarly, during the present study, the amount of metal accumulation differed from one species to other. The planktons collected from Sariyar dam reservoir in Turkey showed the metal accumulation in an order of Pb > Cr > Cd > Hg [52]. The significant accumulation of Cr and tolerance to other metals and metalloid present in the water bodies/effluent showed by various GA and BGA forms indicates that an interplay of various strategies like synthesis of metal binding ligands e.g. metallothioneins [53] or phytochelatins [54], an induction of cysteine synthesis [55] and secretion of organic compounds [56] might be playing role to cope with toxicity of the elements. Howe and Merchant [57] have reported sequestration of approximately 70% of cystosolic Cd^{2+} by metallothionein III in *Chlamydomonas reinhardtii* and showed that metallothionein III synthesis is related to the degree of pollution in an aquatic environment.

In conclusion, the microalgae growing in Cr-contaminated water bodies exhibited a great degree of diversity, which varied from one season to other and with the microalgal form, such as BGA or GA. Different species of both BGA and GA accumulated significant quantities of metals, such as Cr and Fe. BGA forms were found to be relatively more tolerant as compared with GA forms and were able to grow in water bodies contaminated with higher Cr concentrations. The maximum amount of Cr accumulation was also found in a BGA (*P. bohneri*). A positive correlation was found between the diversity of BGA and Cr content of the water, while GA diversity showed a negative correlation with Cr concentration. The results suggested towards a possible use of some of the high biomass producing and abundantly growing microalgal forms for developing an effective phytoremediation strategy for contaminated water bodies and selecting biomarkers for Cr toxicity assessment.

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