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Algal Biomass: An Economical Method for Removal of Chromium from Tannery Effluent

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Ever increasing concern about deteriorating environmental conditions has been the main driving force to monitor, assess and remediate pollutants from the ecosystem. In this context, importance of algae in indicating and managing metal pollution has been widely acknowledged (Burton 1986; Rai et al. 1992; Rai and Agrawal 1995). Some algal forms undergo certain changes and adapt themselves to the stress environment, while sensitive species exhibit toxic responses in a qualitative and quantitative manner and such organisms have been used for indicating the state of the environment (Whitton et al. 1982). Tolerant species grow luxuriantly and dominate the community, which is influenced by seasonal fluctuations in physico-chemical characteristics of the water (Ali et al. 1999). However, the number, type and distribution of organisms in an aquatic habitat are the basic components of the community structure and reflect the environmental conditions of the life support system (Chung 1979). This has been mainly due to their planktonic nature and ability to bioconcentrate pollutants from the ambient medium. The biosorbent potential of algal cells for toxic metals offers an effective and low cost alternative to conventional methods for decontamination of metal containing industrial effluents (Wilde and Benemann 1993; Radway et al. 2001). The removal of metal saturated algae from the medium is found to be an economical method for removing toxic metals from waste water resulting in high quality reusable effluent water (Filip et al. 1979) and algal biomass could be used for production of biogas and the composted biomass could be used as fertilizer after amendment with alkaline material to check mobility of metal present therein (Fang and Wong 1999).

Despite the large scale algal ponds being used in several countries as an effective means of sewage treatment (Oswald 1972; Shelef et al. 1978), use of algal biomass in recovery of metals from industrial waste has amply been demonstrated. Besides, the biosorption potential depends upon various factors like; pH, biomass, metal species, competing ions and type of algal species involved (Lau et al. 1999; Wang and Dei 1999). Further, periodicity of various algal forms comprising diverse taxonomic groups has been observed in the pristine aquatic environments (Tarar and Bodkhe 1999; Dwivedi et al. 2004); however, frequency, dominance *vis-à-vis* seasonal variation has not been demonstrated under polluted habitats. Therefore for developing a viable and effective bioremediation system, the information on metal bioaccumulation and seasonal variations of tolerance natural algal flora is needed. The present study deals with metal accumulation potential of major algal species collected from tannery effluent and periodicity of other potential strains in different seasons. These strains could be

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utilized either for removal of metals from the tannery effluent or as a biomonitoring device for Cr contamination.

MATERIALS AND METHODS

The algal samples were collected seasonally from the settling tank of combined effluent treatment plant (CETP), Unnao, Uttar Pradesh, India in 250 ml plastic bottles using sterile forceps. The samples were examined immediately for the type of algal forms after bringing it to the laboratory in living condition. For bluegreen algal (BGA) strains staining was done with 1% aqueous methylene blue solution to bring out mucilaginous envelope, while green algae were stained with iodine solution. Algal samples were also fixed in 4% formaldehyde solution for proper identification in the laboratory. Major algal species were collected in bulk in polythene carry bags (2 kg capacity), washed thrice with distilled water and oven dried at 90°C to constant weight. The different metals viz., Cr, Fe, Cu, Mn and Zn present in dried samples of high biomass producing algae were analyzed after material was digested with $HNO_3/HClO_4$ (3:1, v/v) mixture at 80°C and absorbance was recorded on atomic absorption spectrophotometer (GBC AVANTA Σ). Detection limit for Cr, Fe, Cu, Mn, and Zn were 0.05, 0.05, 0.025, 0.02 and 0.005 mg l^{-1}, respectively. Analytical data quality of metals was ensured through repeated analysis (n = 5) of EPA quality control samples in water and the results were found to be within ±3.05% of certified values. For algae recoveries of metals from tissue were found to be more than 98%. The blanks were run in triplicate to check the precision of the method with each set of samples.

The tannery effluent in different season was collected in five-liter acid washed plastic container for estimation of various physico-chemical parameters and metal content (APHA, 1992). Some physico-chemical parameters like; pH, temperature, electrical conductivity (EC) and total dissolved solid (TDS) were recorded on the spot by using portable water analysis kit (Century CMK, 731). The biochemical oxygen demand (BOD) of the effluent was estimated using nutrient buffer pillow and lithium hydroxide manometrically (model 2173B, Hach, USA), while chemical oxygen demand (COD) was determined according to reaction digestion method using DR 3000 spectrophotometer (Hach, USA). Rest of the parameters like chloride, sulphide, and sulphates were determined using ion selective electrodes (model 960, Orion, USA), while total metal concentrations in effluent were detected after digestion in acid mixture as described above.

Identification of various BGA strains collected from a chrome polluted environment was done by using taxonomic keys given in Desikachary (1959), Prasad and Srivastava (1992), while different forms of green algae were identified with the help of Prescott (1951), Philipose (1967) and Prasad and Mishra (1992). The diatoms were identified using Prasad and Srivastava (1992). Five number (n =5) algal samples were collected during each of the three seasons. All the determinations were carried out by taking five replicates in each case.

RESULTS AND DISCUSSION

The physico-chemical parameters of effluent water collected seasonally during the year 2003-2004 from settling tank of CETP, Unnao were analyzed, and the results have been depicted in Table 1. The various physico-chemical properties of effluent water varied from one season to other. During the investigation temperature ranged between 20.7-32.5°C; pH, 7.8-8.4; EC, 20.84-22.44 μΩ; total solids, 16,562-16,856 mg l^{-1}; TDS, 5865-6065 mg l^{-1}; BOD, 1192-1285 mg l^{-1}; COD, 16620-16823 mg l^{-1}; chloride, 5021-5290 mg l^{-1}; sulphide, 22-26.17 mg l^{-1}; sulphates, 156-165 mg l^{-1}; Cr, 12.2-16.1 mg l^{-1}; Cu, 0.011-0.018 mg l^{-1}, Zn, 0.24-0.31 mg l^{-1}; Mn, 0.045-0.052 mg l^{-1} and Fe, 1.11-1.28 mg l^{-1}.

Ten algal species belonging to eight genera were found growing in effluent water (Table 2), out of which four of each class belonging to BGA and green algae, while the remaining of the two species represented the class bacillariophyceae. It is interesting to note that non-heterocystous BGA population comprised the dominant community. Although most of the algal forms encountered during summer months except *Ulothrix* sp., but *Oscillatoria tenuis* occurred predominantly in pre-summer and summer months, while *Phormedium bohneri* was found through out the year and makes a thin mat on the wall of settling tank. Green algae like, *Chlorococcum humicolo* and *C. vitiosum* were found growing only during summer months and absent in all the seasons, while *Ulothrix* sp. was found in winter seasons. Based on number of algal forms belonging to different classes, and dominance of *O. tenuis* the treated effluent seems to be of eutrophic nature which supports the view of Rao (1972), that the frequent presence of genus *Oscillatoria* is indicative of a high degree of eutrophication in water bodies. During rains abundance of *Chlamydomonas angulosa* was observed and its bloom formation conforms the observation of Munawar (1974) who stated that members of volvocales developed and multiplied quickly in large numbers after short spells of rains during monsoon.

Table 1. Seasonal physico-chemical characteristics of the collected effluent from settling tank of CETP, Unnao.

Parameters	**Summer (Feb. -June)**	**Rainy (July - Spt.)**	**Winter (Oct. - Jan.)**
Color	Blackish brown	Dark brown	Dark brown
Odor	Foul smell	Foul smell	Foul smell
Temperature (°C)	32.5±0.26	24.8±0.51	20.7±1.01
pH	8.4±0.15	7.8±0.23	8.2±0.95
EC (μΩ)	21.02±1.00	22.44±1.11	20.84±1.32
Total solids	16856±1.8	16686±2.15	16562±2.03
T DS	6065±84.53	5980±66.34	5865±72.11
BOD	1285±47.59	1192±36.87	1220±33.21
COD	16726±69.00	16620±75.26	16823±61.32
Chloride	5290±65.50	5021±42.31	5110±53.38
Sulphide	25.5±0.45	22±1.02	26.17±1.34
Sulphates	156±2.64	158±5.31	165±4.94
Cr	16.1±0.55	12.2±0.94	15.6±0.75
Cu	0.018±0.002	0.011±0.001	0.016±0.004
Zn	0.29±0.004	0.24±0.005	0.31±0.003
Mn	0.05±0.001	0.045±0.007	0.052±0.005
Fe	1.25±0.014	1.11±0.023	1.28±0.011

All the values (mg l^{-1}), are mean ± SE (n=5) otherwise stated

An overview of the various physico-chemical characteristics with algal diversity showed that most of the parameters are auto-correlated. However, it is interesting to note that the trace metals contamination of the effluents generally favoured the growth of bluegreens, while Cr, Cu and Fe concentration favoured diatoms populations.

Table 2. Seasonal variation of ten algal species present in the effluent.

Genus/species	**Growth/dominance**		
	Summer (Feb. - June)	**Rainy (July - Spt.)**	**Winter (Oct. - Jan.)**
Blue green algae			
Aphanocapsa grevillei	+	-	-
Oscillatoria animalis	+	-	-
Oscillatoria tenuis	+++	-	++
Phormedium bohneri	++	+	+
Green algae			
Chlamydomonas angulosa	+	+++	-
Chlorococcum humicolo	+	-	-
C. vitiosum	+	-	-
Ulothrix sp.	-	-	++
Diatoms			
Navicula cuspidata	+	-	++
Nitzschia linearis	++	-	+

+++: dominant; ++ : common; + : rare; - : absent

In contrast only Zn was found to favor the growth of green algae (r =0.928 at 5 %). The sulphide content of effluent had negatively affected the population of all the groups of algae. Similarly, sulphide had been reported to affect adversely the population of volvocales (Munawar 1974). The density of *C. angulosa* has been found low when sulphide content of effluent water was high during summer and winter seasons in the settling tank. Interestingly both the chlorococcalean members viz., *C. humicolo* and *C. vitiosum* present during summer months in the investigated tanks when effluent water having high temperature (32.5 - 34.4°C) with high hydrogen ion concentration support the view of Zafar (1967), that the temperature and pH play an important role in the periodicity of chlorococcales. However, restricted occurrence of both the alga may be due to toxicity of chromium present in the effluent. It is interesting to note that both the diatoms species were absent during rains, while found in other seasons of the year. *Navicula cuspidata* was found commonly during winter, while *Nitzschia linearis* occurred predominantly during summer season. These observations support the earlier finding of Munawar (1974) and Kant and Kachroo (1973) that diatoms occurred in significant number throughout the summer season, while second peak population was observed in October. Because only Cr tolerant algal species were able to grow in the tannery effluent, seasonal fluctuations in physico-chemical properties had less influence on periodicity of occurrence of different groups of algae.

High biomass producing algal strains encountered in the effluent water belongs to the genera *O. tenuis, P. boheneri, Ulothrix* sp. and *C. angulosa*, which accumulated significant quantities of toxic metals in the order Cr>Fe>Zn>Mn>Cu (Table 3). Although, significant amount of these trace metals were accumulated by algal forms, it was maximum for Cr. Highest amount of chromium was found accumulated in *P. bohneri* (26284.00 μg g^{-1} dw) followed by *O. tenuis* (20142.75 μg g^{-1} dw), *Ulothrix* sp. (15356.80 μg g^{-1} dw) and *C. angulosa* (8790.69 μg g^{-1} dw). Iron was found highest in *Ulothrix* sp. (2084.72 μg g^{-1} dw) followed by *P. bohneri* (950.55 μg g^{-1} dw), *C. angulosa* (565.66 μg g^{-1} dw), and *O. tenuis* (185.91 μg g-1 dw). The highest metal accumulation amongst BGA and green algae was found in *P. bohneri* which may be ascribed to the presence of thin mucilage sheath over trichome as reported by earlier workers (Mohamed 2001; Tien 2002). Further algal surfaces have multiple binding sites and isolated mucilaginous sheath comprising of polysaccharides and some small amount of protein, which are able to bind large amount of heavy metals.

Table 3. Metal accumulation potential of major algal species collected from settling tank of CETP, Unnao.

Metals	**Metal accumulation (μg g^{-1} dw) in different strains**			
	O. tenuis	***P. bohneri***	***Ulothrix* sp.**	***C. angulosa***
Cu	16.60± 0.85	37.32±2.43	68.67±1.68	15.56±0.42
Mn	22.37±0.76	42.36±3.12	107.30±3.61	86.02±94.81
Cr	20142.75±386.47	26284.00±338.43	15356.80±216.92	8790.69±186.46
Zn	109.40±6.11	215.61±7.87	316.80±13.97	482.11±7.22
Fe	185.91±4.68	950.55±13.96	2084.72±141.30	565.66±6.62

Values are mean ± SE (n=5)

The hyperaccumulation of chromium and resistance to other metals shown by BGA strains during the present study support the previous work of Fiore and Trevors (1994) who reported BGA to be relatively more tolerant to heavy metals and found suitable organism for use in bioremediation of metal pollutants. Besides, number of factors like plasmid borne resistance, extracellular binding or precipitation, impermeability, exclusion, internal detoxification and transformations affects Cr accumulation in BGA (Fiore and Trevors 1994; Thompson et al. 2002). However, the study to explore genes involved in chromium resistance (Thompson et al. 2002) in these organisms remains to be done. In contrast, green algae adopted a variety of strategies to reduce the toxicity of metals. *Chlorella* secretes organic material under copper stress and decreases the concentration of free copper ions in the medium (Prasad et al. 1998; Boswell et al. 2002). Cadmium resistance in *C. reinhardtii* was found to be due to nuclear mutation (Collard and Matagne 1990). Lemaire et al. (1999) have reported involvement of thioredoxin gene in the defense mechanism against heavy metal toxicity. Since bloom forming *C. angulosa* tolerated elevated Cr concentration (12.2-16.1 mg l^{-1}) and demonstrated substantial accumulation potential (8790.69 μg Cr g^{-1} dw) with out any morphological changes, the alga could be considered as a strong indicator of preexisting genetic adaptation. Further, *Ulolthrix* sp. accumulated appreciable amounts of Cr (15356.8 μg g^{-1} dw) followed by Fe (2084.72 μg g^{-1} dw), Zn (316.8 μg g^{-1} dw), Mn (107.3 μg g^{-1} dw) and Cu (68.67 μg g^{-1} dw), which could be used for bioremediation of tannery effluent. Chromium, Fe, Mn, Zn and Cu accumulation in all the algal species was linearly related with ambient metal concentration in the effluent Similarly, natural population of *Hydrodictyon reticulatum* have shown substantial accumulation of toxic metal and was found suitable for treatment of waste water (Rai and Chandra, 1992; Rai et al. 1995).

Results of the study showed potential of different groups of algae to grow in tannery effluent having high Cr (12.5-16.1 mg l^{-1}) concentrations and some major algal species were found to accumulate substantial amounts of Cr in their tissues, which could be used in developing a bioremediation strategy for pollution abatement. However, population density, their nature of mixing with effluent, volume of effluent in treatment tank under low and high discharges and amount of optimum algal biomass is to be determined before attempting field application. The development of an interdisciplinary approach involving engineering skills along with bioaccumulation and waste treatment to develop a viable technology for Cr removal from tannery effluent is currently underway.

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