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Dominance of Algae in Ganga Water Polluted Through Fly-Ash Leaching: Metal Bioaccumulation Potential of Selected Algal Species

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Coal is used as a principal source of commercial energy in many countries. After burning of coal by thermal power plants, fly-ash is generated as a solid waste. In India, 75% of energy supply is coal-based and this is expected to continue during the next decades. It is estimated that about 64% of coal produced in India is used for power generation (Sahu 1998; Tripathi et al. 2004). There are about 82 utility thermal power stations, in addition to several captive power plants, where bituminous coal is being used (with ash contents >30%) and these produce approximately 120 million tons of fly-ash per annum. Bituminous coal ash production is believed to increase at an annual rate of around 8-10% of which between 85-90% is deposited in the vicinity of the power plants (Singh and Siddiqui 2003). Leachates of fly-ash have high concentrations of metals such as Cu, Zn, Fe, Ni, Mn, B, Hg and Al which negatively affect surface water quality (Carlson and Adriano 1993; Gupta et al. 2006) resulting in killing of sensitive plant and animal species and general changes in ecological relationships.

Algae play an important role in maintaining the equilibrium of aquatic ecosystems (Campanella et al. 2000) and are good indicators of water pollution. They are often used in phytotoxicity tests for environmental monitoring (Boswell et al. 2002). Several cyanophycean algae have been reported to accumulate significant amounts of toxic metals from fly-ash contaminated surface water (Rai et al. 2000; Mohamed 2001, Rai et al. 2005). Green algae also absorb heavy metals from the surrounding environment (Rai and Chandra 1992; Terry and Stone 2002) and are considered as indicators (Fityanos et al. 1999) and bioremediators of heavy metals in aquatic bodies (Gamila and Naglaa 1999; Nakanishi et al. 2004). However, the abundance and tolerance of bluegreen (BGA) and green (GA) algae in polluted habitats, especially fly-ash contaminated waters, vis-à-vis their metal bioaccumulation potential, has not been thoroughly investigated. Therefore, the present work has been carried out near the National Thermal Power Corporation, Unchahar, Raebareli, U.P., India, where fly-ash is disposed off near the bank of the river Ganga. The purpose of this study was to identify sensitive and tolerant species of algae to fly-ash toxicity as well as screening of potential algal strains for the removal of metals from fly-ash contaminated sites. Strains that show a large potential metal accumulation may proof useful as phytoremediation tools to improve water quality by harvesting their biomass.

MATERIALS AND METHODS

During the study, three sites, i.e. Kharauli, Arkha and Kalakankar were selected for sampling due to their varying distance from fly-ash dikes (Fig. 1). In this survey,

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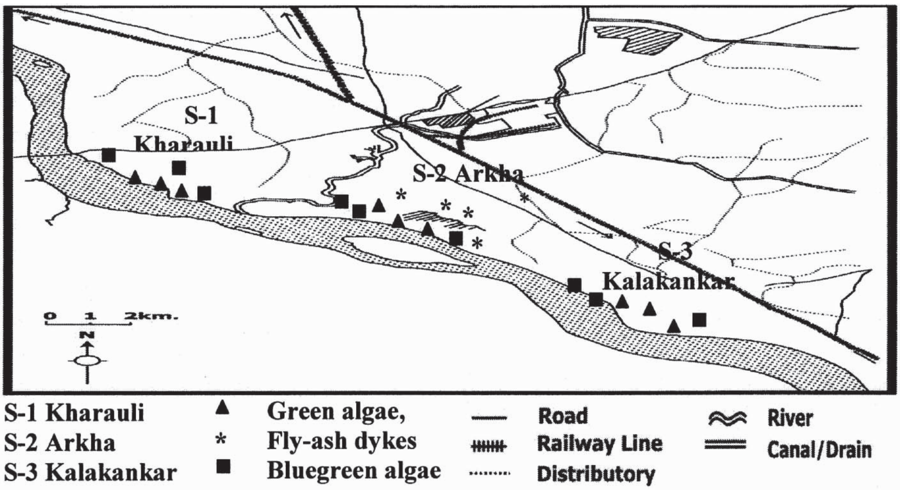

Figure 1. Map showing the location of sampling sites in the vicinity of NTPC, Unchahar, Raebareli, India.

Kharauli village (situated upstream of Arkha) was selected as a control, non-polluted site (ups). Arkha village was treated as highly polluted site (hps) because fly-ash dikes are present around the village on the riverbank, while Kalakankar (situated down stream of Arkha) is considered as moderately polluted (mps). The surface water (Ganga river) quality of selected sites was monitored thrice in a year during summer, rainy and winter seasons. Ganga water samples (250 ml) were collected in triplicate from each site in plastic containers. Temperature was measured on the spot by a thermometer. Dissolved Oxygen (DO), pH, electrical conductivity (EC), and total dissolved solids (TDS) were analyzed with a Portable Water Analysis Kit (Century CMK, 731), while other parameters like biochemical oxygen demand (BOD), chemical oxygen demand (COD) and phosphorus were estimated as per procedures given in (APHA 1985). The phytoplankton and biomass of the algae were collected from Ganga water periodically using Wisconsin plankton net (28µm mesh) in 100 ml plastic bottles with the help of sterilized forceps. The material was examined immediately after bringing it to laboratory in living condition and photomicrographs were taken with a Nikon microscope SZ1450. The occurrence frequency of algal species was determined using a heamocytometer based on the percent occurrence of an individual species by considering the total no of species present in the samples. Individual algal species were classified as dominant (>70%), common (40-70%) and rare (<40%) based on their frequency of occurrence. The bluegreen algae (BGA) samples were stained with 1% aqueous methylene blue solution to stain the mucilage envelope, while green algae were stained with iodine solution. Detailed line drawings were made and accurate measurements were taken for species level identification. Various algal taxa were identified following the taxonomic keys provided by Prescott (1951), Desikachary (1959), and Philipose (1967).

Bloom forming filamentous forms were collected in bulk using a plankton net and stored in polythene bags (2 kg capacity), then washed thrice with distilled water and oven dried at 80^0C to constant weight. The concentrations of different metals i.e. Cu, Mn, Zn, Fe, Ni and Pb in dried algal biomass and surface water were analyzed following wet digestion in

Table 1. Distribution and dominance of bluegreen algae in polluted and unpolluted sites of river Ganga (Raebareli).

Taxon	Habitat	Occurrence	Seasons
Merismopedia elegans	ups, mps	C	w,r
Arthrospira jenneri	hps	C	w
Spiruliana luxa	ups	R	r
Oscillatoria curviceps	ups,mps	D	w
O. amoena	hps,mps	D	w
O. vizagapatansis	hps	C	w
O. prolifica	hps	R	r
O. nigra	hps,mps	C	w
Oscillatoria sp.	hps, mps	R	w
Phormedium papyraceum	hps	D	s, w,r
P. bohneri	hps	R	w
Lyngbya hicronymusii	hps	C	w
Nostoc muscorum	hps	D	s
Anabaena doliolum	hps	D	w
Nodularia sp.	hps	R	w
Calothrix fusca	hps	R	w
Wholia sp.	hps	D	w, s
Westiliopsis sp.	hps	R	w, s

ups = unpolluted site; mps = moderately polluted site; hps = highly polluted site, D = dominant (>70%); C=common (40-70%); R = rare (<40%); s = summer; r = rainy; w = winter.

HNO_3: $HClO_4$ (3:1 v/v) at 90°C. The metal content was estimated in filtered samples using an Atomic Absorption Spectrometer (GBC Avanta Σ, Australia). The mean recovery was 96±5%, 97±7%, 95±6%, 98±5%, 96±6% and 97±5% for Cu, Mn, Zn, Fe, Ni and Pb, respectively. The detection limits of Cu, Mn, Zn, Fe, Ni and Pb were 0.001, 0.02, 0.005, 0.02, 0.02 and 0.06 mg l^{-1}, respectively. The relationship amongst various physico-chemical properties of the surface water and the presence of specific algal strains in different seasons was tested using a linear correlation coefficient test (significant at 5%). All the determinations were carried out taking 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 (1984). The diversity richness and similarity indices were calculated according to Ludwig and Reynolds (1988).

RESULTS AND DISCUSSION

Most of the physico-chemical properties of the surface water varied significantly (DMRT <0.05) from site to site and from one season to another (Fig.1 A-C). The mean value of these parameters at the various sites showed annual variation as follows: temperature, 24.9-26.1°C; pH, 7.4-7.9; EC, 0.602-0.680 mmhos cm^{-2}; DO, 3.8-4.04; BOD, 37.84-62.88; COD, 53.82-113.83; TDS, 0.337-0.554 and phosphorus, 0.329-0.509 mg l^{-1}. The low values of DO observed in this study are in conformity with the results of Tare et al. (2003) who also noticed low DO values in different stretches of river Ganga. Similarly, the concentration (mg l^{-1}) of heavy metals varied significantly; Cu ranged between 0.022-0.039; Mn, 0.277-0.378; Zn, 0.116-0.232; Fe, 13.12-23.91, Ni, 3.45-5.31 and Pb, 0.08-0.24 (DMRT <0.05) (Fig.1 A'-C').

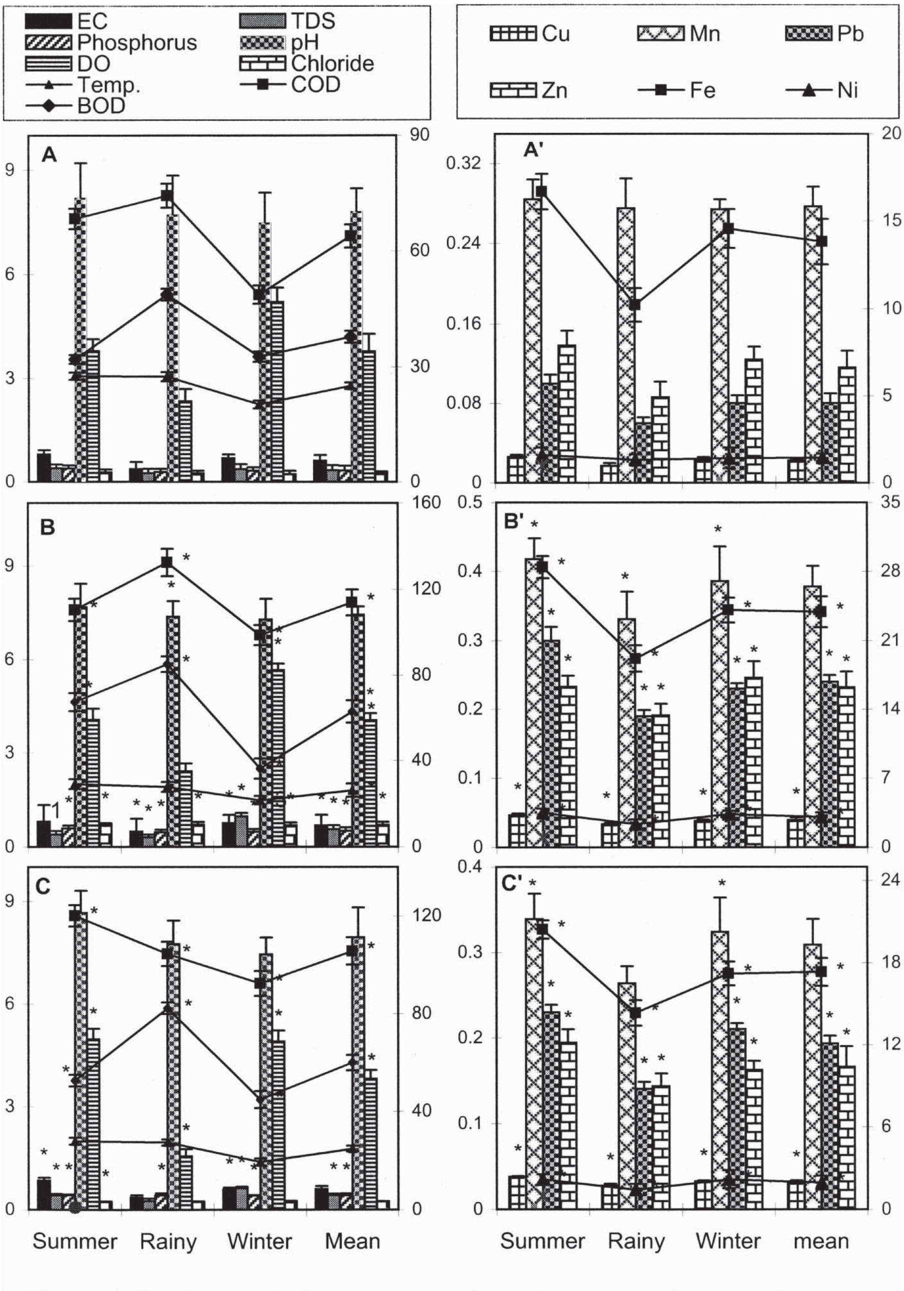

Figure 2. Physio-chemical parameters and metal contamination of surface water (Ganga river) of ups (A and A'), hps (B and B') and mps (C and C') in different seasons, Values are given in mg l^{-1} except for pH and Temp. (^{o}C).Values are mean±SD. * denote significant seasonal difference for parameters from control site (ANOVA<0.01, DMRT<0.05).

Table 2. Distribution and dominance of green algae in polluted and unpolluted sites of river Ganga (Raebareli).

Taxon	Habitat	Occurrence	Seasons
Hydrodictyon reticulatum	mps	C	w
Dictyospherium pulchellum var. *minutum*	ups	R	w
Pediastrum clatratum	ups	C	w
P. tetras var. *tetraodon*	hps,mps	R	w
P. tetras	ups	R	w,s
P. duplix var. *subgranulatum*	ups	R	w
Coelastrum subgranulatum	ups	C	w,s
Scenedesmus bijugatus	ups	R	w
S. quadricauda var. *westii*	ups	C	w
S. longus var. *naegelii*	ups	C	w
S. quadricauda var. *maximum*	ups	R	w
S. quadricuada var. *longispina*	hps,mps	C	s,w
S. bijugatus var. *alternans*	mps	C	w
S. armatus	ups	C	w
S.dimorphus	mps	R	w
S. quadricuada var. quadrispina	hps, mps	C	w,s
Oedogonium sp.	hps	D	s
Spirogyra sp.	hps	D	s
Cosmarium furcatospermum	hps	R	s
Euastrum inermius var. *burmense*	hps	R	s

ups = unpolluted site; mps = moderately polluted site; hps = highly polluted site, D = dominant (>70%); C=common (40-70%); R = rare (<40%); s=summer; w=winter.

A total number of 38 phytoplankton taxa were present at polluted and unpolluted sites. These taxa included 18 BGA (cyanophyceae) and 20 GA (chlorophyceae). A wide variation was observed among BGA and GA with respect to their habitat, occurrence, seasonal variation, metal tolerance and metal accumulation potential. During the investigation, it was observed that the diversity of green algae occurred at ups, while BGA dominated at the hps. Cyanobacterial forms such as *Merismopedia elegans* and *Spirulina luxa* occurred at ups. Interestingly all the six heterocystous (BGA) strains viz., *Anabaena doliolum, Nostoc muscorum*, *Nodularia* sp. *Calothrix fusca, Wholia* sp. and *Westiliopsis* sp. were found at the hps (Table 1). These finding are in accordance with the earlier reports that N_2 fixing taxa have tolerance to high salinity and boron and are the most successful early colonizers in fly-ash contaminated areas (Carlson and Adriano 1993; Gupta et al. 2002). Fly-ash disposal in aquatic ecosystem increases the salinity (Carlson and Adriano 1993), which is directly correlated with the increase in population of BGA (Sellner et al. 1988). In the present study also, the Whittaker beta-diversity index for BGA was highest at the hps where maximum fly-ash leaching was occurring. Besides, boron, present in the fly-ash, is essential for N_2 fixation by heterocystous BGA (Bonilla et al. 1990), which supports the growth of these strains. As fly-ash is deficient in N, BGA were able to grow due to their N_2 fixing ability, consequently increasing the N status of fly-ash (Rai et al. 2000). The presence of non-heterocystous forms clearly indicated that these must be capable of fixing atmospheric N_2 under fly-ash stress conditions as reported by other workers for anaerobic and microaerobic conditions (Ohki et al. 1992; Tiwari et al. 2000). Furthermore, waste of various industries causing eutrophication in river systems

Table 3. Metal content in major algal species collected from Ganga water near fly-ash dikes.

Genus/Species	Site	Metals accumulation (µg g^{-1} dw) in different species					
		Cu	Mn	Zn	Fe	Ni	Pb
Bluegreen algae							
Oscillatoria curviceps	mps	36.68^{cd} ± 7.29	11901.18^{a} ± 271.29	198.42^{c} ±7.99	1409.95^{b} ± 29.55	512.06^{b} ± 36.11	142.61^{b} ± 8.51
O. amoena	hps	292.60^{a} ± 16.75	7565.45^{b} ± 120.30	277.06^{b} ± 12.36	1396.19^{b} ± 81.01	724.61^{a} ± 6.78	238.12^{a} ± 13.11
Phormidium papyraceum	hps	15.36^{d} ± 2.58	3625.30^{c} ± 54.87	15.64^{e} ± 1.65	1062.71^{bc} ± 87.03	368.17^{c} ± 3.57	14.80^{cd} ± 1.70
Nostoc sp.	hps	42.75^{bcd} ± 4.85	39.97^{e} ± 7.40	52.89^{de} ± 12.96	19.44^{e} ± 4.50	12.12^{e} ± 2.72	8.20^{d} ± 1.08
Anabaena doliolum	hps	62.52^{bc} ± 3.61	69.72^{e} ± 8.92	178.08^{c} ± 13.16	856.86^{c} ± 65.12	416.11^{c} ± 6.60	12.08^{d} ± 1.66
Wholia sp.	hps	285.11^{a} ± 12.78	46.78^{e} ± 6.04	68.12^{d} ± 5.44	383.00^{d} ± 16.51	165.17^{d} ± 4.62	18.11^{cd} ± 2.06
Green algae							
Hydrodictyon reticulatum	mps	78.00^{b} ± 8.75	202.00^{e} ± 12.23	544.00^{a} ± 17.45	7491.00^{a} ±154.89	23.49^{e} ± 3.58	224.87^{a}± 11.22
Spirogyra sp.	hps	8.92^{d} ± 1.21	1663.25^{d} ±23.76	28.65^{e} ± 4.20	71.82^{de} ± 6.42	234 .00^{d} ±12.72	46.24^{c} ± 3.21

hps=highly polluted site, mps= moderately polluted site; Values are means of three replicates ± SD, Different symbols denote significant difference in metal accumulation (ANOVA<0.01; DMRT<0.05).

resulted in increased populations of *Oscillatoria* sp. and *Phormidium* sp. (Sudhakar et al. 1991).

Out of 20 GA species, 16 species represented the order chlorococcales followed by three of the conjugales and one of the order of oedogoniales. The diversity of chlorococcalean GA was largely found upstream from the polluted site, while some species viz., *Pediastrum tetras* var. *tetraedon, Scenedesmus sp., S. quadricuada var. longispina* and *S. bujugatus* were found rarely at mps. GA like *Dictyospherium pulchellum* var. *minutum*, *Pediastrum clatratum* and *Coelastrum subgranulatum* were restricted in their occurrence and, though frequently present in the ups, they were absent at other sites (Table 2). Some other GA like, *Oedogonium sp., Spirogyra sp.* were dominant at hps, while *Cosmarium furcatospermum* and *Euastrum inermium* var. *burmense* occurred rarely at this site during the summer season.

The frequent occurrence of chlorophytes at ups and their absence at hps during spring and summer seasons showed that these taxa were sensitive to metal pollution caused by fly-ash leaching at hps. Similarly, Srivastava and Sahai (1976) found a direct correlation between frequency of phytoplankton and metal pollution in lake Chilka. But, abundance of *P. tetras* var. *tetraodon*, *S. quadriquada* var. *longispina* and *S. quadricuada* var. *quadrispina* at hps showed that these taxa have remarkable tolerance to metals present in Ganga water as also recorded by Fityanos et al. (1999). Colonies of *Scenedesmus* sp. were used for monitoring of various metal pollutants in river basins in Thailand and the Federal Republic of Germany (Chandra and Sinha 2000).

The matrix analysis of correlation coefficients between various physico-chemical

Table 4. Correlation coefficient values between physico-chemical parameters, metals content and algal forms from three selected sites.

	Physico-Chemical Parameters/ Metals							
	Temp.	**pH**	**EC**	**DO**	**BOD**	**COD**	**TDS**	**Phosph.**
BGA	0.988*	-0.961*	0.989*	0.997*	0.592	0.626	0.901*	0.859
GA	-0.617	0.516	-0.626	-0.776	-0.982*	-0.989*	-0.954*	-0.977*

	Cu	**Mn**	**Zn**	**Fe**	**Ni**	**Pb**
BGA	0.848	0.950*	0.902*	0.939*	0.895	0.740
GA	-0.981*	-0.906*	-0.953*	-0.920*	0.958*	-0.999*

Tabular r =0.900 significant at p<0.1% at df= 2(n-1).

characteristics and metal contents of Ganga water with the occurrence frequency of algal forms from three selected sites is depicted in Table 4. The physico-chemical variables have a great influence on frequency and distribution of both green and bluegreen algal forms (p<0.1%; df=2). The DO, EC and temperature have a positive influence on BGA forms, while pH of the water affected the distribution of these forms negatively. The green algal forms seem to be more susceptible to the variation in parameters such as pH, TDS, temperature, BOD and COD, showing a negative correlation between these parameters and the distribution of GA (p<0.1%; df=2). It is interesting to note that increases in the concentration of metals viz., Cu, Mn, Zn, and Fe favoured the growth of BGA forms in Ganga water, while these metals have a negative influence on the distribution of green algal forms. It is clear from table 5 that site 2 is rich in algal species according to the species richness index and Margalef's index. All the three sites showed far less similarities with respect to species composition and species turnover according to the Jaccard similarity index and Whittaker's beta-diversity index.

Out of 38 species, only 8 were found to produce high biomass. The metal accumulation potential of these strains varied from species to species under field conditions. The maximum accumulation of Zn, Cu and Ni was shown by *Oscillatoria amoena,* which was 277.06, 292.60 and 724.61 μg g^{-1} dw, respectively. This species also showed appreciable accumulation of Fe (1396.19 μg g^{-1} dw) and Mn (7565.45 μg g^{-1} dw), but maximum accumulation of Fe (1409.95 μg g^{-1} dw) and Mn (11904.18 μg g^{-1} dw) was shown by *O. curviceps*. Although Hg was present (0.107μg g^{-1}) in raw fly-ash, it was below the detection limit in Ganga water and algal samples collected from the selected sites during the study. The metal accumulation sequence of *O. amoena* depicted the order of Mn>Fe>Ni>Cu>Zn>Pb. *O. curviceps* showed an accumulation order of Fe>Mn>Ni>Zn>Pb>Cu. The BGA viz., *N. muscorum, Wohlia* and GA like *Spirogyra,* were dominant forms at hps but higher metal concentrations were detected in the biomass of non-heterocystous forms such as *O. curviceps, O. amoena* and *P. papyraceum* probably due to the presence of a mucilaginous sheath which is able to bind large amounts of metals (Wong et al. 1998; Tien 2002). Further, species of *Oscillatoria* and other aquatic plants in the ash basin ecosystem has been earlier demonstrated to accumulate considerably high amounts of trace and toxic metals including Hg (Adriano et al. 1980).

Among GA, *Hydrodictyon reticulatum* accumulated appreciable amounts of metals (μg g^{-1} dw); Fe (7491.00) followed by Zn (544.00), Pb (424.87), Mn (202.00), Cu (78.00) and Ni (23.49). Earlier, Rai and Chandra (1992) found metal accumulation by *H. reticulatum* in an order of Fe>Mn>Pb>Cu under different sets of field

Table 5. Diversity indices and similarity index of algal communities at different selected sites.

Diversity indices		**Site-1**		**Site-2**		**Site-3**
Species richness		13		22		11
Margalef's index		1.9392		3.0396		1.5807
Simpson's Index		0.9117		0.9397		0.8989
Shannon's Index		2.4657		2.9019		2.3229
Jaccard similarity index				**Whittaker's beta-diversity**		
	Site-1	**Site- 2**	**Site-3**	**Site-1**	**Site-2**	**Site-3**
Site 1	1	0	0.09	0	1	0.83
Site 2	0	1	0.22	1	0	0.64
Site 3	0.09	0.22	1	0.83	0.64	0

conditions. However, our studies demonstrated maximum accumulation of Mn by *Spirogyra* sp., which confirms our earlier observation that *S. adnata* accumulated the highest level of Mn from lake Nainital water (Ali et al. 1999). The correlation between physico-chemical properties of Ganga water with phytoplankton density and abundance in different seasons was established. Cyanobacterial dominant forms present in hps viz. *O. curviceps, O. amoena, N. muscorum* and *Wohlia sp.* and chlorophycean species viz. *Oedogonium* and *Spirogyra* seem to work as biomonitoring agents of fly-ash leachate pollution in river Ganga. Further, the high metal accumulation potential shown by some characterized algae showed the possibility of their use as a bioremediation tool for decontamination of surface water polluted with hazardous metals derived from fly-ash leachates.

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