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Screening of native plants and algae growing on fly-ash affected areas near National Thermal Power Corporation, Tanda, Uttar Pradesh, India for accumulation of toxic heavy metals

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Abstract

The present investigation was carried out to screen native plants growing in fly-ash (FA) contaminated areas near National Thermal Power Corporation, Tanda, Uttar Pradesh, India with a view to using them for the eco-restoration of the area. A total number of 17 plants (9 aquatic, 6 terrestrial and 2 algal species) were collected and screened for heavy metal (Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As) accumulation. Differential accumulation of various heavy metals by different species of plants was observed. *Hydrilla verticillata* was found to be the most efficient metal accumulator among 9 aquatic plants, *Eclipta alba* among 6 terrestrial plants and *Phormedium papyraceum* between 2 algal species. In general, the maximum levels of most metals were found in terrestrial plants while the lowest in algal species. However, translocation of the metals from root to shoot was found to be higher in aquatic plants than terrestrial ones. These results suggest that various aquatic, terrestrial and algal species of plants may be used in a synergistic way to remediate and restore the FA contaminated areas.

Keywords: Algae; Aquatic plants; Fly-ash; Phytoremediation; Terrestrial plants

1. Introduction

Fly-ash (FA), a resultant of combustion of coal at high temperature, has been regarded as a problematic solid waste [1]. The global generation of coal FA is estimated to be above 600 million tonnes [2]. In India only, annual production of FA is about 120 million tonnes by 82 power plants, which is expected to cross the figure of 150 million tonnes in the coming years [3]. Since, Indian coal is rich in ash content [4]; it has posed serious disposal and ecological problems and caused large tracts of arable and non-arable land to become unusable. Dumped FA may also adversely affect the environment by mobilization of its fine particles and hazardous constituents contaminating air, water, soil and vegetation [5]. Despite negative environmental impact of FA, coal continues to be a major source of power production in India and therefore FA disposal is a major environmental issue. In recent times, attempts have been made to utilize FA for various purposes, such as for manufacturing of bricks [6] and as a soil amender [7]. However, these practices utilize only limited proportion (<15%) of FA and huge amounts remains unutilized.

Fly-ash is largely alkaline in nature and contains many essential elements like S, B, Ca, Mg, Fe, Cu, Zn, Mn and P along with toxic metals, such as Cr, Pb, Hg, Ni, As and Cd [8,9]. FA application to soil at a low rate has been reported to promote the growth of the plants [10] through improvement of soil conductivity, organic carbon, microbial activity [6], soil porosity and water holding capacity [11]. This raises the possibility of using phytoremediation as an applicable technology to remediate the FA contaminated areas as well as to restore these for beneficial purposes [12]. The technology uses plants to remove toxic elements from the contaminated sites through their accumulation in harvestable shoot parts (i.e. phytoextraction) or to immobilize metals in soils or sediments by root uptake, adsorption onto roots or precipitation in the rhizosphere (i.e. phytostabilization) [13]. A number of terrestrial plants particularly the members of Brassicaceae have been found to be good accumulator of

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toxic metals [14,15]. Similarly, the metal accumulating potential of a number of aquatic plants, i.e. *Pistia stratiotes*, *H. verticillata*, *Ceratophyllum demersum*, *Bacopa monnieri*, etc. has been demonstrated [16–20]. In addition, some recent reports demonstrate high metal accumulation ability of algal species like *Oscillatoria* spp., *Phormedium* spp., *Spirogyra* spp., etc. [21,22]. Thus, terrestrial and aquatic plants, and algal species possess the capability to remove the toxicants and may be used for restoration of the degraded ecosystems.

In recent times, restoration of FA affected tracts of land through transplantation of *Dalbergia sisso*, *Eucalyptus*, *Acacia*, *Cassia* and *Tamarindus* has been attempted [1,23]. An initial success in this direction has pushed the researchers to find out other plant species, both aquatic as well as terrestrial, to cover the FA with dense mat of plantation and make the site aesthetically pleasing and to exploit their metal accumulation ability at the same time. According to Yoon et al. [24], plants growing naturally on a contaminated site respond better under stress conditions than plants introduced from other areas in terms of survival, growth and reproduction. Thus, there is a need to evaluate the metal accumulation ability of various native plants growing in FA affected sites to better use them for phytoremediation purposes in future and restoration of degraded lands.

In this backdrop, this present study was undertaken to screen plants growing in FA contaminated areas situated around National Thermal Power Corporation (NTPC), Tanda, Uttar Pradesh, India. The study included the analysis of physico-chemical properties of FA and the evaluation of heavy metals, such as Fe, Zn, Mo, B, Cu, Si, Al, Pb, Cr, Hg, As and Cd in FA, FA effluent as well as in various plants collected from the selected contaminated area.

2. Materials and methods

The samples of FA and FA effluent were collected from FA dumping sites near NTPC, Tanda, Uttar Pradesh, India. After collection, all the samples were brought to the laboratory for analysis. The pH was analyzed by Orion ion meter (USA). Total nitrogen (%) was estimated by Kjeldhal method [25], organic carbon following Walkley and Black [26] and total phosphorus (%) by Olsen method given by Jackson [27]. Level of sulfate, potassium, carbonate, chloride, magnesium and porosity were estimated following standard procedures given in APHA [28]. Water holding capacity was measured by hydrometry.

A total number of 15 plants belonging to different families were collected and kept in plastic bags. One set of healthy plants were pressed in plant press using standard herbarium preparation method. After bringing to laboratory, plant species were identified using the taxonomic keys given by Duthie [29]. The algae were collected from the waterlogged areas near FA dykes using Wisconsin plankton net (28 μm mesh) in 100 ml plastic bottles. The material was examined immediately after bringing it to laboratory in living condition and photomicrographs were taken with a Nikon microscope SZ1450. For identification of algal species, the key provided by Prescott [30] was used. Following aquatic plants; *Marsilia quadrifolia* (Marsiliaceae), *Ranunculus scloralus* (Ranunculaceae), *Ipomoea aquatica* (Convolvulaceae), *Lippia nodiflora* (Verbanaceae), *Potamogeton pectinatus* (Potamogetonaceae), *Eichhornia crassipes* (Pontederiaceae), *Hydrorhiza aristata* (Poaceae), *Hydrilla verticillata* (Hydrocharitaceae), *Ceratophyllum demersum* (Ceratophyllaceae), terrestrial plants; *Parthenium hysterophorus* (Asteraceae), *Solanum nigrum* (Solanaceae), *Limnanthes* spp. (Limnanthaceae), *Equisetum ramosysma* (Equisetaceae), *Saccharum munja* (Poaceae), *Eclipta alba* (Asteraceae) algal species; *Spirogyra biformis* (Chlorophyceae), *Phormidium papyraceum* (Cyanophyceae), were identified.

For heavy metal analysis, the FA samples were extracted with 0.05 M DTPA following the method of Lindsay and Norvell [31]. Plant samples were divided into roots and shoots, washed thoroughly with deionized water for removing the adhering soil/FA particles, blotted and oven dried at 80 °C for 72 h. For analysis of heavy metals (Fe, Zn, Mo, B, Cu, Si, Al, Pb, Cr, Hg and Cd), all the samples (FA, FA effluent and plants) were digested with $HClO_4$:HNO_3 (1:4 v/v) and diluted with Milli-Q water. For As estimation, samples were digested in 1 ml of concentrated HNO_3 on a heating block at 180 °C for 1 h and subsequently at 200 °C to evaporate the samples to dryness [32]. The residue was taken up in 10 ml of 10% (v/v) HCl containing 10% (w/v) KI and 5% (w/v) ascorbic acid. Metal/metalloid concentrations in the diluted samples were determined on the Inductively-Coupled Plasma Mass Spectrometer, PerkinElmer Corporation (ICP Optima 3300 RL).

The standard reference materials of Fe, Zn (BND 1101.02; provided by the National Physical Laboratory, New Delhi, India), Cd, Cr, Cu, Pb (EPA quality control samples; Lot TMA 989), Mo, B, Si, Hg, Al and As (E-Merck, Germany) were used

Table 1
Physico-chemical properties of fly-ash and metal content of fly-ash and effluent. Values are means of triplicates ± S.D.

Parameters	Fly-ash	
pH	7.77 ± 0.06	
Porosity (%)	70.18 ± 4.12	
Water holding capacity (%)	61.47 ± 2.66	
Total nitrogen (%)	0.048 ± 0.001	
Phosphorus (%)	0.66 ± 0.02	
Total organic carbon (ppm)	0.75 ± 0.007	
Sulfate (%)	13.75 ± 0.58	
Potassium (%)	0.97 ± 0.06	
Chloride (%)	2.68 ± 0.18	
Carbonate (%)	2.07 ± 0.26	
Magnesium (%)	1.82 ± 0.09	
Metals (μg g^{-1} dw)	Fly-ash	Effluent
Fe	171.46 ± 4.11	111.73 ± 16.68
Hg	0.023 ± 0.001	0.012 ± 0.001
As	0.002 ± 0.0001	0.052 ± 0.003
Si	189.98 ± 6.13	298.55 ± 26.10
Cu	1.94 ± 0.06	6.94 ± 0.56
Al	46.5 ± 0.26	76.2 ± 0.81
Pb	6.92 ± 0.66	15.25 ± 1.96
Zn	3.60 ± 0.23	1.86 ± 1.77
Cr	0.26 ± 0.11	1.67 ± 0.62
Mo	0.17 ± 0.06	0.81 ± 0.02
B	1.70 ± 0.09	2.47 ± 2.26
Cd	0.41 ± 0.03	4.40 ± 0.86

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 ±2.03% to ±2.95% of certified values. The mean recovery was about 96–98.5% 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 Fe, Zn, Cd, Cr, Cu, Pb, Mo, B, Si, Hg, Al and As were 0.3, 0.2, 0.9, 0.5, 0.9, 1.5, 0.4, 0.1, 0.12, 0.1, 0.9 and 0.3 ppb, respectively.

Two-way analysis of variance (ANOVA) was done on all the data to confirm the variability of data and validity of results. Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments [33].

3. Results and discussion

The physico-chemical analysis of FA and levels of heavy metals in FA and effluent coming through FA dykes have been presented in Table 1. The pH of the FA was in alkaline range, i.e. 7.77. The levels of total nitrogen, phosphorus and organic carbon were low but the porosity and water holding capacity were high. Due to very minute size of particles, FA shows high

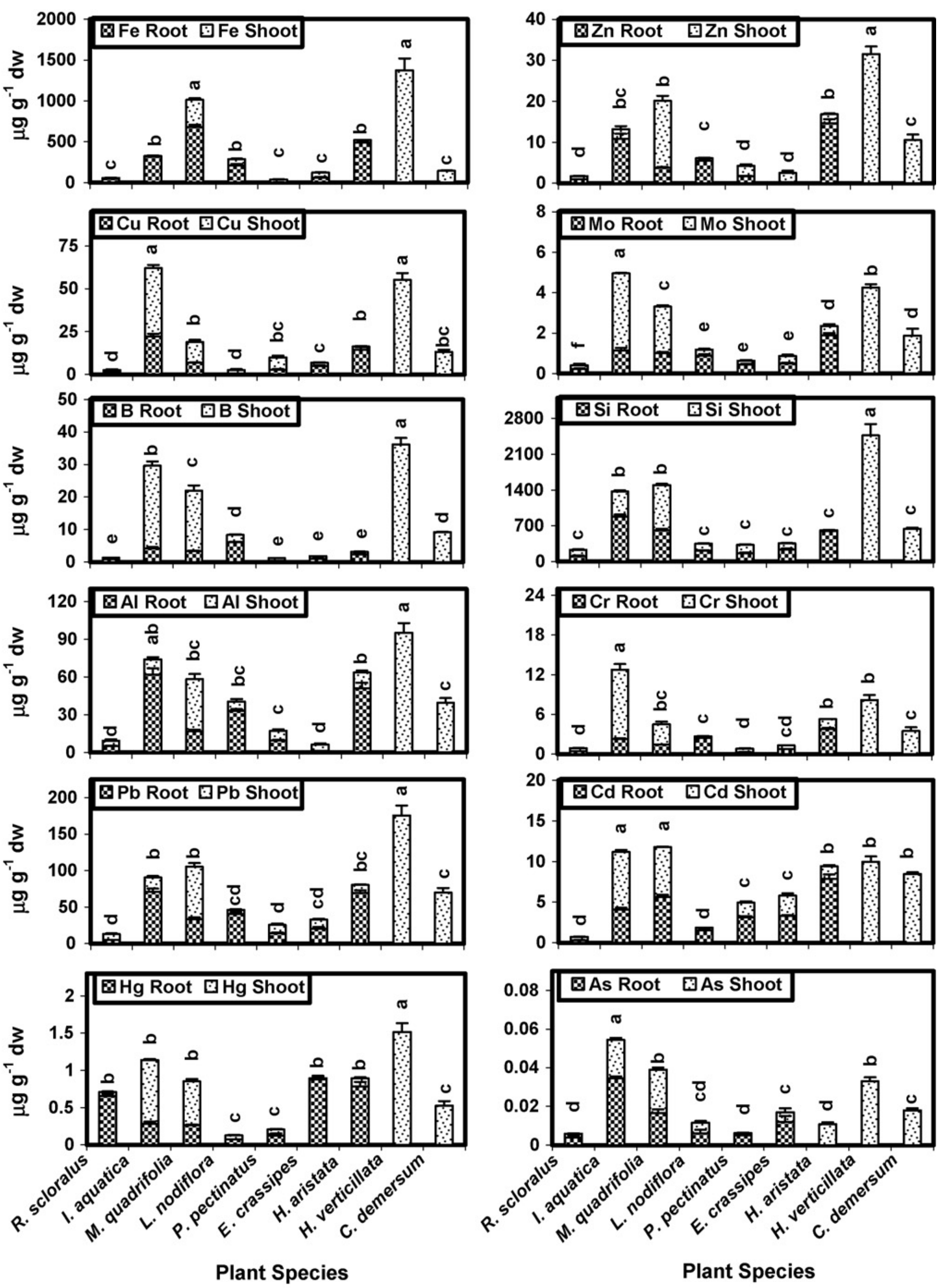

Fig. 1. Accumulation of Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As by different aquatic plants collected from fly-ash affected areas near NTPC, Tanda. Values are means of triplicates ± S.D. ANOVA significant at $p \leq 0.01$. Different letters indicate significantly different values (DMRT, $p \leq 0.05$) for a particular metal.

porosity and water holding capacity [9]. The alkaline pH of FA and low level of nitrogen and phosphorus found in this study are in agreement with the previous reports [7,23,34]. In addition, the level of sulfate, an important nutrient for plants under stress conditions, was also high. Previously, it has been demonstrated that FA may enhance the level of sulfur in plants growing on FA amended soils [1]. Various heavy metals (Fe, Zn, Mo, B, Cu, Si, Al, Pb, Cr, Hg, As and Cd) investigated were present in detectable concentrations in DTPA extracted FA as well as in FA effluent. It has been demonstrated previously that FA has higher availability of various heavy metals except As than that in soil due to their higher concentrations and due to the alkaline pH of FA [35]. Arsenic availability has been demonstrated to increase with decrease in pH [36]. Presumably due to an alkaline pH, As was present at lowest available concentrations in FA in this study.

Plant communities respond differentially to FA environment depending on their ability to accumulate and detoxify various heavy metals [1,9]. A total number of 15 plant species were found to be growing naturally in FA affected areas. Of these, 9 species belonged to aquatic plants while 6 to terrestrial plants. In addition, 2 algal species were also present in dominant form

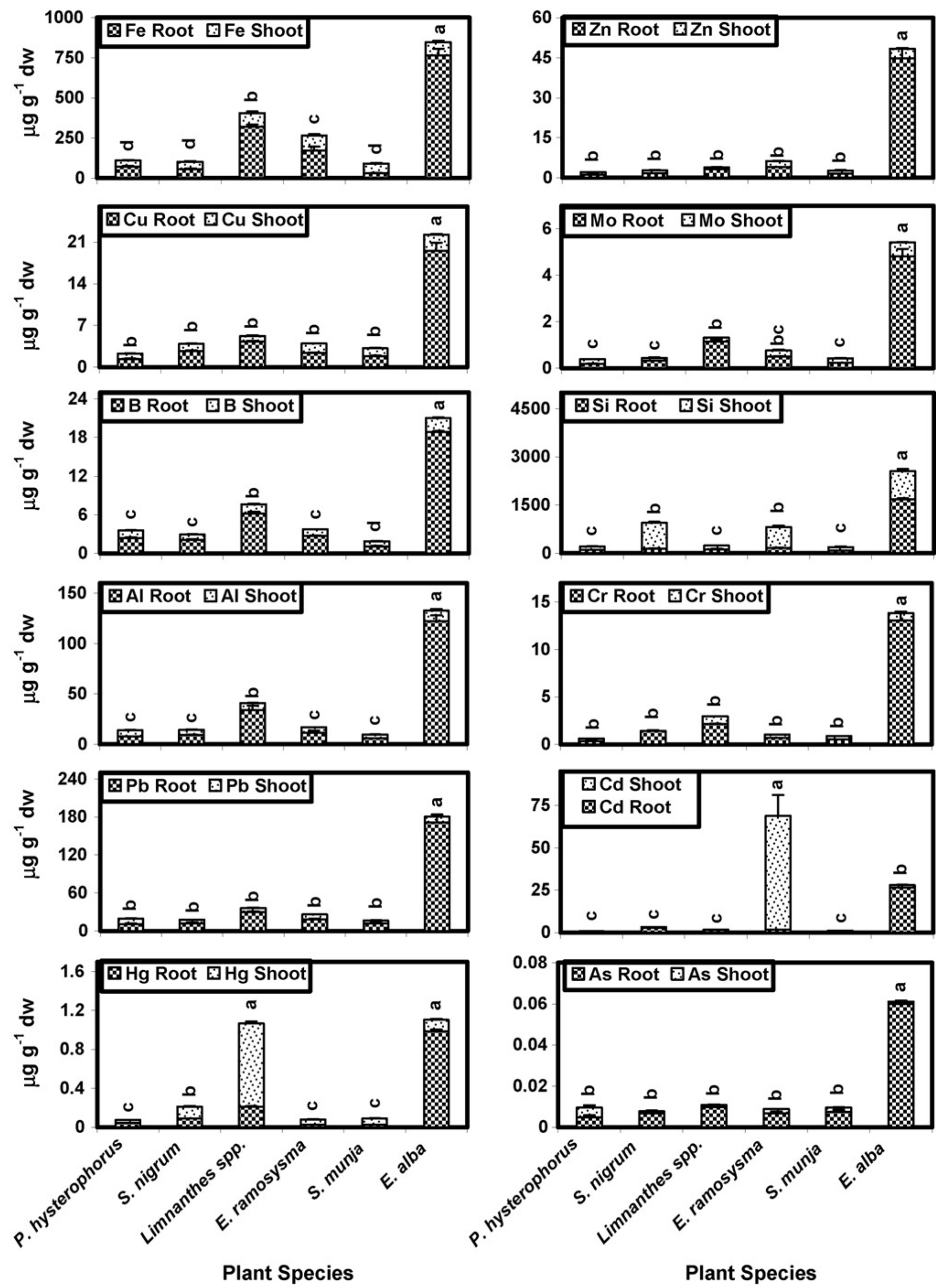

Fig. 2. Accumulation of Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As by different terrestrial plants collected from fly-ash affected areas near NTPC, Tanda. Values are means of triplicates ± S.D. ANOVA significant at $p \leq 0.01$. Different letters indicate significantly different values (DMRT, $p \leq 0.05$) for a particular metal.

in the FA contaminated area. Analysis of accumulation of heavy metals and metalloids by the selected plants depicted differential pattern of accumulation in various species of plants.

In general, with few exceptions, various plants and algae showed the maximum accumulation of Si in their plant parts followed by Fe, Pb and Al. Other metals viz., Zn, Cu, B, Mo, Cd and Cr were detected in low amounts in various plants and algae while As was accumulated in the least amount. A significantly positive correlation was found between the accumulation of various heavy metals in plants and algae and their availability in FA or FA effluent ($p < 0.001$). Among various categories of plants (aquatic, terrestrial and algae), the maximum level of various heavy metals, except Fe, Hg, Cu and B, was detected in terrestrial plants followed by aquatics while the lowest level of all the heavy metals was recorded in algal species. However, in terrestrial plants most of the metals remained concentrated in roots, while aquatic plants showed significant translocation of metals from roots to shoots.

Among various aquatic plants, *H. verticillata* was found to be the best accumulator of various heavy metals (Fig. 1). This plant showed the maximum accumulation ($\mu g\, g^{-1}$ dw) of Fe (1373), Zn (32), B (36), Si (2467), Al (95), Pb (175) and Hg (1.51)

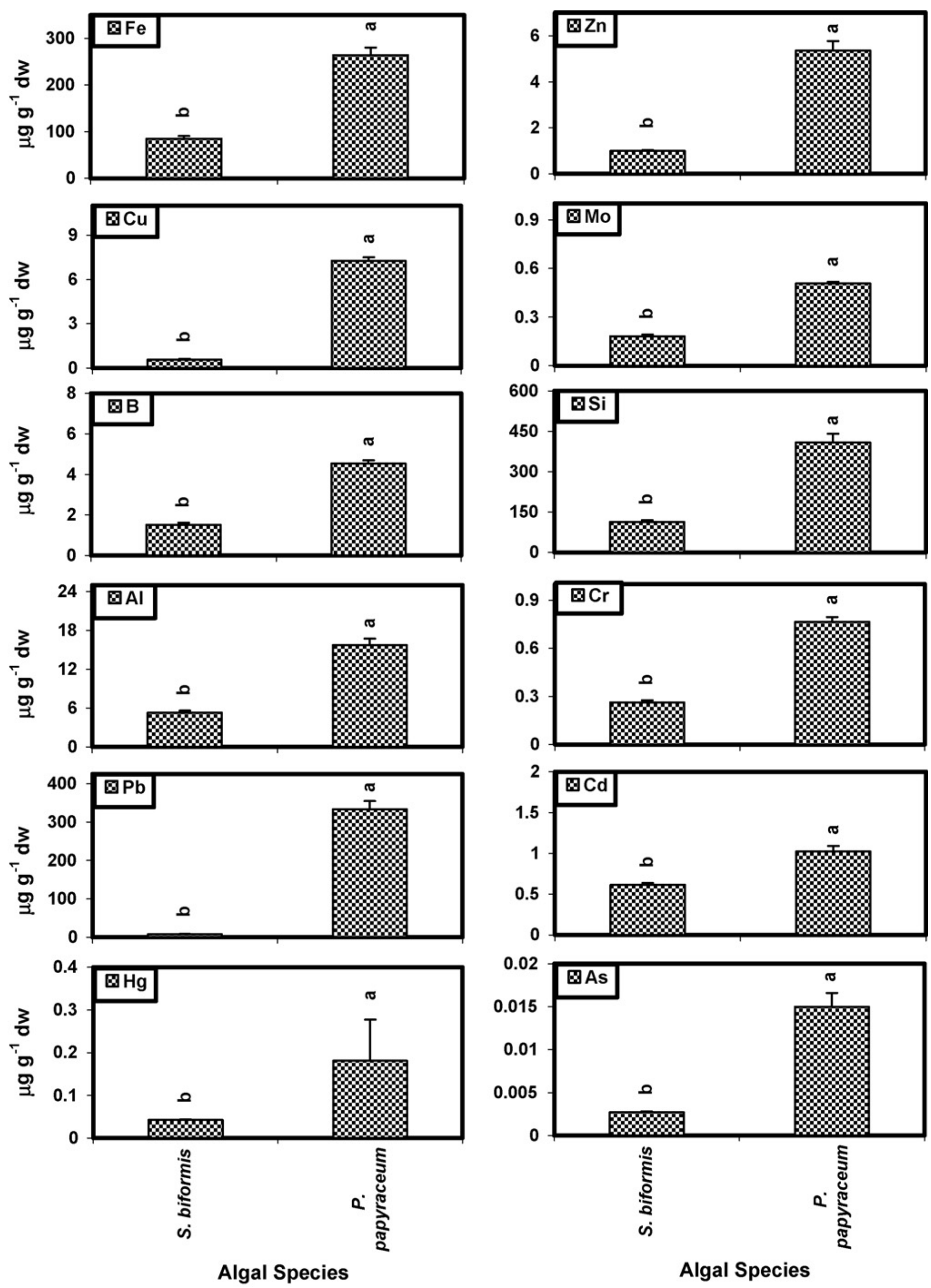

Fig. 3. Accumulation of Fe, Zn, Cu, Mo, B, Si, Al, Cr, Pb, Cd, Hg and As by the 2 algal species collected from fly-ash affected areas near NTPC, Tanda. Values are means of triplicates ± S.D. ANOVA significant at $p \le 0.01$. Different letters indicate significantly different values (DMRT, $p \le 0.05$) for a particular metal.

followed by *I. aquatica* and *M. quadrifolia*. While maximum accumulation of Cu (62), Mo (5) and Cr (13) was demonstrated by *Ipomoea* followed by *Hydrilla* (Cu: 55, Mo: 4.2 and Cr: 8.1 $\mu g\ g^{-1}$ dw). The accumulation order of Cd ($\mu g\ g^{-1}$ dw) was *Marsilia* (12) > *Ipomoea* (11) > *Hydrilla* (10) while that of As was *Ipomoea* (0.05) > *Marsilia* (0.04) > *Hydrilla* (0.03). Aquatic plants like *C. demersum*, *R. scloralus*, *H. aristata* and *E. crassipes* also demonstrated good potential for metal and metalloid accumulation. However, other two plants viz., *P. pectinatus* and *L. nodiflora* showed significantly low accumulation of various metals. One major advantage with *Hydrilla* and *Ceratophyllum* is the presence of a poorly developed/absence of a root system that makes them easily harvestable [17,19,20] and thus suitable as phytoremediator species. For rooted plants, partitioning of metal between root and shoot becomes an important feature to consider them suitable for phytoextraction purposes [15]. In the present study, two good accumulators viz., *Marsilia* and *Ipomoea* had a well-developed root system. *Marsilia* showed a root to shoot translocation factor of >1 for all the metals except Fe while *Ipomoea* showed >1 translocation factors for Hg, Cu, Cr, Mo, B and Cd. Hence, among rooted aquatic plants, *Marsilia* could be considered as the best choice for phytoremediation purposes as compared to *Ipomoea* for various heavy metals. However, Hg and Cr were accumulated in higher amounts by *Ipomoea* and were also translocated to the shoots more efficiently than that by *Marsilea*. Potential of aquatic plants for phytoremediation purposes is well recognized today [16,37,38]. Some aquatic plants including *Hydrilla*, *Ceratophyllum* and *Eichhornia* have been extensively studied by various authors and have been found to accumulate high levels of almost all the heavy metals investigated so far [17,19,20,39–42]. Various other studies demonstrate wastewater treatment and phytoremediation potential of plants like *Ipomoea* and *Marsilia* [43,44].

Among terrestrial plants, *E. alba* showed the maximum accumulation ($\mu g\ g^{-1}$ dw) of all heavy metals but Cd viz., Fe (847), Zn (48), Cu (22), Mo (5), B (21), Si (2560), Al (133), Pb (181), Cr (14), Hg (1.1) and As (0.06) followed by *Limnanthes*. Maximum accumulation ($\mu g\ g^{-1}$ dw) of Cd (69) was found in *E. ramosysma* (Fig. 2). Other terrestrial plants showed comparatively lower potential to accumulate various heavy metals. However, when the translocation factors of heavy metals from root to shoot were taken into consideration, both *Eclipta* and *Limnanthes* did not show >1 translocation factor for any of the metal except Hg, which was translocated to shoots by a factor of >4 in *Limnanthes*. In fact, the translocation factors were very low (<0.5) in both the plants except Si for which the translocation factors were between 0.5 and 1. *Equisetum* showed very high translocation factor of 40 for Cd. Hence, with exception of *Equisetum* for Cd, other terrestrial plants including the potential accumulators viz., *Eclipta* and *Limnanthes*, could not be considered suitable for phytoextraction purposes. There are no studies to the best of our knowledge demonstrating high metal accumulation potential of plants like *Eclipta* and *Limnanthes*, which showed good accumulation of metals in the present study. However metal absorption/adsorption ability of other terrestrial plants viz., *P. hysterophorus* [45], *Saccharum* [46], *E. ramosysma* [47,48] and *S. nigrum* [49,50] have been explored.

Between the 2 algal species viz., *S. biformis* and *P. papyraceum*, *Phormedium* showed the higher accumulation ($\mu g\ g^{-1}$ dw) of all the heavy metals investigated in the study viz., Fe (263), Zn (5), Cu (7), Mo (0.5), B (5), Si (408), Al (16), Pb (334), Cr (0.8), Cd (1), Hg (0.18) and As (0.015) (Fig. 3). Potential of both these algal species for heavy metal (Fe, Zn, Cu, Mn, Pb, Cr and Ni) accumulation has been demonstrated previously during analysis of algal samples collected from the Ganga water polluted through effluent coming from FA dykes of NTPC, Unchahar, Raebareli, India [22] and in algal samples collected from settling tanks of common effluent treatment plant, Unnao, India [21]. Other algal species like *Oscillatoria* and *Anabaena* have also been found to accumulate high levels of heavy metals [21,22].

In conclusion, the potential of terrestrial plants for heavy metal accumulation was found to be lower than that shown by aquatic plants in terms of their low ability to translocate heavy metals from root to shoot. However, as an integrated system these plants may be utilized for both phytoextraction and phytostabilization purposes. Further, their tolerance to FA makes them suitable for plantation and rehabilitation purposes of FA contaminated areas.

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