Environ Sci Pollut Res (2017) 24:22990–23005
DOI 10.1007/s11356-017-9930-4

RESEARCH ARTICLE

Biodiversity variability and metal accumulation strategies in plants spontaneously inhibiting fly ash lagoon, India

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Received: 26 May 2017 / Accepted: 7 August 2017 / Published online: 18 August 2017
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Abstract Out of 29 plant species taken into consideration for biodiversity investigations, the present study screened out *Cyperus rotundus* L., *Calotropis procera* (Aiton) W.T. Aiton, *Croton bonplandianus* Baill., *Eclipta prostrata* (L.) L., and *Vernonia cinerea* (L.) Less. as the most suitable metal-tolerant plant species (high relative density and frequency) which can grow on metal-laden fly ash (FA) lagoon. Total (aqua-regia), residual (HNO_3) and plant available ($CaCl_2$) metal concentrations were assessed for the clean-up of metal-contaminated FA disposal site using naturally colonized plants. The total metal concentration (in mg kg^{-1}) in FA followed an order of Mn (229.8) > Ni (228.4) > Zn (89.4) > Cr (61.2) > Pb (56.6) > Cu (51.5) > Co (41.9) > Cd (9.7). The HNO_3- and $CaCl_2$-extracted metals were 0.57–15.68% and 0.03–7.82% of the total metal concentration, respectively. The concentration of Ni and Cr in FA in the present study was highest among the previously studied Indian and average world power plants and Cd, Ni, and Cr were above soil toxicity limit. The variation in total, residual, and plant-available metal (single extraction) concentration indicated the presence of different proportions of metals in FA lagoon which affects the metal uptake potential of the vegetation growing on it. It has been reported that plant-available metal extractant ($CaCl_2$) is the most suitable extractant for assessment of metal transfer from soil to plant. However in the present study, Spearman's correlation showed best significant correlation between total metal concentration in FA and shoot metal concentration ($r = 0.840$; $p < 0.01$) which suggest aqua-regia as the best extractant for understanding the bioavailability and transfer of metal, and in calculation of BCF for moderately contaminated site. It can be stated that plant-available extractant is not always suitable for understanding the availability of metal, but total metal concentration can provide a better insight especially for moderate or low metal-contaminated sites. Principle component analysis revealed that all the plants showed positive correlation with Co and Cd which suggest its subsequent uptake in root and shoot. The biological indices (BCF, BAF, and TF) revealed that *E. prostrata* (10 mg Cd kg^{-1}) and *C. procera* (3.5 mg Cd kg^{-1}) can be utilized efficiently for the phytoextraction of Cd and phytostabilization of other potentially toxic metals (Pb, Cr, and Co) from FA lagoon. All the plants were tolerant to Pb pollution (TF > 1, BAF > 1, and BCF > 1); hence, there was a negligible translocation of Pb to the aerial tissues of these plants which shows their suitability in phytostabilization. In addition, *V. cinerea* accumulated elevated concentration of potentially toxic Cr (50 mg Cr kg^{-1}) and Ni (67 mg Ni kg^{-1}) which could also help in the phytoremediation of FA lagoon.

Keywords Bioaccumulation factor · Bioconcentration factor · Dominance indices · Metal extractants · Phytoremediation · Heavy metal

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Responsible editor: Elena Maestri

Electronic supplementary material The online version of this article (doi:10.1007/s11356-017-9930-4) contains supplementary material, which is available to authorized users.

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Introduction

An exceptional intensification in the demand of electricity during the past decades has surpassed all projections and has become a serious menace to the environment. In India, thermal power plants relying on combustion of coal meet this increased demand by contributing 59% of the total electricity production in the country (CEA 2017). The global discharge of fly ash (FA) by thermal power plants has reached to an extent of approximately 600 million tons per year (Ram and Masto 2014). The Indian coal is of low grade, having high ash content of the order of 30–45% (average 35%), generating large quantity of fly ash at coal/lignite-based thermal power stations in the country. For generating 1 MW of electricity using Indian coal, approximately 1800 t of FA is produced (CEA 2014), out of which 20% is wasted due to gravity which is removed as bottom ash and the residual part is FA. This FA is accumulated in ash ponds in the form of thin slurry by using hydraulic transportation techniques. These FA ponds empower global environmental issues by contaminating surrounding aquatic, aerial, and terrestrial ecosystems due to seepage, leaching, and wind erosion.

Phytoremediation is a useful technique in restoring the physical, chemical, and biological properties of metal-laden soils (Prasad 2011; Hattab et al. 2014; Sharma et al. 2015; Antoniadis et al. 2017). The identification of metal-tolerant species from plant communities affected by metal pollution through extensive biodiversity survey is a neglected domain though bioaccumulation/phytoextraction studies with wild plant varieties in FA-affected sites are available in recent literatures (Krgovic et al. 2015; Pandey et al. 2016). However, recent works suggest that screening the metal-tolerant plants following biodiversity sampling methods have better and more logical approach than randomly selected plants for bioaccumulation studies. Hernandez and Pastor (2008) have assessed the effect of metal pollution on the grassland biodiversity of abandoned metal mines in Sierra de Guadarrama, Spain, using biodiversity indices and concluded that Zn has the greatest effect on biodiversity followed by Cd, Cu, and Pb. Chowdhury and Maiti (2016) have compared the mangrove biodiversity of polluted and non-polluted sites to screen out four metal-tolerant halophytes (*Avicennia officinalis* L., *Cryptocoryne ciliata* (Roxb.) Fisch. ex Wydler, *Heliotropium curassavicum* L., and *Hemarthria altissima* (Poir.) Stapf & C.E.Hubb.) from a mangrove patch exposed to tannery effluent in Indian sundarbans. Similar ecological region exposed to varied level of pollution stress must affect the succession pattern, dominance, and community structure of the plants favoring pollution-tolerant species in stressed areas and normal assemblage in the pollution-free area. Replenishment of vegetation cover can reduce the horizontal or vertical spreading of the contaminants, erosion, and the risk of leaching of potentially toxic contaminants to groundwater. However, it is important to assess the availability of metal for proper establishment of plant community.

It is repeatedly reported in literature that total metal concentration gives an objectionable indicator of metal availability to plants whereas bioavailable/plant-available metal concentration gives better insight for assessment of metal transfer from soil to plant (Hammer and Keller 2002; Szarek-Lukaszewska and Niklinska 2002; Yoon et al. 2006; Remon et al. 2013; Pratas et al. 2013). The bioavailability of these metals can be assessed by evaluating the concentration of extractable metals that are available to plants for uptake. The various in situ methods for metal extraction to study the bioavailable and exchangeable metal concentration have been used by many researchers for studying the phytoavailability of heavy metals (Margui et al. 2006; Gajic et al. 2016). A variety of extractants have been used for accounting exchangeable, carbonate-bound, Fe–Mn oxides bound; organically bound; and residual metals present in FA (Jiao et al. 2016; Dar et al. 2017). $CaCl_2$ is known to be a suitable extractant for assessing the bioavailability of metals (Pueyo et al. 2004). Nitric acid being a strong extractant extracts metals from all fractions excluding the insoluble residual fraction (Lavado and Porcelli 2000).

In the present study, it was hypothesized that metal pollution must have selection pressure on a similar community, so it would affect the abundance of the species. And, this process could be used to identify/screen pollutant-tolerant plants by comparing a pollution-affected community with a non-polluted site. In addition, it is checked in the study whether really bioavailable extractant is the most suitable for assessment of metal transfer from soil to plant. Various researchers have assessed the total and bioavailable metal concentrations in FA lagoons/deposits of different thermal power plants (Kumari et al. 2016; Pandey and Mishra 2016). Considering the prominence of environmental problems caused by FA generated by thermal power plants, the present study aims at assessment of total, residual, and bioavailable/plant-available metal concentration present in the rhizospheric soil of different plants growing on the FA lagoon, ascertaining spontaneously colonized metal-tolerant plant species growing on FA lagoons by comparing the biodiversity of polluted site with control site. And finally, by using correlation matrix and biological indices, metal-tolerant plants were identified for phytoremediation of metal-contaminated FA lagoon.

Materials and methods

Study site description

Chandrapura thermal power station (CTPS) is located in the Chandrapura town of Bokaro district in the Indian state of Jharkhand (23°44′23.8″ N, 86°06′46.3″ E; Elevation:

222 m) and operated by Damodar Valley Corporation (Fig. 1). The CTPS has an installed capacity of 920 MW with five units under operation, out of which the first unit was commissioned in October, 1964. It covers an area of 750 ha and is bounded by Damodar river on the south. The climate is humid and sub-tropical characterized by hot and dry summer (maximum temperature reaches upto 46 °C) from March to October and cold winter from November to February.

Biodiversity and dominance assessment

A total of 14 quadrate plots (5 m × 5 m) were randomly selected out of which 7 were located in the control site (CS) and 7 in the FA-polluted site (Fig. 2). In the polluted site, quadrates were selected in the waste land, i.e., in the FA pond, whereas in the control site, they were randomly laid across the vegetation, i.e., the agricultural land in the vicinity of FA pond (about 30 m distance). Work done by Chowdhury and Maiti (2016) highlighted the importance of comparison of plant biodiversity between the community compositions of control and polluted site, prior to the investigation of metal accumulation in selected plant species. In the present study, the same methodology has been followed by comparing the diversity to screen out the plant varieties that have the highest tolerance to a particular stress. A total of 29 different plants from FA and CS were collected and identified, and the five most dominant plant species were selected based on relative density (RD) and frequency (F) for search of a phytostabilizer (in the present

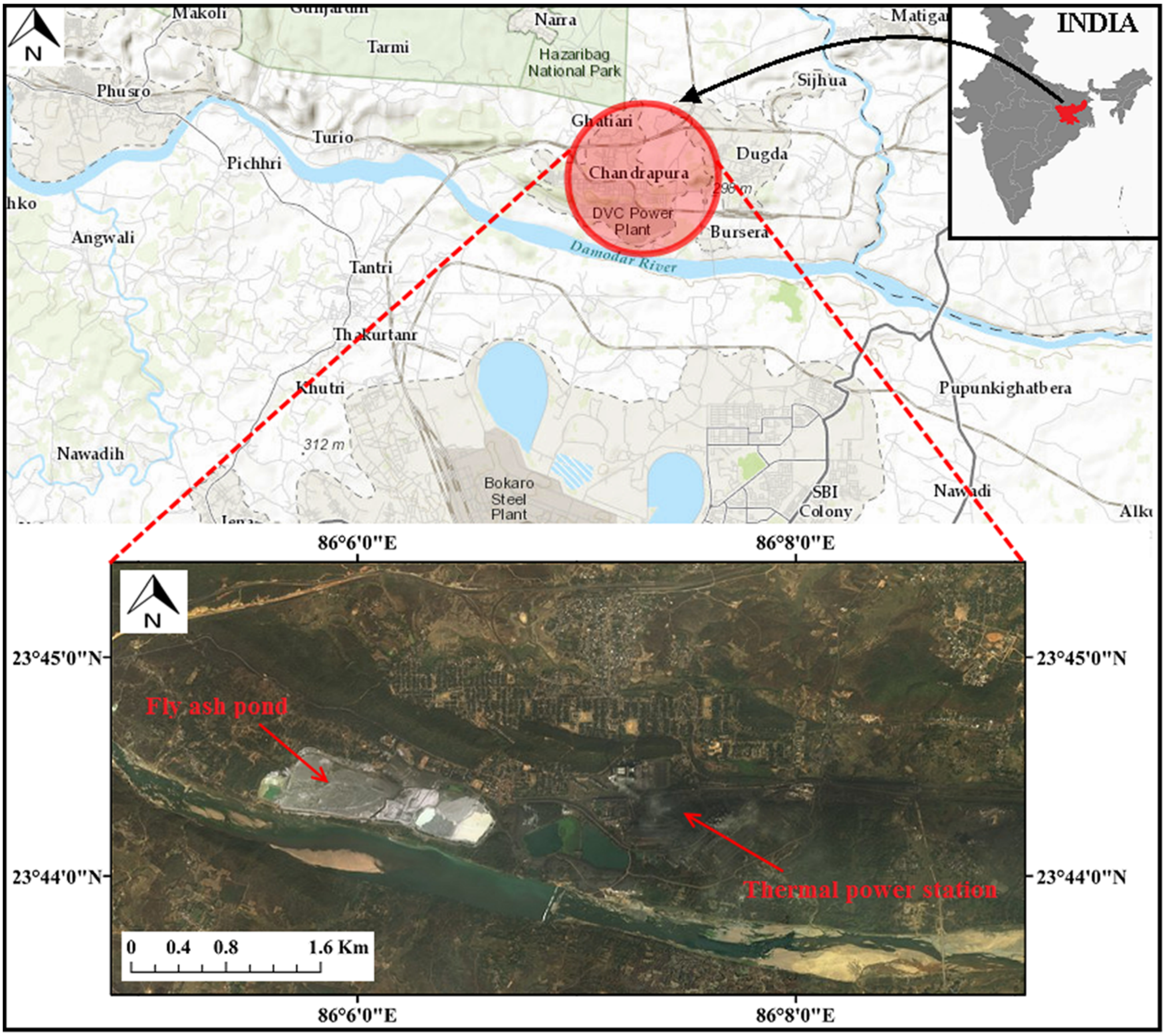

Fig. 1 Location map showing fly ash disposal site at Chandrapura Thermal Power Station, Jharkhand (India)

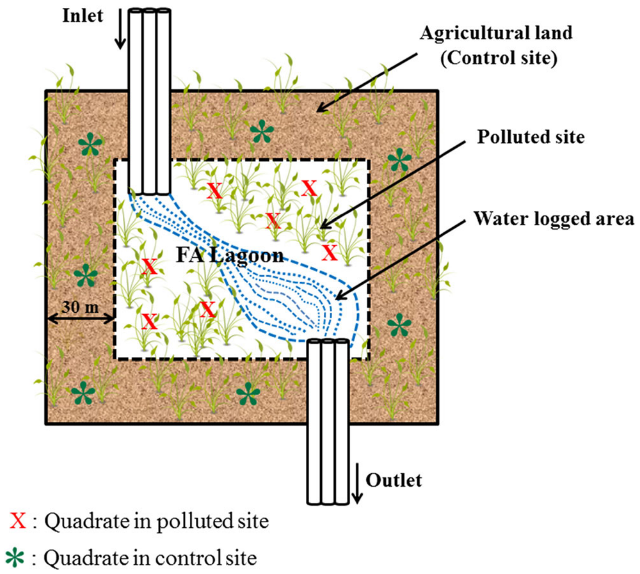

Fig. 2 Layout showing the sampling design from polluted and control site

study, plants having RD > 10% and F > 85% were considered).

Understory species and ruderals were only considered for biodiversity sampling. The relative density (%) of ruderals and biodiversity indices (Simpson's Index of Dominance and Diversity, Shannon-Weiner index) were calculated to access the community structure and biodiversity due to heavy metal pollution. Relative density (RD) is an estimate of the numerical strength of a species in relation to all the individuals of all the species, calculated as

$$\mathit{Relative\ Density}\ (\mathit{RD}\%) = \frac{\mathit{Number\ of\ individuals\ of}\ \text{a}\ \mathit{species}}{\mathit{Number\ of\ individuals\ of}\ \text{all}\ \mathit{the\ species}} \times 100$$

Frequency (F) is the distribution or dispersion of an individual species, estimated as percentage of occurrence:

$$\mathit{Frequency}\ (F\%) = \frac{\mathit{Number\ of\ quadrates\ the\ species\ occured}}{\mathit{Total\ number\ of\ quadrates\ studied}} \times 100$$

These estimators gives an idea of the status of a particular species in relation to the whole community, which was used in this study to understand the changes in distribution of the plant species between the control and FA-polluted site. Similarity in species composition between the control and polluted areas was calculated by Sorenson's coefficient of community (CC) (Smith and Smith 2012). This is a widely used index to study the similarity of two communities based on the presence/absence of a particular species, calculated as

$$\mathit{Sorenson}'\text{s}\ \mathit{coefficient\ of\ community}\ (\mathrm{CC}) = \frac{2C}{S1 + S2}$$

where C = common species in both the patches (i.e., polluted and control site), S1 = number of species in polluted site, and S2 = number of species in control site.

The health of the ecosystem was assessed using the Simpson's index for dominance (D), Simpson's index of diversity (D′) and Shannon Weiner Index (H′) (Simpson 1949).

Simpson's index for dominance (D) indicates whether the plant community is evenly distributed or has a biased distribution towards a particular or a group of species (plant association):

$$\mathit{Simpson}'\text{s}\ \mathit{index}\ \text{for}\ \mathit{dominance}\ (D) = \sum_{i=1}^{s} \frac{ni\ (ni-1)}{N\ (N-1)}$$

Due to the reciprocal relationship between diversity and dominance, the diversity of a particular species increases as the dominance decreases and vice versa. Thus, Simpson's index of diversity is calculated as

$$\mathit{Simpson}'\mathit{sindex\ of\ diversity}\ \left(D'\right) = 1-\mathrm{D}$$

where ni = total number of individual in each species; *N* = Total number of individual of all species; *S* = total number of species.

Shannon-Weiner Index (H′) evaluates the health and entropy of the ecosystem which is defined as

$$\mathit{Shannon-Weiner\ Index}\ \left(H'\right) = -\sum_{i=1}^{s} pi \times lnpi$$

where pi = ni/N and ln denotes the natural logarithm.

Collection and chemical characterization of FA and CS

A total of 25 FA samples were collected (five samples from the rhizosphere of each plant species) randomly from the top layer (0–15 cm) of FA lagoon in air tight polyethylene zipper bags and transported to the laboratory. The collected FA samples were air dried at room temperature (30–35 °C) for 1 week, homogenized using a mortar and pestle, and then passed through a 2-mm sieve for further analysis. The pH of FA was measured in FA: water suspension of 1:1 and 1:2.5 (*w*/*v*), electrical conductivity (EC) in 1:2.5 (*w*/*v*) FA: water suspension (stirring time: 2 h) by using a pH meter (HI 2020, HANNA Instruments, Woonsocket, Rhode island, USA) and conductivity meter (HI 2030, HANNA Instruments, Woonsocket, Rhode island, USA), respectively. Organic carbon was determined as per Walkley and Black (1934) and available nitrogen as per Subbiah and Asija (1956).

Metal analysis of FA

The bioavailability of metals in FA was determined by performing single extraction using 0.01 M $CaCl_2$ and 1 N HNO_3 with FA/extractant ratio of 1:10 *w*/*v* and shaking time of 2 h (Gupta and Sinha 2007a; Maiti 2007) and total metal concentration in FA was determined by digesting 0.5 g of homogenized air-dried FA sample using a mixture of HNO_3 (69%; EMPARTA) and HCl (~ 37%; EMPARTA) (3:1) (Maiti and Jaiswal 2008) in a microwave digester system (ETHOS 1, Milestone SrL, Sorisole, BG, Italy). Then, 1% HNO_3 solution was added to the digested mass to make up a volume of 50 mL and filtered using Whatman no. 42 filter (pore size 2.5 μm). The samples were refrigerated until analysis for the determination of metal (Cu, Mn, Zn, Pb, Ni, Cr, Co, and Cd) concentration using flame atomic absorption spectrophotometer (FAAS-GBC Avanta PM, Melbourne, Australia). When the concentration of metals was found below detection limit, it has been concentrated to 1:10. The detection limits were 0.001 mg L^{-1} for Cu, 0.0015 mg L^{-1} for Mn, 0.008 mg L^{-1} for Zn, 0.06 mg L^{-1} for Pb, 0.01 mg L^{-1} for Ni, 0.003 mg L^{-1} for Cr, 0.004 mg L^{-1} for Co, and 0.0004 mg L^{-1} for Cd.

Collection and metal analysis of naturally colonized plant species

Out of 29 different plant species, 5 most dominant species (identified on the basis of RD and F)—*Cyperus rotundus* L., *Calotropis procera* (Aiton) W.T. Aiton, *Croton bonplandianus* Baill., *Eclipta prostrata* (L.) L., and *Vernonia cinerea* (L.) Less.—were collected each in five replicates from the FA lagoon and transported to the laboratory. The collected plant samples were washed with running tap water and then put in an Ultrasonicator (Branson 2200, Bransonic Ultrasonic Corp. Danbury, CT, USA) with deionized water to remove the adhered FA particles. The aboveground and belowground tissues were separated and dried in a hot-air oven (RDHO 80, REMI Ltd., Mumbai, India) overnight at 80 °C to constant weight. The dried tissues were homogenized using a mortar and pestle for the processing of digestion. The dried and powdered portion of 0.5 g of plant tissue was digested with a mixture of HNO_3 and $HClO_4$ (5:1, *v*/*v*) (Maiti and Nandhini 2006) in a microwave digester system. After that, the volume was made up to 50 mL using 1% HNO_3 and filtered using Whatman no. 42 filter (pore size 2.5 μm). The samples were refrigerated until analysis. Metal (Cu, Mn, Zn, Pb, Ni, Cr, Co, and Cd) concentrations in plant tissues were measured using atomic absorption spectrophotometer.

Biological indices

The plant–metal interaction and metal uptake potential of different plant species was assessed using biological indices (Rani et al. 2017; Kumar et al. 2017). Biological concentration factor (BCF) of metals is calculated as the ratio of the metal concentration in root of the plant to the metal concentration in FA. The biological accumulation factor (BAF) is calculated as the ratio of the metal concentration in root and shoot of the plant to the metal concentration in FA. The translocation factor (TF) is defined as the ratio of the metal concentration in shoot to the metal concentration in root.

Quality assurance and quality control

All analytical grade reagents and calibrated glassware were used. Reagent blanks, duplicates, and spiked samples were used. Standard Reference Materials (AccuTrace, Accu Standard Inc., USA; Matrix 2–5% Nitric acid; CRM uncertainty ± 5%) were used for the preparation and calibration of each analytical batch. The recovery percentage of metals in the samples of soil and plant tissues ranged from 81 to 107%. Calibration coefficients were maintained at a high level ≥ 0.99. Limit of detection (LOD) values were calculated as three times the standard deviation of the blank samples for each measured element.

Statistical analyses

Data were checked for normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test). Kruskal-Wallis test was used to check the statistical significant difference between the mean values. Spearman's correlation coefficient (r) was performed at $p < 0.01$ and $p < 0.05$ level of significance. Principal component analysis (PCA) was performed for the concentrations of total metal accumulated in the roots and shoots of the plants to assess the behavior/relationship of metals with each other. Normalized variables (original variables) were transformed into the rotated components to extract significant principal components (PCs) by suppressing the contribution of variables with minor significance, after Kaiser–Meyer–Olken (KMO) test (> 0.7) and Bartlett's test (< 0.1) that determines the appropriateness of data reduction through PCA analysis. Furthermore, these PCs were subjected to varimax rotation with loading coefficients (> 0.1) to generate PC factors/groups. There is always a debate between the uses of orthogonal versus oblique rotation methods. Orthogonal rotation classifies the components/factors with the assumption that they are unrelated to each other. But in the case of metal clusters, unrelated components are easy to read, so varimax orthogonal rotation was used in the current study. All statistical calculations were performed using software SPSS, IBM statistics version 16.0 package and graphs were prepared using Origin 8.

Results and discussion

Effect of metal pollution on plant community composition

Understory and ruderal species are the pioneer species to colonize in a stressed and denuded landscape. Sorenson's coefficient of community (CC) value of 0.8 affirmed that both the FA-polluted site and control site possessed similar plant assemblage. A total of 29 species were recorded in the study site of which 10 species were absent in the polluted site (Table 1). The Dhoob grass (*Cynodon dactylon* (L.) Pers.) had highest RD of 10 with a 100% frequency. *Ageratum conyziodes* L., had a relative density of 7.76 and frequency 100%, followed by *Lantana camera* L. (RD = 6.9, F = 71%). All the three species generally showed invasive habit but in the control site, a low Simpson's index for dominance value (D = 0.04) indicated that the understory vegetation around the lagoon was not under invasion stress. In contrast in the polluted site, *E. prostrata* was the most abundant (RD = 18.9, F = 100%) followed by *C. bonplandianus* (RD = 12.6, F = 86%), *C. procera* (RD = 10.5, F = 86%), and *V. cinerea* (RD = 10.5, F = 86%). *C. procera* is an invasive species reported in Brazil and is highly tolerant to anthropogenic stress (Sobrinho et al. 2013; Frosi et al. 2013). The Simpson's index for dominance value (D) for the polluted site was 0.1, which was greater than that of the control site (0.04) and so the control site has showed more diversity than the polluted site. Similarly, control site has higher Simpson's index for diversity (D′ = 0.96) in comparison to polluted site (0.90).

Shannon-Weiner Index (H′) indicates the health of the ecosystem where the control site has a better H′ value of 3.04 compared to 2.18 of the polluted site. The control site has a healthier community structure than the polluted site. Many hypothesis are used to understand the changes in forest community structure in response to environmental factors that include geomorphological factors, climatic variability, physio-anatomical variations due to changing abiotic parameters, biotic factors such as herbivory, predation, parasitism, and animal plant interactions (Aston and Macintosh 2002; Smith and Smith 2012). Dominant species generally achieve their majority in the ecosystem at the expense of the non-competitive species (Smith and Smith 2012). Here, five species were seen to have the highest relative density (RD) in the polluted site (*C. procera*, *C. bonplandianus*, *C. rotundus*, *E. prostrata*, and *V. cinerea*). So, they must have tolerance towards metal pollutants at the vicinity of the FA lagoon and hence these five species have been selected for bioaccumulation studies. *C. rotundus* (family: Cyperaceae), a perennial grazable grass and a most invasive weed in tropical and temperate regions. *C. procera* (family: Apocynaceae) is a flowering plant and possess green globes which are hollow and contains toxic milky sap. *C. bonplandianus* (family: Euphorbiaceae), a medium-sized shrub which grows wild as a weed. *E. prostrata* (family: Asteraceae) is a small herbaceous plant finding extensive application in folk medicines and is found in tropical and sub-tropical regions of the world. *V. cinerea* (family: Asteraceae) is a medicinal plant having antibacterial and anti-inflammatory properties (Iwalewa et al. 2003).

Chemical characterization, total, residual, and bioavailable metals in FA

The $pH_{H2O}(1:1)$ (*w*/*v*) and $pH_{H2O}(1:2.5)$ (*w*/*v*) of rhizospheric FA was circumneutral (ranged between 5.31–7.04 and 5.54–7.39, respectively) (Table 2). The FA is generally alkaline in nature attributing due to the presence of sulfur in coal, hydroxides, and carbonates of calcium and magnesium. However, the slight alkaline nature of FA could be due to its weathering also. The EC_{H2O} (1:2.5) (*w*/*v*) ranged between 24.20 and 42.60 μS/cm. Taking an account of organic carbon and organic matter content in FA is vital as they impact other physical, chemical, and biological properties of the FA. The organic matter content also influences the availability of various metals for the uptake of plants. The organic matter and organic carbon content in the collected FA was below 1%. The low organic matter content can be attributed to the absence of plant litter as there was colonization of limited plants tolerant to anomalous metal concentration. The FA was found to be deficient in the N content. Overall, the control soil was better in nutrient content as compared to FA; however, in spite of low nutrient content, majority of plant species were spontaneously established on FA lagoon.

The rhizospheric FA showed anomalous concentration of metals when extracted using three different extractants. The total metal concentration (in mg kg^{-1}) in FA was in the order of Mn (229.8) > Ni (228.4) > Zn (89.4) > Cr (61.2) > Pb (56.6) > Cu (51.5) > Co (41.9) > Cd (9.7). In the present study, the average total metal concentration at CTPS, Jharkhand (India), along with the usual total metal concentration present in the FA generated around the world is compared in Table 3. A list of total metal concentration (mg kg^{-1}) in FA generated in different regions of India is also tabulated in Supplementary Table. The concentration of Ni and Cr in the present study was highest among the listed power plants. Concentration of Cd, Cr, and Ni were found above critical soil total concentration/permissible level (Alloway 1990, 2013; Kumar et al. 2017; González-Miqueo et al., 2010). Many researchers had

Table 1 Occurrence of understory and ruderal species in control and fly ash-polluted site along with their relative density (RD) and frequency (F)

Species	Family	Control site		Polluted site	
		RD	F	RD	F
Achyranthes aspera L.	Amaranthaceae	1.7	57	0	0
Ageratum conyzoides L.	Asteraceae	7.76	100	5.26	71
Amaranthus spinosus L.	Amaranthaceae	3.45	43	2.11	29
Amaranthus viridis L.	Amaranthaceae	3.45	43	1.05	29
Argemone mexicana L.	Papaveraceae	4.31	29	1.05	29
Azolla caroliniana Willd.	Azollaceae	0.86	14	6.32	71
Calotropis procera L.	**Asclepiadaceae**	**4.31**	**43**	**10.53**	**86**
Cassia tora L.	Caesalpiniaceae	4.31	86	1.05	29
Chenopodium album L.	Chenopodiaceae	3.45	43	0	0
Croton bonplandianus Baill.	**Euphorbiaceae**	**1.72**	**29**	**12.63**	**86**
Cynodon dactylon (L.) Pers.	Fabaceae	10.34	100	2.11	43
Cyperus rotundus L.	**Cyperaceae**	**2.59**	**29**	**16**	**100**
Datura metel L.	Solanaceae	1.72	43	0	0
Desmodium triflorum (L.) DC.	Fabaceae	5.17	86	0	0
Eclipta prostrata (L.) L	**Asteraceae**	**3.45**	**43**	**18.95**	**100**
Eragrostis tenella (Linn.) P Beauv.	Poaceae	0.86	29	0	0
Euphorbia hirta L.	Euphorbiaceae	1.72	71	1.05	29
Ipomoea carnea Jace.	Convolvulaceae	1.72	29	0	0
Lantana camara L.	Verbenaceae	6.9	71	5.05	71
Leucas aspera L.	Lamiaceae	6.03	86	1.05	71
Parthenium hysterophorus L.	Asteraceae	5.17	86	1.05	57
Phyla nodiflora (L.) Greene	Verbenaceae	2.59	57	0	0
Physalis minima L.	Euphorbiaceae	2.59	29	0	0
Saccharum spontaneum L.	Poaceae	4.31	71	2.11	43
Sida cordifolia L.	Malvaceae	2.59	29	1.05	14
Solanum nigrum L.	Solanaceae	1.72	29	1.05	29
Tephrosia purpurea (L.) Pers.	Fabaceae	0.86	14	0	0
Tridax procumbens L.	Asteraceae	0.86	29	0	0
Vernonia cinerea (L.) Less.	**Asteraceae**	**3.45**	**43**	**10.53**	**86**

Plants with high "RD" and "F" were annotated in bold

RD relative density in percentage, *F* frequency in percentage

reported that estimation of total metal concentration does not always provide the exact information about mobility, bioavailability of metals, and potential toxicity and hence it is important to analyze bioavailable metal concentrations using different extractants such as $CaCl_2$ and nitric acid (Remon et al. 2013; Wojcik et al. 2014). Nevertheless, this justification mainly suits for highly metal-contaminated sites. In case of low or moderately contaminated sites like FA lagoon, it is essential to check the correlation between the metal concentration in substrate and in plant tissues.

To identify the best extractant suitable for assessment of bioavailability of metal in substrate and its subsequent transfer from substrate to plant, three different extractants (aqua-regia, HNO_3 and $CaCl_2$) were used. The $CaCl_2$-extracted fraction was very less and accounted for 0.34–0.51% for Mn, 0.86–1.75% for Ni, 0.28–1.29% for Zn, 0.55–0.72% for Cr, 5.28–7.80% for Pb, 0.03–0.06% for Cu, 0.40–2.15% for Co, and 0.19–1.79% for Cd of the total metal concentration and does not demonstrate any correlation with metal concentration in plant tissues. Similarly, HNO_3 showed better extracting capability than did $CaCl_2$ and accounted for 6.15–10.38% for Mn, 1.89–2.33% for Ni, 3.15–3.90% for Zn, 0.57–0.83% for Cr, 8.4–15.6% for Pb, 5.39–6.48% for Cu, 2.81–3.85% for Co, and 0.57–2.50% for Cd and showed a low level significant correlation with metal concentration in shoot. The total metal concentration extracted by aqua-regia (total) was much greater than the bioavailable metal fractions obtained by using HNO_3 and $CaCl_2$ (Table 4). Spearman's correlation showed best significant correlation ($r = 0.786$; $p < 0.05$) between total metal

Table 2 Chemical characteristics of fly ash and control soil collected from rhizosphere of plant species growing at CTPS, Jharkhand (India), and control site

Substrate	Parameter	*C. rotundus*	*C. procera*	*C. bonplandianus*	*E. prostrata*	*V. cinerea*
Flyash	pH_{H2O} (1:2.5) (*w*/*v*)	7.17 ± 0.15a (7.00–7.39)	6.23 ± 0.15b (6.06–6.45)	6.96 ± 0.09a (6.88–7.11)	7.16 ± 0.12a (7.04–7.33)	6.03 ± 0.35b (5.54–6.36)
Control soil		6.24 ± 0.06a (6.18–6.32)	5.87 ± 0.09bc (5.76–5.99)	6 ± 0.12b (5.85–6.14)	6.16 ± 0.07a (6.05–6.23)	5.80 ± 0.14c (5.65–5.98)
Fly ash	EC_{H2O} (1:2.5) (*w*/*v*); μS/cm	35.92 ± 3.69b (30.20–39.50)	40.57 ± 1.71a (38.07–42.60)	28.46 ± 2.24c (27.20–32.10)	28.92 ± 2.98c (24.20–32.20)	30.92 ± 1.97c (27.80–33.20)
Control soil[a]		71.31 ± 7.75 (62.99–83.12)	77.68 ± 7.01 (69.32–87.34)	74.15 ± 7.86 (65.93–83.12)	70.99 ± 5.85 (63.98–78.38)	76.76 ± 9.53 (67.21–90.23)
Fly ash	Organic carbon (%)	0.51 ± 0.03b (0.47–0.56)	0.52 ± 0.02b (0.51–0.56)	0.49 ± 0.02b (0.47–0.52)	0.37 ± 0.02c (0.35–0.41)	0.58 ± 0.02a (0.57–0.62)
Control soil[a]		0.59 ± 0.12 (0.43–0.73)	0.61 ± 0.09 (0.47–0.71)	0.60 ± 0.09 (0.51–0.73)	0.58 ± 0.12 (0.43–0.73)	0.62 ± 0.11 (0.47–0.78)
Fly ash	Organic matter (%)	0.89 ± 0.06b (0.81–0.97)	0.91 ± 0.05b (0.84–0.97)	0.85 ± 0.03b (0.81–0.90)	0.67 ± 0.04c (0.60–0.71)	1.01 ± 0.03a (0.98–1.07)
Control soil[a]		1.03 ± 0.21 (0.74–1.25)	1.06 ± 0.16 (0.81–1.22)	1.04 ± 0.16 (0.87–1.25)	1.01 ± 0.20 (0.74–1.25)	1.07 ± 0.20 (0.81–1.34)
Fly ash[a]	Available N (mg kg^{-1})	103.50 ± 11 (91.46–118.34)	110.60 ± 12.46 (96.23–124.38)	105.20 ± 11.77 (92.74–119.22)	117.30 ± 9.79 (105.43–129.54)	108.80 ± 12.87 (96.67–128.43)
Control soil		331.60 ± 15.45c (310.11–347.22)	382.80 ± 9.74a (371.99–398.21)	344.50 ± 15.96c (326.45–362.12)	391.50 ± 13.99a (371.89–404.18)	362.60 ± 10.87b (349.12–378.62)

Values are represented as mean ± standard deviation of five replicates. Values in parenthesis represent the range. EC is electrical conductivity. Different alphabetical letters in the same row indicate significant difference at $p < 0.05$;

[a] Represents statistical similarity of a parameter in a row

concentration in FA and shoot metal concentration which suggest aqua-regia as the best extractant for understanding the bioavailability and transfer of metals, and in calculation of BCF for moderately contaminated site (Table 5). Strong significant correlation ($r = 0.929$; $p < 0.01$) observed between metal concentration in roots and shoots shows

Table 3 Total metal concentration (mg kg^{-1}) in FA generated around the world, India, and in the present study

Location of TPP	Metal concentration								Reference
	Mn	Zn	Cu	Pb	Cd	Co	Ni	Cr	
Pocerady, North Bohemia, Prague	Na	261	69.2	30.2	1.21	28.4	28.4	52.6	Kapicka et al. (1999)
Nikola Tesla thermal power station, Serbia	812	125	225	126	4	na	na	na	Pavlovic et al. (2004)
Can Thermal Power Plant, Turkey	na	117.5	72	38.1	0.3	31.5	26.5	na	Baba et al. (2008)
India average	76.5–328.2	15.8–165.0	18.8–115.0	10.7–120.0	1.5–42.5	0.5–49.0	20.8–204.8	12.5–40.3	–
World average	na	14–13,000	30–3020	3.1–1600	0.1–250	na	na	na	Tripodi and Cheremissinof (1980); Baba (2000)
Chandrapura Thermal Power Station (Jharkhand), India	229.8	89.4	51.5	56.6	*9.7*	41.9	*228.4*	*61.2*	Present study
Critical soil total concentration/-permissible level of heavy metal	1500–3000	200	63	70	1.3	25–50	100	60	Alloway (1990, 2013), Kumar et al. (2017); González-Miqueo et al. (2010)

TPP thermal power plants

Table 4 Metal concentration (mg kg^{-1}) extracted using aqua-regia, nitric acid, and calcium chloride in FA samples of CTPS, Jharkhand (India) (mean ± SD)

Plant species	Cu	Mn	Zn	Pb	Ni	Cr	Co	Cd
Aqua-regia-extracted metal concentration								
C. rotundus	51.44 ± 0.64b (50.60–52.10)	269.70 ± 3.88a (264.20–274.80)	84.86 ± 2.13^{a} (82.40–87)	53.26 ± 2.14c (50.2–55.3)	218.62 ± 16.37^{a} (199.60–236)	59.92 ± 2.30c (56–62)	45.52 ± 2.61a (43–49.40)	10.76 ± 0.95a (9.2–11.6)
C. procera	52 ± 2.23b (49–55)	239.34 ± 10.83b (224.60–253.70)	88.02 ± 3.82^{a} (82.40–93)	56.98 ± 1.98ab (54.3–59.4)	214.72 ± 25.28^{a} (186.80–249.60)	55.32 ± 0.54d (54.80–56)	39.96 ± 3.74b (36–45.20)	7.8 ± 0.44b (7.20–8.40)
C. bonplandianus	47.36 ± 1.77c (45–49.80)	234.44 ± 8.69b (224.10–246.60)	84.7 ± 11.00^{a} (66.20–92.40)	59.78 ± 2.40a (57.3–62.9)	241.88 ± 16.52^{a} (217.40–257.40)	60.10 ± 2.49c (57.40–63.80)	46.14 ± 2.00a (43.20–48)	10.14 ± 1.04a (9–11.60)
E. prostrata	56.16 ± 0.86a (55–57.20)	201.16 ± 10.54c (182.60–208.40)	98.12 ± 13.38^{a} (86–118.40)	55.72 ± 2.97bc (52.3–59.3)	236.58 ± 20.11^{a} (216.70–257.40)	68.04 ± 1.68a (66 .0–70.0)	41.92 ± 0.67b (41.20–42.80)	9.72 ± 0.67a (9.0–10.40)
V. cinerea	50.88 ± 2.38b (47–53.40)	204.34 ± 4.68c (199.60–211.20)	91.76 ± 3.70^{a} (86.20–95.20)	57.52 ± 2.62ab (54.3–60.9)	230.54 ± 8.37^{a} (219.60–239.40)	62.84 ± 2.30b (60–66)	36.28 ± 3.03c (31.80–39.60)	10.52 ± 0.22a (10.20–10.80)
HNO$_3$-extracted metal concentration								
C. rotundus	3.2 ± 1.05^{a} (2.20–4.80)	16.60 ± 6.75c (9.94–23.66)	3.02 ± 1.47^{a} (1.96–5.52)	5.20 ± 1.33b (4.28–7.50)	4.54 ± 0.78b (3.96–5.92)	0.44 ± 0.01c (0.42–0.46)	1.39 ± 0.15^{a} (1.14–1.52)	na
C. procera	3.30 ± 0.16^{a} (3.10–3.50)	22.42 ± 0.75a (21.60–23.52)	3.44 ± 0.02^{a} (3.42–3.48)	8.94 ± 1.63a (6.24–10.24)	4.11 ± 0.85b (2.74–4.92)	0.32 ± 0.02d (0.30–0.36)	1.25 ± 0.08^{a} (1.16–1.36)	0.20 ± 0.02a (0.18–0.24)
C. bonplandianus	3.07 ± 0.12^{a} (2.90–3.21)	17.33 ± 0.63bc (16.54–18.10)	2.71 ± 0.22^{a} (2.48–3.00)	5.03 ± 0.35b (4.62–5.56)	4.58 ± 0.21b (4.28–4.82)	0.50 ± 0.02b (0.48–0.53)	1.30 ± 0.06^{a} (1.22–1.36)	0.11 ± 0.02b (0.08–0.14)
E. prostrata	3.03 ± 0.14^{a} (2.86–3.20)	17.81 ± 1.42bc (15.98–19.74)	3.1 ± 0.33^{a} (2.68–3.52)	4.97 ± 0.22b (4.68–5.22)	4.34 ± 0.30b (4.00–4.76)	0.54 ± 0.01a (0.52–0.56)	1.36 ± 0.07^{a} (1.26–1.44)	0.08 ± 0.01c (0.07–0.10)
V. cinerea	3.00 ± 0.17^{a} (2.80–3.22)	21.22 ± 1.30ab (19.70–22.84)	3.33 ± 0.27^{a} (3.02–3.70)	6.00 ± 0.35b (5.42–6.28)	5.38 ± 0.28a (4.90–5.64)	0.52 ± 0.03ab (0.48–0.56)	1.40 ± 0.06^{a} (1.30–1.46)	0.06 ± 0.01d (0.04–0.08)
CaCl$_2$-extracted metal concentration								
C. rotundus	0.03 ± 0.01^{a} (0.02–0.05)	0.94 ± 0.35ab (0.51–1.46)	1.01 ± 0.12a (0.93–1.22)	4.17 ± 0.79a (3.24–5.40)	3.25 ± 0.32a (2.82–3.72)	0.41 ± 0.07^{a} (0.33–0.50)	0.63 ± 0.03b (0.60–0.69)	na
C. procera	0.03 ± 0.01^{a} (0.02–0.05)	0.72 ± 0.04b (0.65–0.77)	0.31 ± 0.12 cd (0.21–0.50)	3.73 ± 0.32ab (3.36–4.07)	3.77 ± 0.38a (3.32–4.31)	0.40 ± 0.07^{a} (0.35–0.53)	0.86 ± 0.12a (0.66–0.99)	0.14 ± 0.03a (0.11–0.20)
C. bonplandianus	na	1.16 ± 0.09a (1.02–1.28)	0.40 ± 0.05c (0.33–0.47)	3.16 ± 0.12b (3.03–3.35)	2.09 ± 0.24b (1.73–2.32)	0.36 ± 0.03^{a} (0.32–0.40)	0.34 ± 0.06c (0.27–0.42)	0.02 ± 0.008b (0.02–0.08)
E. prostrata	0.02 ± 0.01^{a} (0.01–0.03)	1.04 ± 0.21a (0.68–1.23)	0.56 ± 0.09b (0.47–0.68)	3.71 ± 0.43ab (3.20–4.40)	2.62 ± 0.66b (1.79–3.39)	0.38 ± 0.02^{a} (0.36–0.41)	0.20 ± 0.05d (0.15–0.27)	0.15 ± 0.04a (0.11–0.21)
V. cinerea	0.03 ± 0.007^{a} (0.02–0.04)	0.70 ± 0.04b (0.63–0.74)	0.26 ± 0.03d (0.24–0.32)	3.83 ± 0.23a (3.47–4.07)	3.22 ± 0.44a (2.45–3.54)	0.38 ± 0.04^{a} (0.33–0.44)	0.42 ± 0.08c (0.29–0.53)	0.16 ± 0.01a (0.14–0.18)

Values are represented as mean ± standard deviation of five replicates. Different alphabetical letters in the same column for the same extraction method indicates significant difference at $p < 0.05$. Values in the parenthesis indicates the range

CS control site, *na* not analyzed

[a] Not significant at 0.05 level of significance

Table 5 Spearman's correlation coefficient between mean metal concentrations in root, shoot with extracted mean metal concentrations by aqua-regia (AR), nitric acid (NA), and $CaCl_2$ (CC) in fly ash (FA)

	Root	Shoot	FA (AR)	FA (NA)	FA (CC)
Root	1	0.929[b]	0.667	0.667	0.690
Shoot		1	0.786[a]	0.738[a]	0.619
FA (AR)			1	0.667	0.595
FA (NA)				1	0.690
FA (CC)					1

[a] Correlation is significant at the 0.05 level of significance

[b] Correlation is significant at the 0.01 level of significance

transfer of metals from root to shoot and phytoextraction ability of plant. A low significant correlation was also observed between HNO_3-extractable concentration and shoot metal concentration.

Metal accumulation by five dominant plant species

A total of 29 plant species belonging to 17 different families were observed on control site, out of which the same 20 metal-tolerant plant species from 16 different families were naturally colonized on FA lagoon. Five plant species were selected on the basis of its higher relative density and frequency and analyzed for metal accumulation capacity to check its suitability in phytoremediation of FA lagoon. The root and shoot portions of the plants growing on FA lagoon accumulated varying concentration of different metals probably due to plant's metabolism and mechanisms driving the uptake of metals from the FA (Table 6).

In the case of potentially toxic but essential metals (Cu, Mn, Zn, and Ni), among all five plants, *C. rotundus* had accumulated maximum concentration of 28.50 mg Cu kg^{-1}; however, in the case of shoot, it was maximum for *C. procera* (20.28 mg Cu kg^{-1}). The Cu concentration in the root or shoot portions was within the safe limits as per Alloway (1990) and Kabata and Pendias (2001). The critical Mn concentration in plants range between 300 and 500 mg kg^{-1} above which toxic effects are likely to occur (Alloway 1990); however, all the plants were below toxicity levels and observed maximum in the shoot portion of *V. cinerea* (106.16 mg kg^{-1}). Availability of Zn to plants depends upon pH, organic matter, moisture regime, microbial activity, and adsorption sites and the normal concentration of Zn in plants ranges between 10 and 300 mg kg^{-1} (Alloway 1990). The present study revealed the highest Zn concentration in the roots of *E. prostrata* (152.62 mg kg^{-1}), and its concentration in all the plants were within safe limits. Due to deficiency of organic matter and slightly acidic pH, the Zn was not much accumulated by plants and TF_{Zn} < 1 for

Table 6 Total metal accumulation (mg kg^{-1}) in root and shoot of five dominant plant species growing on FA (Mean ± SD; $n = 5$)

Metal	*C. rotundus*		*C. procera*		*C. bonplandianus*		*E. prostrata*		*V. cinerea*	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Cu	28.50 ± 2.38a	13.14 ± 2.59B	11.84 ± 2.26c	20.84 ± 2.41A	5.18 ± 0.83d	9.82 ± 1.02C	28.48 ± 1.55a	19.26 ± 2.28A	18.56 ± 2.26b	10.44 ± 1.45 BC
Mn	17.40 ± 3.61b	69.40 ± 9.26B	18.62 ± 3.92b	43.18 ± 4.70C	18.18 ± 2.11b	23.06 ± 3.02D	21.68 ± 3.20b	41.62 ± 2.56C	40.12 ± 5.86a	106.16 ± 7.55A
Zn	61.20 ± 4.21b	40.58 ± 4.02D	25.66 ± 3.06d	57.66 ± 5.10C	59.12 ± 4.05b	42.44 ± 2.89D	152.62 ± 7.04a	68.82 ± 4.40B	45.16 ± 3.38c	77.86 ± 2.95A
Pb	26.94 ± 2.13b	18.54 ± 1.43B	38.56 ± 2.76a	25.50 ± 2.10A	26.08 ± 2.77b	24.08 ± 3.26A	17.60 ± 2.09d	6.70 ± 1.33D	22.38 ± 3.29c	15.44 ± 2.43C
Ni	71.62 ± 10.66a	25.84 ± 2.55C	38.38 ± 3.15b	66.24 ± 2.57 A	67.46 ± 3.64 a	57.12 ± 4.12B	63.58 ± 4.55a	65.56 ± 3.90A	67.36 ± 3.30a	66.50 ± 3.17A
Cr	4.06 ± 0.47c	2.18 ± 0.41C	2.40 ± 0.28c	4.10 ± 0.52B	4.32 ± 0.17c	2.64 ± 0.20C	14.60 ± 1.72b	4.24 ± 0.50B	34.28 ± 4.66a	16.32 ± 2.01A
Co	15.42 ± 1.81b	10.86 ± 1.04B	5.56 ± 1.55d	9.88 ± 1.01B	10.84 ± 1.19c	10.92 ± 1.16B	35.61 ± 3.43a	11.20 ± 1.07B	11.36 ± 1.24c	13.12 ± 1.19A
Cd	5.30 ± 0.48a	4.04 ± 0.61B	1.44 ± 0.15d	2.32 ± 0.14C	3.94 ± 0.45bc	4.28 ± 0.62B	3.48 ± 0.49c	6.70 ± 1.02A	4.06 ± 0.20b	2.74 ± 0.32C

Different small and capital letters in the same row represent significant difference between the five dominant plants for root and shoot, respectively, at $p < 0.05$

C. rotundus, *C. bonplandianus*, and *E. alba* further suggests its hindered mobility in plant shoot. TF > 1 for Mn in all the studied plants and in most of the plants in case of Zn suggests its transfer from root to shoot which is essential for plant growth. The mobility of Ni in the soil increases with a decrease in the pH and cation exchange capacity. Tripathi et al. (2004) demonstrated Ni accumulation in *Cassia siamea* Lamk growing on FA between 60 and 120 mg Ni kg^{-1}, and the normal and critical concentration range of Ni in plants reported by Alloway (1990) were 10–50 and 10–100 mg kg^{-1}, respectively. In the present study, the range of Ni concentration in plants was between 25 and 72 mg Ni kg^{-1} which was within the safe limits. Overall, all the potentially toxic but essential metals (Cu, Mn, Zn, and Ni) were accumulated in significant quantity but within the safe limits which further helps in plant growth and development.

In case of other potentially toxic metals (Pb, Co, Cr, and Cd), maximum concentration was for Pb followed by Co > Cr > Pb. The normal range of Pb in plants, i.e., 0.2–20 mg kg^{-1} (Alloway 1990), and the critical Pb concentration (30–300 mg kg^{-1}) above which toxicity effects are likely to occur has not been surpassed by any of the plant species (Alloway 1990). However, significant concentration of metal was accumulated in plants with maximum concentration of Pb in the roots of *C. procera* (38.56 mg kg^{-1}). Cr was found to have its maximum concentration in the roots of *V. cinerea* (34.28 mg kg^{-1}). Along with this, shoots of *V. cinerea* (16.32 mg kg^{-1}) and roots of *E. prostrata* (14.60 mg kg^{-1}) exhibited metal toxicity due to Cr as per limits defined by Shanker et al. (2005). Cr concentration above 5 mg kg^{-1} sometimes results in the necrosis, chlorosis, and detrimental effects on the plants (Shanker et al. 2005; Kumar et al. 2017). Maximum concentration of Co was in the roots of *E. prostrata* (35.61 mg kg^{-1}) which indicates all the plant species are within safe limits. Cadmium is an effective inhibitor of photosynthesis (Krupa et al. 1993) so it becomes toxic if its concentration is high. The maximum Cd concentration was in shoot of *E. prostrata* (6.70 mg kg^{-1}) while the lowest Cd concentration in roots of *C. procera* (1.44 mg kg^{-1}).

Principal component analysis (PCA) was performed only for more potentially toxic metals (Pb, Cr, Co, and Cd) which could be possibly harmful for human health if exposed in higher concentration. The PCA revealed three principal components with eigenvalues > 1 were extracted, together explaining about 74.13% of total variability in the data (Fig. 3). PC1 has high positive correlation with Cd and Co and negative correlation with Cr and Pb. Except for *V. cinerea* and *E. prostrata*, all the shoots and roots of other three studied plant species showed high positive correlation with Cd and Co. However, all the plants showed positive correlation with Co and Cd which suggest its subsequent uptake in root and shoot. PC2 shows high positive correlation of Cr with Cd and Co. Among shoot part of all five plants, maximum accumulation of Cd was found in *E. prostrata* with high correlation of Cd and its subsequent uptake in shoots of *E. prostrata.*

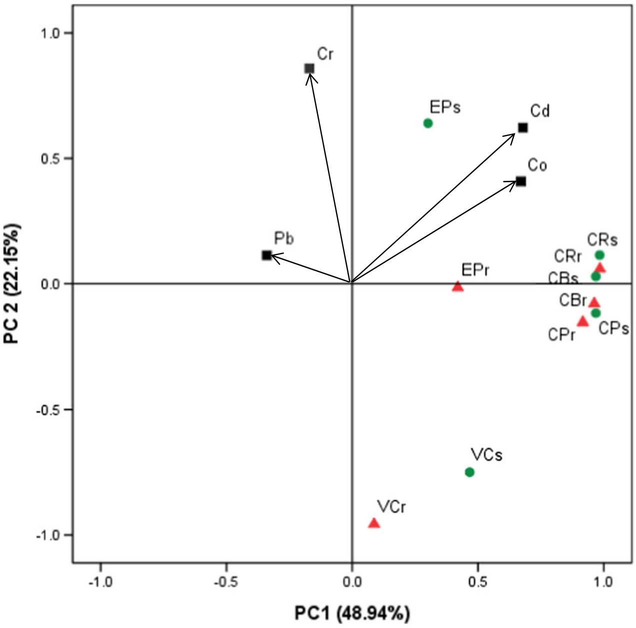

Fig. 3 Principal component (PC) analysis ordination diagram grouping five plant species with respect to their metal concentration (Pb, Cr, Co, and Cd) in shoot and root. Numbers in the parenthesis show percent of explained variance. *CRr C. rotundus* root. *CRs C. rotundus* shoot. *CPr C. procera* root. *CPs C. procera* shoot. *CBr C. bonplandianus* root. *CBs C. bonplandianus*. *EPr E. prostrata* root. *EPs E. prostrata* shoot. *VCr V. cinerea* root. *VCs V. cinerea* shoot

Biological indices for assessment of potentiality of studied plants in phytoremediation

To understand the potentiality of plants in phytoremediation of metal-polluted sites, three different factors are generally evaluated, bioconcentration factor (BCF) of root, biological accumulation factor (BAF) of shoot, and translocation factor (TF) (Figs. 4a, b and 5). The mobility of toxic and non-essential metals from belowground tissues to aboveground tissues is restricted as they are not much required by the plants and gets sequestered in the vacuoles of the roots which favor the plant's behavior against heavy metal toxicity. However, various other properties of the rhizospheric FA drive the uptake of metals by plant roots such as pH, electrical conductivity, plant-associated bacteria, mycorrhizal fungi, etc. (Redon et al. 2009; Alloway 2013). The BCF of metals in root portion of a plant indicates the phytoextraction potential of plants from the FA. The BCF values were in the range of 0.1–0.5 for Cu, 0.1–0.2 for Mn, 0.7–1.6 for Zn, 0.5–0.7 for Pb, 0.2–0.4 for Ni, 0.1–0.6 for Cr, 0.2–0.9 for Co, and 0.2–0.4 for Cd. In the case of BAF, the values ranged between 0.3 and 0.8 for Cu, 0.2 and 0.7 for Mn, 0.9 and 2.3 for Zn, 0.4 and 1.1 for Pb, 0.4 and 0.6 for Ni, 0.1 and 0.8 for Cr, 0.4 and 1.1 for Co, and 0.5 and 1.0

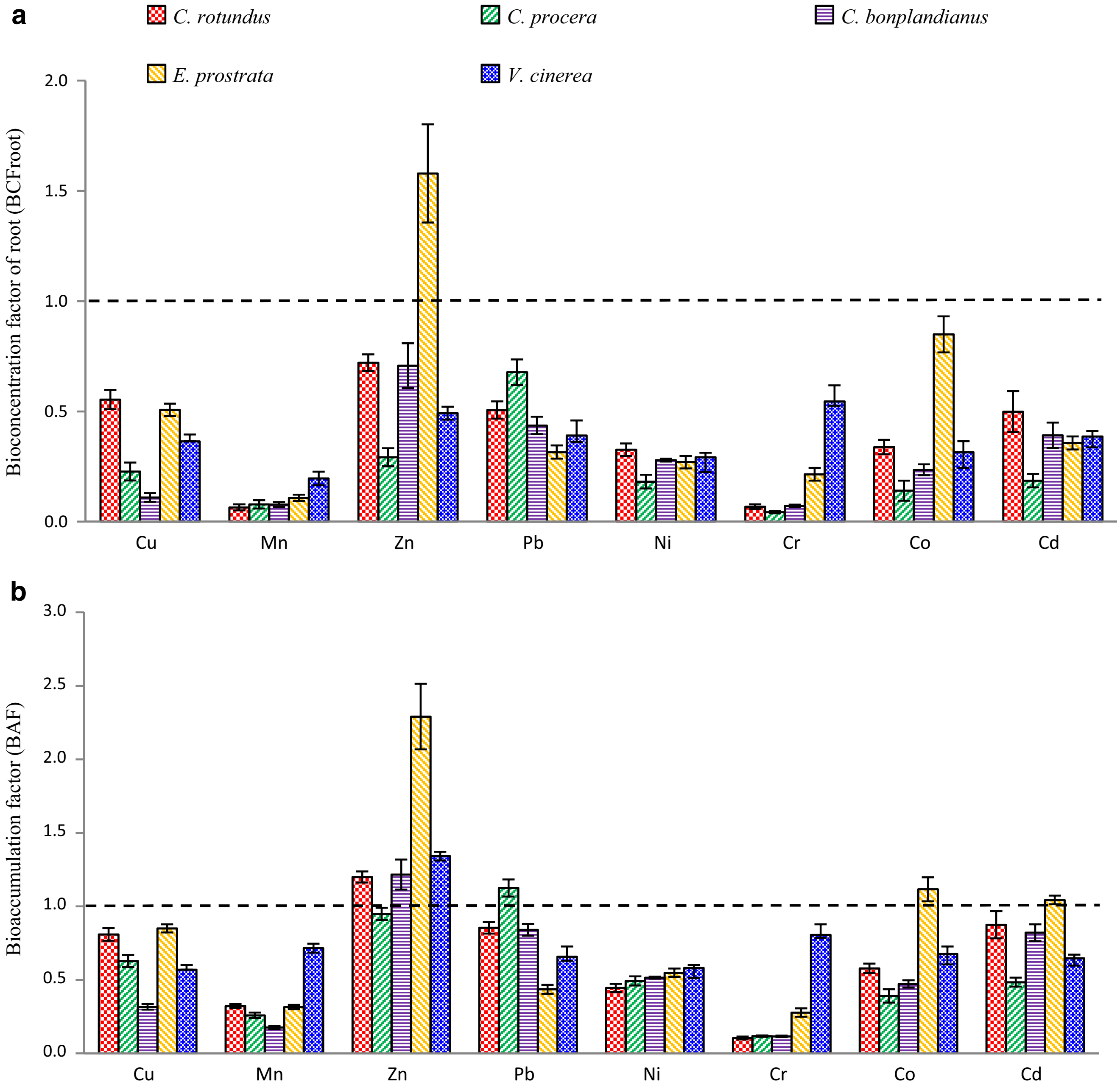

Fig. 4 **a** Bioconcentration factors of root (BCF) and **b** bioaccumulation factor (BAF) of metals in the five most dominant plant species growing on FA lagoon (*n* = 5; mean ± standard deviation)

for Cd. The TF of different metals in plants under investigation ranged between 0.30 (Cr in *E. prostrata*) to 4.12 (Mn in *C. rotundus*). The highest BCF_{root} (0.55) of Cu was in *C. rotundus*, and the highest BAF (0.85) of Cu was observed in *E. prostrata*. The accumulation of Cu in shoots of *C. procera* have been confirmed by several researchers (Gupta and Sinha 2007b, 2008; Ahmad et al. 2011; Kumar et al. 2013) which was confirmed in the present study by considerable BAF (0.63) of Cu in *C. procera* in spite of low BCF_{root} (0.23). Except for Zn, such low BCF and BAF values clearly indicate the excluding strategy in all plant species naturally inhabiting the waste deposits studied. In most of the plant species, low BCF of Mn and Zn was found due to its translocation to the shoot tissue (TF > 1). In fact, BCF and BAF is a function of both the metal transfer from the soil to the plant root and root to shoot translocation efficiency, and thus, it depends not only on the metal concentration in the growth medium but also on its mobility (Wojcik et al. 2014). BCF_{root} of Cr in *C. rotundus*, *C. procera*, *E. prostrata*, and *V. cinerea* was found high in comparison to BAF due to its low

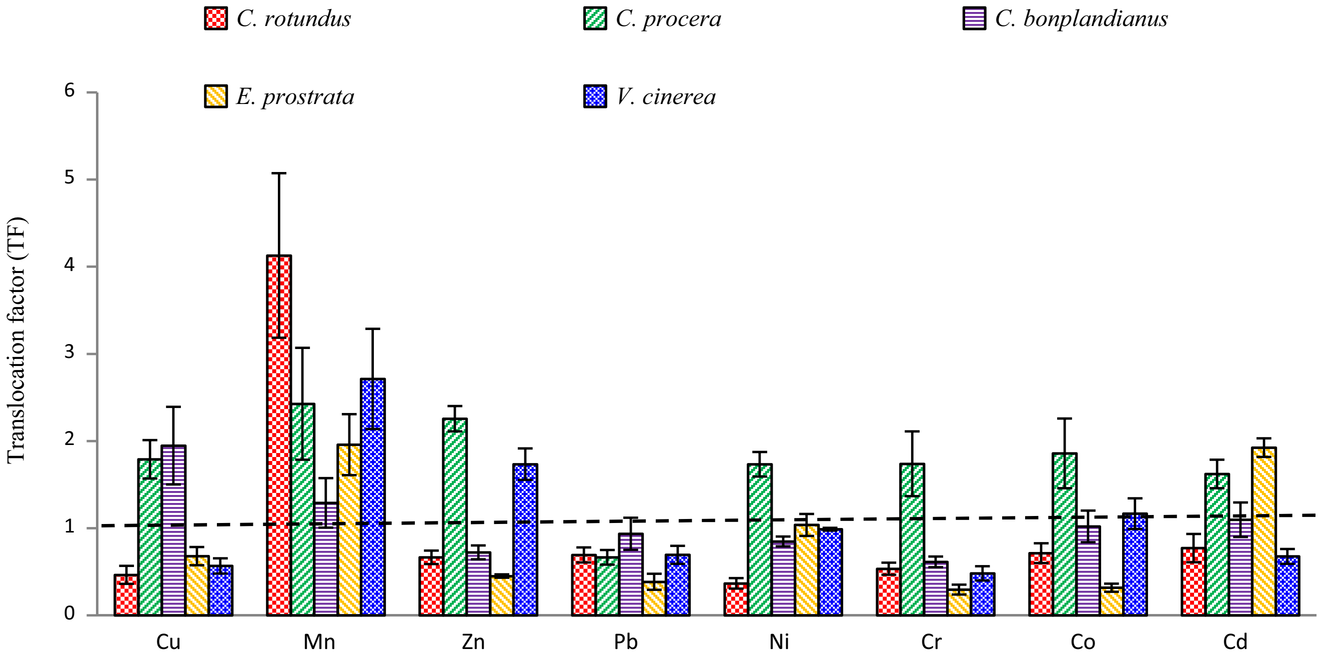

Fig. 5 Translocation factor (TF) of metals in five most dominant plant species growing on FA lagoon ($n = 5$; mean ± standard deviation)

translocation to shoot tissue of the plants. Similar findings in studies conducted on *C. rotundus* (Rai 2009), *C. procera* (Kumar et al. 2013; Kumar et al. 2017), *E. prostrata* (Dwivedi et al. 2008; Ahmad et al. 2011), and *V. cinerea* (Jaison and Muthukumar 2017) have also supported this behavior of these plants towards Cr. Because of low mobility of Pb, its TF was at minimum as compared to other metals for all the studied plants. Zn and Cd are highly mobile and get easily transported from soil to root to shoot. Zn is a competing ion with Cd (Mengel and Kirkby 1978) as the transportation of both the metals is facilitated by a common carrier at the root plasma membrane which has comparatively higher affinity for Cd than Zn (Hart et al. 2005). Both the metals showed higher BAF values for most of the plant species. Except for Pb, *C. procera* was able to translocate (TF > 1) all the studied metals from root to shoot. In addition, it also showed higher BAF and BCF values for Pb as compared to other plants which suggest its applicability in phytoextraction. The TF of Co was observed in the order of *C. procera* (1.86) > *V. cinerea* (1.17) > *C. bonplandianus* (1.02) > *C. rotundus* (0.71) > *E. prostrata* (0.32). The high TF in three of the plant species can be attributed to the vital role that Co plays in biological N fixation (Alloway 1990). The TF of Cd in *E. prostrata* was high (1.93) despite its toxicity. However, *E. prostrata* has also shown high TF for Cd (4.93) in a study conducted by Ahmad et al. (2011).

Phytoextraction is definitely not an option for decontamination of mine tailings or smelter wastes as was comprehensively explained by Ernst (2005). However, it may be applied to soils with low and moderate concentrations of metals. FA upholds moderate concentration of metals and could be toxic when leached into the environment. *E. prostrata* was found to have BAF and TF > 1 which suggests its phytoextraction ability for Cd which is also evidenced from PCA and can act as a suitable plant for phytoremediation of FA lagoon. In addition, it can also act in phytostabilization of other highly toxic metals such as Pb, Co, and Cr. *V. cinerea* accumulates high concentration of Cr in root (above toxicity limit without showing any detrimental effect) with BCF, BAC, and TF < 1which suggests its applicability in phytostabilization of FA lagoon. *C. procera* accumulated significant concentration of all the metals and could be used in phytoremediation of moderately contaminated sites.

Conclusions

The present study strongly encourages the assessment of biodiversity between the polluted and control site in bioaccumulation studies while identifying the plant species which are tolerant to metal contamination and could help in restoration of fly ash lagoon. Moderate concentration of metals was found in rhizospheric fly ash. However, the concentration of Ni and Cr in the present study was highest among the previously studied Indian and world power plants. After the comparison of biodiversity between polluted and control site, the polluted site (i.e., flyash lagoon) was found to have lesser biodiversity and higher dominance indicating a stressed ecosystem facilitating the proliferation of pollution-tolerant plant species. Out of 29 plant species taken into consideration for biodiversity investigations, the present study screened out *C. rotundus*, *C. procera*, *C. bonplandianus*, *E. prostrata*, and *V. cinerea* as the most suitable metal-tolerant plant species which can grow on metal laden fly ash lagoon. The variation in total, residual, and plant-available metal concentration indicated the presence of different proportions of metals in fly ash lagoon which affects the metal uptake potential of the vegetation growing on it. It is being reported in literature that bioavailable/plant-available metal concentration is the most suitable for assessment of metal transfer from soil to plant. However, in the present study, positive correlation was observed between total metal concentration and shoot metal concentration which suggests importance of correlation study for selection of extractant for calculation of biological indices. It can be stated that total metal concentration can provide a better insight for moderately or low metal-contaminated sites. The biological indices (BCF, BAF, and TF) revealed that *E. prostrata* and *C. procera* can be utilized efficiently for the phytoextraction of toxic metals from FA lagoon. Multivariate analysis (PCA) indicated that Cd was found to have an independent route of transport to shoots whereas all the plants were tolerant to Pb pollution hence there was a negligible translocation of Pb to the aerial tissues of the plants. *V. cinerea* accumulated elevated concentration of potentially toxic Cr and Ni which could also help in the phytoremediation of FA lagoon. The study concluded that five species (*C. rotundus*, *C. procera*, *C. bonplandianus*, *E. prostrata*, and *V. cinerea*) can be used for phytoremediation of FA lagoons. and these species are commonly found in India.

Future perspectives and recommendations

Use of different extractants and correlation study are needed to identify the best suitable extractant for calculation of biological indices such as bioconcentration and bioaccumulation factor to know exactly the strategy (phytoextraction, phytostabilization, hyperaccumulation, etc.) of the plant species for moderate or low metal-polluted sites. Sequential extraction can be done for better insight for assessment of metal mobility and immobilization of metals in soil.

Acknowledgements This research was financially supported by the Ministry of Human Resources and Development of the Government of India. The authors are grateful to Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand (India), for providing laboratory and e-research facilities for carrying out this study.

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