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An Assessment of Metal in fly Ash and Their Translocation and Bioaccumulation in Perennial Grasses Growing at the Reclaimed Opencast Mines

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ABSTRACT: Rapid expansion of the industrial sector adds more effluent into the agricultural land and in the vicinity of industry, which possesses the major threat of land contamination and environmental degradation. The present study was conducted to determine toxic metal concentration in fly ash and metal accumulation potential of the *Saccharum munja* and *Cynodon dactylon*, the two main grass species growing naturally on fly ash lagoon for Fe, Ni, Zn, Cu, Mn, Pb and Cd. Accumulation of metals were found in the order of Fe > Zn > Mn > Cu > Ni > Pb and Cd. In spite of significant accumulation of Fe in the root and shoot, the BCF and BAC were found very low (<1) in both the grasses. The metal excluding properties in shoots of both the grasses with low translocation factor (<1 for Fe, Ni and Pb), high bioconcentration factor (>1 for Zn and Pb) and low bioaccumulation coefficient (<1 for Fe, Mn, Ni, Cu and Cd) suggests its suitability for phytoremediation of fly ash lagoons. Findings of the study suggest that weed species may be used for the stabilization of toxic metals contaminated land and to empower the rural economy.

Key words: Fly ash; *Saccharum munja*; *Cynodon dactylon*; Phytoremediation, Translocation factor

INTRODUCTION

Coal-fired energy generation is the major sources of power supply for the developing countries. As it is growing at an average rate of 1.8 % per annum, it will be the largest source of world power energy till 2040 (IEO, 2013). The darkest side of electricity generation is the generation of fly ash (FA) as a by-product. Moreover, the present generation of FA from coal based thermal power plants in India is 131 MT/year and it is expected to increase to 300-400 MT/year by 2016-17 (FAU, 2013). The deposition of FA in the vicinity of power plants may cause ecotoxicological contamination and pollution in the environment globally. Indeed, probably 70% of fly ash being produced worldwide is disposed in landfills (Haynes, 2009).

Increase in abandoned FA deposits and lagoons holding an ample amount of FA causes air, soil and water pollution and may be harmful to living organisms (Pandey *et al*., 2009; Ansari *et al*., 2011). In addition, low water holding capacity, unavailability of nitrogen and phosphorus, low organic carbon and toxic metals restrict the plant succession, results in sparse vegetation growth on the bared FA dumps (Haynes, 2009). However, few plants and grass species were found growing luxuriantly on FA lagoons may because of their low nutrient requirement, drought resistance and metal tolerance capacity (Pandey *et al*., 2009). Research has been mainly carried out to restore and enhance the productivity of sodic lands using *Cynodon dactylon* (Gupta et al., 2002; Singh et al., 2013). However, very few researches have been carried out to evaluate the potential of different plants, herbs and shrubs for the remediation and stabilization of FA lagoons. However, few studies were carried out on wild grass species because of its insufficiencies in high biomass production, growth rate, root penetration and longer lifespan. Some promising plant species (*Calotropis procera, Typha latifolia* and *Saccharum spontanum*) capable to tolerate the adverse conditions are useful in restoration of degraded lands (Gupta & Sinha, 2008; Maiti & Jaiswal, 2008; Pandey *et al*., 2012).

S. munja and *C. dactylon* are the two most common perennial and palatable grasses naturally growing on the FA lagoons. Both the grasses are self propagating, drought resistant, soil binder and metal tolerant which can withstand in adverse environmental conditions.

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Likewise, *S. munja* has high biomass with deep root penetration system, which upholds the FA particles and prevent from wind erosion into the surroundings. On the other side, *C. dactylon* is low biomass producing grass species which have less root penetration system. Moreover, it propagates with rhizomes, stolen and seeds which remains adhered even after its removal from the main site. Because of the serpentine nature with multiple auxiliary roots which binds the top surface of FA, forms a thick turf on the barren areas and prevents from airborne pollution. Revegetation and stabilization of FA lagoons with *S. munja* and *C. dactylon* can be the most appropriate, cost-effective and eco-friendly technique to combat with this problem. In addition, implementation of eco-engineering technology (planting of dense cuttings) for higher biomass production of *S. munja* can be used for strengthening of the rural economy and socio-economic development of the wider area (Pandey *et al*., 2012). The objectives of the present study were to: (a) determine the chemical properties along with total metal concentrations of FA; (b) comparison of metal uptake and its distribution in root and shoot part of *S. munja* and *C. dactylon*; and (c) calculation of translocation factor (TF), bioconcentration factor (BCF) and bioaccumulation coefficient (BAC) of each metal for *S. munja* and *C. dactylon*. Present research will also help to assess the magnitude of metals transferred by naturally growing grasses and the selection of species for in situ revegetation and stabilization of FA lagoons.

MATERIALS & METHODS

Study of natural vegetation growing on FA lagoons is located (N 23º 472 , E 86 º 132) at Dhanbad district of Jharkhand, India (Fig. 1). The climate is tropical, characterized by 80-85% of annual rainfall (June-September). The average annual rainfall is 1241mm and relative humidity of the area is found to be 55% - 87%. There were three lagoons filled up, with a total capacity of approx. 68,000 m^3 and evacuation was done by shovel and loaded into the trucks. *S. munja* and *C. dactylon* are the abundant and naturally growing grasses on the FA lagoons. *S. munja* (Graminae) is a perennial tropical grass, generally available in arid regions in India. It has a potential for land stabilization, acts as animal feed and its fibers can be used to manufacture handicrafts, rope and furniture (Vasudevan *et al*., 1984). It is a drought resistant, forms thick clump with high biomass tufts and prevents soil erosion (Pandey *et al*., 2012). It is a choice species used for revegetation and stabilization of erosion prone rugged slopes and their conversion into biologically productive sites of high socio-economic and aesthetic values (Sharma *et al*., 2005).

C. dactylon commonly known as Bermuda grass is an important warm-season turf grass naturally grown

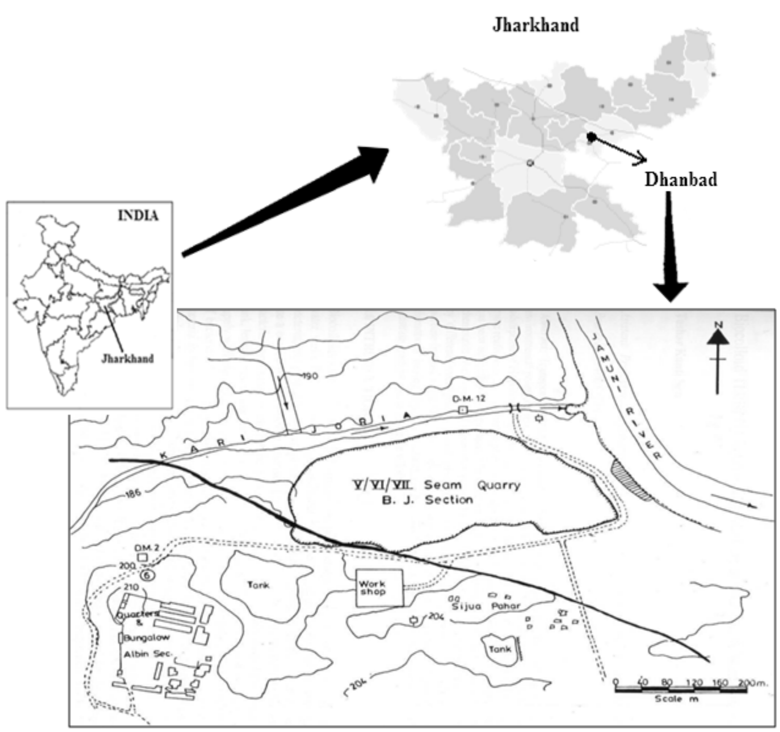

Fig. 1. Layout of the opencast mine voids used for backfilling with fly ash

Fig. 2. (a) Wet disposal of Fly ash (b) Natural colonization of *S. munja* and *C. dactylon* (c) Flow of fly ash slurry (d) Collection of fly ash and plant samples

in the early stages of succession and remains occupied permanently. In addition, the grass can tolerate wide range of pH and temperature variations (Li *et al*., 2011), drought resistant (Shi *et al*., 2014) and controls air-borne suspension of FA. It is a source of metabolites; commonly use treats urinary tract and microbial infections (Annapurna *et al*., 2013). It is considered as the most economic, easily available and palatable fodder grass in India. Randomly 14 numbers of surface samples (0-15 cm depth) were collected from the FA filled mine pit. Fly ash samples were air dried for 3 days, homogenized, ground to pass through a 2 mm sieve and completely dried in hot air oven for 12 hr at 105^{o} C. The pH and electrical conductivity (1:2.5; soil: deionised water) were measured in a soil–water suspension using a pH meter (Cyberscan 510) and conductivity meter (EI 601), respectively; organic carbon (Walkley & Black, 1934); available nitrogen (Subbiah & Asija, 1956) were determined. Cation exchange capacity was determined using 1 N ammonium acetate extraction method (Jackson, 1973) using a flame photometer (Systronics 128) (Das & Maiti, 2009). From each sampling unit, FA and both the grass samples were also collected to study the bioavailable metals in FA and its accumulation in plant parts (Fig. 2). Total metal concentrations in FA were determined by digesting 0.5 g of sample using conc. HNO_3 and HCl (3:1, v/v) in a microwave digester. The collected grass samples were rinsed thoroughly with tap water and finally with millipore water. The grass samples were oven dried at 80 °C until a constant weight was achieved and ground with mortar and pestle to get homogenized powder. Total digestion of 0.5 g powdered plant sample was carried out in microwave digester by adding 10 mL conc. HNO_3. All the FA and grass samples were analyzed in triplicate to determine toxic metals concentration using Flame Atomic Absorption Spectrophotometer (FAAS, GBC Avanta 200, Australia). Biological factors are indices to estimate the plant-metal interaction in the FA. Several researchers have described various biological factors (Kumar & Maiti, 2014a; Pandey *et al*., 2012) as follows:

The translocation factor (TF) indicates plant's ability to transfer metal from root to aerial part. TF >1 indicates that plant translocate metals effectively from root to shoot.

$$TF = \frac{\text{Concentration of metal in shoot}}{\text{Concentration of metal in root}} \qquad (1)$$

Biological concentration factor (BCF) indicates metals uptake by roots from the fly ash.

$$BCF = \frac{\text{Concentration of metal in root}}{\text{Total metal concentration in fly ash}} \qquad (2)$$

Biological accumulation coefficient (BAC) was calculated as the ratio of metal concentration in aerial parts of plant to those in fly ash/topsoil.

$$\text{BAC} = \frac{\text{Concentration of metal in shoot}}{\text{Total metal concentration in fly ash}} \qquad (3)$$

Analytical grade chemical and reagents (Merck) were used during metal analysis. All the samples were kept in zip polyethylene bags previously rinsed with 1M HNO_3 and Millipore water and stored at 4°C before analysis. The blanks were run in triplicate to check the precision of the method and instruments with each set of samples. Standard Reference Materials (AccuTrace, AccuStandard Inc., USA; Matrix 2-5% nitric acid; CRM uncertainty ± 5%; verified against NIST SRM# 3108 for Cd; 3126a for Fe; 3136 for Ni; 3128 for Pb; 3132 for Mn; 3168a for Zn; 3114 for Cu) were used for the preparation and calibration of each analytical batch. Calibration coefficients were maintained at a high level e" 0.99. The recovery percentage of metals in the SRM-samples for fly ash and plants ranged from 80%-102% and 83%-121%, respectively. Precision and accuracy of the analysis were checked by means of duplicate analysis of the selected samples (less than 10% relative variation). Inter batch variations were monitored by repeated analysis of selected samples in various analytical batches (less than 10% relative variation). The accuracy of the analytical procedure adopted for FAAS analysis was checked by running standard solutions after every 5 samples.

Translocation factor (TF), biological accumulation factor (BAF) and biological accumulation coefficient (BAC) values were calculated using mean values of the measurement in MS-Excel 2007 (Microsoft Inc.). The Pearson's correlation analysis was performed using SPSS, IBM statistics version 20.0 (South Asia Pvt. Ltd) package.

RESULTS & DISCUSSION

Chemical parameters of the FA filled in lagoon are shown in Table 1. The pH of the fly ash was found slightly acidic (6.17 ± 0.12) which is due to the geo-climatic condition of the region. Low EC (97.43 ± 33µS m^{-1}) and organic carbon (0.49 ± 0.15 %) was found in FA due to absence of plant debris. However, cation exchange capacity of fly ash was found slightly moderate due to presence of calcium and magnesium ions. Available nitrogen was found <10 mg kg^{-1}.

Total metal concentrations in fly ash, root and shoot of *S. munja* and *C. dactylon* are presented in Fig. 3. Concentration of Fe ranges between 13165 - 14185 mg kg^{-1} in the FA followed by other metals in the order of Mn > Zn > Ni > Cu > Pb and Cd. Since, some of the metals are mobile in nature which can easily leached from the main site and ash weathering during rehabilitation process results in decrease in metal concentration in fly ash (Maiti & Nandhini, 2006; Haynes, 2009). In addition, plant also accumulates significant amount of metals in their root and shoot for their growth and development.

Metal accumulation in plants depends on various factors such as type of plant, age, pH, form and type of available metals in substrate and climatic conditions (Maiti & Jaiswal, 2008). Accumulation of metals were studied in both the grass species, and were found in the order of Fe > Zn > Mn > Cu > Ni > Pb and Cd. Maximum accumulation of Fe was found in the roots of *C. dactylon* (1396 ± 401 mg kg^{-1}) whereas minimum accumulation for Cd was found for *S. munja* (0.47 ± 0.13). If metals are accumulated in aboveground tissues, they may pass on the contaminants to herbivores (Kumar & Maiti, 2014b). High concentration of Fe, Pb and Ni were found in root than shoot whereas, high concentration in shoot were found for Cd > Zn > Mn in both the grass species. It was found that *C. dactylon* accumulate high concentration of Zn and Cd in their shoot as compared to *S. munja*. Plant uses common carrier for the absorption and translocation of Zn and Cd at the root plasma membrane. Since the affinity of Zn transportation is higher than Cd, more concentration of Zn is being transported from root to shoot (Hart *et al.*, 2005). Different biological factors such as, translocation factor (TF), biological concentration factor (BAF) and biological accumulation coefficient (BAC) were evaluated to access the plant-metal interaction growing on fly ash and to assess the plants potential for phytoremediation.

Translocation factors indicate the extent of metal transfer from root to shoot. It is concluded from various

Table 1. Chemical characteristics of fly ash used in the mine fill site. Mean ± SD (Min - Max)

Parameter	Fly ash (n = 14)
pH (1: 2.5, w/v)	6.17 ± 0.12 (5.65 – 6.92)
Electrical conductivity (µS m^{-1}) (1:2.5, w/v)	97.43 ± 33 (49.80– 281)
Organic carbon (%)	0.49 ± 0.15 (0.12 – 1.30)
Available N (mg/kg^{-1})	< 10
Cation exchange capacity (CEC) [cmol (p+) kg^{-1}]	6.50 ± 1.82 (4.32 - 7.60)

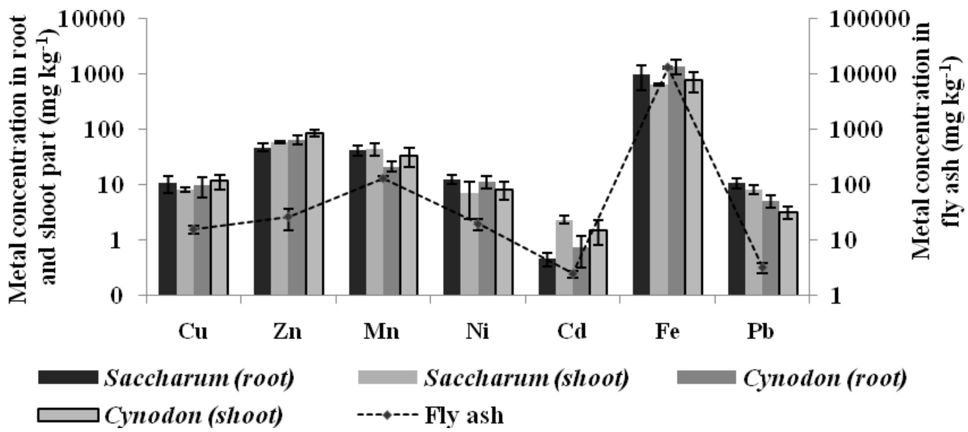

Fig. 3. Heavy metal concentration in fly ash, root and shoot of *S. munja* and *C. dactylon* growing naturally on fly ash filled lagoons

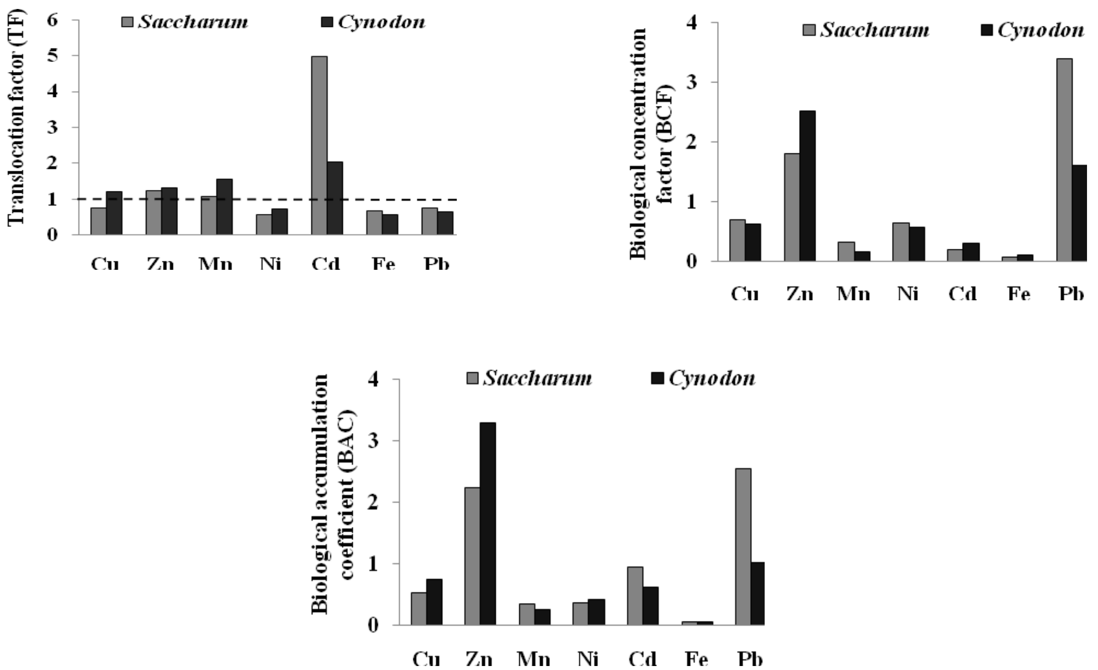

Fig. 4. (a) Translocation factor of metals from root to shoot. (b) Biological concentration factor of metals in the root. (c) Biological accumulation factor of metals in the shoot of *S. munja* and *C. dactylon* naturally growing on fly ash filled lagoons

studies that transfer of metals from soil to plants is complex physiochemical process although it is also affected by physiological and environmental factors (Rafati *et al*., 2011). TF values for *S. munja* has lower than *C. dactylon* are shown in Fig. 4a. High TF (>1) were found for Cd, Mn, Zn, and Cu in *C. dactylon* whereas low for Ni, Fe and Pb. Similarly, higher TF values were found only for Zn and Cd in *S. munja*. Inspite of low availability of Cd in fly ash, maximum translocation of Cd was found in both the grass species on the other hand, low TF (<1) indicates lower translocation of metals from root to shoot.

Biological concentration factor (BCF) is the ratio of the total metal concentration in plant root to that in the substrate are shown in Fig. 4b. It is well reported that BCF greater than 1 indicates phytoremediation property of the plant (Zhang *et al*., 2002; Ali *et al*., 2013). BCF value except for Zn and Pb, found <1 for most of the metals in both the grass species, concluding its lower accumulation of metals in roots and can be considered as a metal excluder species of heavy metal growing on fly ash. Minimum accumulation in root further lowers the minimum

Table 2. Correlation coefficient (r) between fly ash vs root and shoot metal concentration in naturally colonized *S. munja* and *C. dactylon* growing on flyash filled lagoon

	S. munja		*C. dactylon*	
Metals	**Root**	**Shoot**	**Root**	**Shoot**
Cu	-0.977	-0.965	0.887	0.136
Zn	-0.546	-0.701	0.909	-0.659
Mn	-0.557	0.961	-0.054	0.835
Ni	-0.932	-0.167	0.922	-0.774
Cd	-1.000**	-0.928	0.845	-0.901
Fe	0.955	-0.526	0.550	-0.578
Pb	-0.550	-0.500	-0.280	0.256

** Significant correlation at 0.01 level (2 tailed) according to Pearson's correlation.

Table 3. Correlation coefficient between the roots and shoot metal concentration in naturally colonized *S. munja* and *C. dactylon* growing on flyash filled lagoon

Plant	Metals	Cu (root)	Zn (root)	Mn (root)	Ni (root)	Cd (root)	Fe (root)	Pb (root)
S. munja	Cu (shoot)	0.838	0.598	0.226	0.934*	0.991	-0.882	-0.502
	Zn (shoot)	0.476	0.884*	-0.188	0.822	0.469	-0.847	-0.224
	Mn (shoot)	-0.864	-0.72	-0.271	-0.985**	-0.911	0.934*	0.545
	Ni (shoot)	-0.04	0.879*	-0.728	0.519	0.082	-0.437	-0.51
	Cd (shoot)	0.51	0.619	0.182	0.64	-0.553	-0.599	-0.422
	Fe (shoot)	0.565	-0.222	0.739	0.214	0.058	-0.17	-0.225
	Pb (shoot)	-0.236	-0.395	0.306	-0.485	-0.3	0.249	0.746
C. dactylon	Cu (shoot)	0.609	0.19	-0.055	-0.309	0.613	0.297	0.102
	Zn (shoot)	0.133	0.543	0.533	0.054	-0.28	0.23	-0.753*
	Mn (shoot)	-0.03	0.058	-0.375	-0.295	-0.144	0.169	-0.241
	Ni (shoot)	-0.03	0.058	-0.281	-0.545	0.07	-0.106	-0.492
	Cd (shoot)	-0.53	-0.113	0.208	-0.773	-0.461	-0.341	-0.043
	Fe (shoot)	0.145	0.002	0.244	-0.662	0.18	0.284	0.509
	Pb (shoot)	-0.297	-0.532	0.343	-0.287	-0.245	-0.019	0.789*

translocation in shoot. It was found that the BAF for *C. dactylon* was lower than that of *S. munja*.

Biological accumulation coefficient (BAC) is the ratio of the total metal concentration in the plant shoot to that in substrate (fly ash) are shown in Fig. 4c. BAC in both the grasses was found low for most of the metals. Because of the higher BCF (>1) of Zn and Pb may resulted in increase of BAC values in *S. munja* and *C. dactylon*. Fe was the major metal accumulated by both the grass species. In spite of significant accumulation of Fe in the root and shoot, the BCF and BAC were found very low (<1) in both the grasses.

To assess the negative and positive translocation and allocation of metals, correlation coefficient (r) is an important statistical tool to determine the phytoavailability of different metals in fly ash. Elemental properties of fly ash and nature of the plant species are the two most important factors which determine the phytoavailability of heavy metals (Maiti & Nandhini, 2006). Correlation between the plant metal growing on fly ash and metal in root and shoot of *S. munja* and *C. dactylon* are presented in Table 2 The positive and negative correlation shows the suitability and non-suitability of metal accumulation in plants respectively (Maiti & Nandhini, 2006). It was found that most of the metals in root and shoot of *S. munja* and *C. dactylon* negative correlation with metal concentration in fly ash. In case of Cd, significant negative correlation (-1.000; $p < 0.01$) was found between fly ash and roots of *S. munja*. All the negative results show the metal excluding nature of both the grass species and classify them into metal-excluder species for fly ash.

Metals in trace amount are essential for the growth of plant species. However, increase and excess in its concentration results in detrimental effect, like chlorosis, necrosis, toxicity and stunted growth of plants (Maiti, 2013). The possibility of easy availability and movement of metals between root and shoot is usually determined by correlating the metal concentration in different plant parts (Pandey *et al.*, 2012). Table 3 depicts the correlation between metal accumulation in root and shoot of *S. munja* and *C. dactylon*. Strong negative and positive correlation was found between different metals. Positive significant correlation ($r = 0.884$, $p < 0.05$) was found between Zn concentration in root and Zn concentration in shoot of *S. munja*. It also shows a positive significant correlation ($r = 0.879$, $p < 0.05$) between Zn(root) vs Ni(shoot).

Positive significant correlation ($p = 0.05$, $r = 0.934$) was found between Cu(shoot) vs Ni(root) and Mn(shoot) vs Fe(root). Strong negative correlation ($r = -0.985$, $p < 0.01$) was found between Mn(shoot) and Ni(root). In case of *C. dactylon*, positive significant correlation ($r = 0.789$, $p < 0.05$) was found between Pb concentration in root and shoot which signifies the translocation of metal from root to shoot. Similarly, negative significant correlation ($r = -0.753$, $p < 0.05$) between Zn concentration in shoot and Pb concentration in root. However no significant correlation was found for Cd in both the grasses.

CONCLUSIONS

The study concludes that the grass species, *S. munja* and *C. dactylon* are the naturally colonized, perennial grass species growing luxuriantly on the FA filled lagoons. Vigorous growth, high biomass, drought resistant characteristics of both the grasses shows its natural tendency to adjust and grow under different ecological conditions. Lower translocation, bioconcentration, bioaccumulation and negative correlations between different metals categorize these grasses under metal excluder (non accumulator) species. Because of the commercialization of the huge biomass, *S. munja* can be used to strengthen the rural economy and socio-economic development of surrounding villages. Further, *C. dactylon* helps to produce green turf, which prevents airborne erosion of FA from the site and provides easily available and natural palatable fodder for cattle. Since both the grasses, *S. munja* and *C. dactylon* are perennial, self propagating and metal excluder in nature with no maintenance, together acts as best suitable eco-phyto-engineers for the revegetation and stabilization of the fly ash lagoons by enhancing the aesthetic value and attenuating health hazards of the environment.

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