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Management of Fly Ash Landfills with *Cassia surattensis* Burm: A Case Study

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In India, the majority of thermal power plants are coal fired (Khan and Khan 1996), as a result fly-ash, the principal by-product, is produced in huge amount (about 15-30% of the burnt coal, depending on the ash content). The disposal of fly-ash is great problem and most of the ash generated (85-95%) due to combustion of fossil fuel is being dumped to nearby areas as fly-ash land fills or dykes. Although fly-ash contains many essential nutrients for plants, it is deficient in N and P content. Besides, fly-ash also contains high levels of certain toxic metals (Mehra et al. 1998) which limit the survival of plants and subsequently suppress growth of the plant (Wong and Wong 1986; Singh et al. 1997). Application of nitogen fixing cyanobacteria inoculants to enhance N and P status and in reducing metal toxicity of fly- ash has recently been reported (Rai et al 2000).

Symbiotic N_2 fixation the association between the genus *Rhizobium* and legumes, has most agronomic potential for improving soil N status, and is therefore of great interest. Further, *Rhizobium* has been reported to be key element for plant establishment under xeric and nutrient unbalanced conditions (Requena et al. 1996; Barea et al. 1996). Leguminous plants have ability to fix atmospheric N_2 due to presence of *Rhizobium* in their root nodules and exhibit tolerance towards environmental stresses. It has been reported that leguminous plants could grow well on fly-ash amended soils without manifestation of any injury symptoms (Singh et al. 1997). However, the best solution for management of fly-ash landfills is to vegetate them by these plants to arrest the fly-ash particles.

Cassia surattensis Burm (Leguminosae), an ornamental, medicinal and economically important plant was selected for the present study and a series of laboratory experiments were performed by comparing plant growth performance on fly-ash and garden soils before using it as a most suitable plant species in the management of fly-ash landfills by vegetating the area. Impact of fly-ash on growth, chlorophyll content and nodule number along with metal accumulation capability of plant has been evaluated. Besides, a fly-ash tolerant strain (CT-1) was isolated from the plant growing on fly-ash contaminated soil and performance of *C. surattensis* on fly-ash after inoculation with *Rhizobium* strain (CT-1) was studied. Results of these experiments are reported in this paper.

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MATERIALS AND METHODS

Plants of *Cassia surattensis* Burm. were raised from seeds at National Botanical Research Institute, Lucknow. Sixty days old plants were transplanted in nine earthen pots containing fly-ash and nine pots filled with garden soil (control). Plants were kept in natural conditions and irrigated with tap water at regular intervals avoiding leakage of water from pots. Plants of three pots grown in garden soil and three pots grown in fly-ash were harvested at 15,30 and 60 d of transplantation. Harvested plants were washed thoroughly thrice with double distilled water and biomass of whole plant, roots and shoots were measured. Length of the roots/shoot, branching pattern of roots and leaf area (by area measurement system; Delta T Devices, U.K.) were recorded. Physico-chemical analyses of soil and fly-ash were done as per procedures described by Piper (1966). The toxic metal contents in fly-ash, garden soil and plant tissues were estimated in oven dried (80°C) fly-ash/garden soil/plant samples by digesting in HNO_3:$HClO_4$ (v/v; 3:1). The concentration of metals (Cu, Zn, Mn, Fe and Ni) in digested samples was determined with a Atomic Absorption Spectrophotometer (Perkin Elmer 2380) using specific hallow cathode lamps. The standard reference materials of metals (E- Merck, Germany) were used for calibration and quality assurance for each analytical batch.

Chlorophyll content was estimated after extraction in 80% chilled acetone as per procedures of Arnon (1949) and carotenoids following the method of Dexbury and Yentsch (1956).

Fly-ash tolerant strain of *Rhizobium* was isolated on Yeast Mannitol Agar (YEMA) medium. Plant growing in fly-ash (amended with soil and farmyard manure) was uprooted, washed thoroughly and healthy nodules were detached from roots. Nodules were surface sterilized with 5% H_2O_2 (5 min), rinsed with sterile distilled water and crushed in sterile double distilled water. Serial dilutions (1:10 to 1:1000) of nodule extract were plated on YEMA plates. Pure cultures of (CT-1) were maintained on yeast mannitol broth. *C. surattensis* seedlings were raised in Jensen nitrogen free agar medium (Jensen, 1942). Before sowing, seeds were surface sterilized with H_2O_2 (5%) for 10 min then rinsed five times with sterile deionized water. Seeds were soaked overnight in sterile tap water, then germinated on filter paper. After germination seedlings were transferred to 150 ml conical flask (1 in each) containing 25 ml Jensen nitrogen free liquid medium. Fifteen days old seedlings were inoculated with 1 ml ($4.3x10^5$ cells ml^{-1}) and 2 ml ($8.6x10^5$ cells ml^{-1}) of *Rhizobium* (CT-1) culture. Plants were placed under controlled conditions (light/dark cycle 14:10 h, temperature 28±2°C, 115 μ $mol^{-2}s^{-1}$ illumination provided through day fluorescent tube light) and observed for initiation of nodulation. Such nodulated plants were transferred in 6" plastic pots containing sterilized fly-ash (fly-ash was steam sterilized for one hour on three consecutive days) after 7 d of inoculation. These pots were placed under field condition. Uninoculated plants grown in fly-ash served as control. Plants were uprooted from both the treatments after 30 d, washed thrice with double distilled water, nodule number was counted and biomass of the plants was recorded.

Variability of the data and validity of results were checked employing students 't'-test (Schefler 1969).

RESULTS AND DISCUSSION

Physico chemical analysis of fly-ash used in the experiment showed high pH and low total nitrogen and phosphorus content (Table 1) and was enriched with high concentration of toxic metals viz., Cu, Mn, Zn, Fe, Ni and Pb. Garden soil used as control during the experiment has slightly alkaline pH (7.7) and has sufficient amount of nitrogen and phosphorus for supporting plant growth.

Table1. Physico-chemical characteristics of fly-ash and garden soil used for experimentation

Parameters	Garden Soil	Fly-ash
Physico-chemical		
PH	7.7 ± 0.280	9.60 ± 0.380
EC m mhos cm^{-1}	1.016 ± 0.001	8.60 ± 0.031
CEC [meq $(100\ g)^{-1}$]	1.20 ± 0.080	1.58 ± 0.054
Total nitrogen (%)	0.090 ± 0.002	0.02 ± 0.001
Total phosphorus (%)	0.222 ± 0.011	0.02 ± 0.001
Organic matter (%)	1.483 ± 0.068	1.172 ± 0.048
Metal ($\mu g\ g^{-1}$)		
Cu	38.4 ± 1.821	58.6 ± 2.80
Mn	45.8 ± 2.292	70.0 ± 3.212
Zn	22.6 ± 1.091	82.0 ± 3.260
Fe	2850 ± 138.521	4150 ± 207.50
Ni	23.8 ± 1.160	204 ± 10.20
Pb	30.16 ± 1.230	40.1 ± 1.630

Mean ± SD (n = 3), student's t test.significant ($p< 0.05$) as compared to control.

Fly-ash affected the growth of *C. surattensis* adversely as indicated by lowered biomass of whole plant, roots and shoot (Table 2). Plants grown in fly-ash have lesser number of nodules in comparison to garden soil after 15 d of exposure. An increase in nodule number of fly-ash grown plants in comparison to garden soil was recorded after 30 d of exposure which further decreased with increasing time of exposure (60 d) in comparison to garden soil Fly-ash inhibited the formation of lateral roots and root biomass. Fly-ash inhibited length of root and shoot. Similarly, fly-ash decreased the leaf number and photosynthetic area of the plant.

Total chlorophyll content was not much affected by fly-ash (Table 3). An increase in total chlorophyll content was recorded at initial growth phase which was reduced slightly by 13% after 60 d of exposure. However, carotenoids were more affected by fly-ash as about 20% inhibition in carotenoid was recorded after 30 d of growth on fly-ash. *C. surattensis* accumulated considerable amount of toxic metals (ANOVA : $P<0.05$). However, the accumulation of various metals

Table 2. Effect of fly-ash on biomass, nodule number, photosynthetic area and leaf number of the *C. surattensis*

Parameters		Treatment duration (d)		
		15	30	60
[A] Biomass (g DW)				
Whole plant	Control	0.917 ± 0.045	1.197 ± 0.048	1.236± 0.042
	Fly-ash	0.758 ± 0.038	0.629 ± 0.031	0.530± 0.024
Shoot	Control	0.568 ± 0.022	0.614 ± 0.026	0.647± 0.027
	Fly-ash	0.549 ± 0.020	0.326 ± 0.009	0.286± 0.011
	Control	0.349 ± 0.017	0.583 ± 0.026	0.589± 0.025
Roots	Fly-ash	0.209 ± 0.012	0.303 ± 0.015	0.244± 0.012
[B] Plant length (cm)				
Whole plant	Control	55.4 ± 2.4	64.3 ± 2.8	70.8 ± 2.8
	Fly-ash	38.0 ± 1.7	50.7 ± 2.3	58.8 ± 2.5
Shoot	Control	30.4 ± 1.5	34.0 ± 1.7	41.2 ± 1.9
	Fly-ash	20.0 ± 0.9	27.5 ± 1.4	29.6 ± 1.3
Roots	Control	25.0 ± 1.2	30.3 ± 1.5	40.0 ± 2.1
	Fly-ash	18.0 ± 0.6	23.2 ± 0.8	29.2 ± 2.5
[C] Nodule number (per plant)				
	Control	25.0 ± 1.00	77.0 ± 3.08	42.0 ± 2.1
	Fly-ash	5.0 ± 0.15	115.0± 3.70	24.0 ± 1.2
[D] Photosynthetic area (cm^2)				
	Control	216.6 ± 10.8	225.3 ±10.81	1579.0± 75.0
	Fly-ash	36.0 ± 1.30	89.0 ± 4.40	781.0 ± 23.4
[E] Number of leaves / plant				
	Control	32.0 ± 1.40	35.0 ± 1.50	43.0 ± 1.72
	Fly-ash	12.0 ± 0.60	18.0 ± 0.70	26.0 ± 1.30

Mean ± SD (n = 3), student's t test.significant ($p < 0.05$) as compared to control.

Table 3. Effect of fly - ash on photosynthetic pigments (mg g^{-1} FW) of *Cassia surattensis*

Parameters		Treatment duration (d)		
		15	30	60
Chlorophyll a	Control	1.14± 0.106	1.37± 0.294	1.37± 0.290
	Fly-ash	1.45± 0.309	1.03± 0.107	0.94± 0.142
Chlorophyll b	Control	0.409 ± 0.019	0.411 ± 0.104	0.411 ± 0.404
	Fly-ash	0.565 ± 0.147	0.353 ± 0.043	0.340 ± 0.056
Total chlorophyll	Control	1.55± 0.122	1.78± 0.206	1.89± 0.205
	Fly-ash	2.01± 0.455	1.38± 0.144	1.28±0.194
Carotenoids	Control	7.50 ± 0.911	7.86 ± 0.614	7.88 ± 0.613
	Fly-ash	6.75 ± 1.25	5.93 ± 0.80	6.53 ± 0.660

Mean ± SD (n = 3), student's t test.significant ($p < 0.05$) as compared to control.

increased progressively with growth of the plant (Table 4). The plant tissues accumulated metals in the order Fe>Mn>Ni>Zn>Cu. Root accumulated maximum amount of metals followed by leaves and stem. The plants grown in garden soil under control conditions have also shown lesser amounts of these metals. *Rhizobium* (CT-1) inoculation increased the growth of *C. surattensis* in fly-ash (Table 5). An increase in biomass of intact plant, shoot and roots was observed after 30 d of transplantation under field condition.

Table 4. Heavy metal accumulation by different plant tissues of *C. Surattensis*

Treatment	Metal accumulation (μ g g^{-1} DW)				
	Cu	Zn	Mn	Fe	Ni
[A] Leaves					
15 d	61.5 ± 3.1	187.5 ±7.5	220.5± 9.0	703.5± 8.7	138.8±3.0
30 d	67.0 ± 2.0	189.0±7.6	225.0± 9.0	1224 ±12	285.0±6.9
60 d	80.5 ± 4.0	192.0± 5.8	232.5± 10	1960 ± 12	747.0±9.0
[B] Stem					
15 d	82.5±4.1	174.8±7.0	171.8± 6.6	762.0± 9.0	199.5± 4.8
30 d	85.0±2.5	180.0± 7.2	187.5±7.5	915.0± 9.0	372.0±7.5
60 d	87.0± 2.4	189.0± 4.8	223.5± 9.0	997.5± 9.6	592.5± 13
[C] Roots					
15 d	123.0± 7.5	267.7±13	194.3± 5.8	1837 ± 12	267.0±6.0
30 d	140.0± 5.0	277.5± 13	241.5±12	2574±15.0	462.0±8.4
60 d	150.0± 6.0	297.8± 12	560.0±15	4470 ± 16	577.7±13

Mean ± SD (n = 3), student's t test.significant ($p< 0.05$) as compared to control.

Table 5. Effect of *Rhizobium* on growth performance of *C. surattensis* saplings growing on fly-ash

Treatments	Biomass (g DW)			Nodule number
	Root	Shoot	Leaf	
Control (uninoculated)	2.50±0.10	2.75±0.14	2.30±0.09	0.0
Inoculated ($4.3x10^5$ cell ml^{-1})	2.73±0.13	2.96±0.15	3.00±0.12	12.0±0.6
Inoculated ($8.6x10^5$ cell ml^{-1})	5.43±0.27	3.43±0.17	7.17±0.29	26.0±1.0

Mean ± SD (n = 3), student's t test. significant ($p< 0.05$) as compared to control.

Results showed potential of *C. surattensis* to grow on fly-ash without any visible phytotoxic symptoms. However, a decrease in biomass of plant was recorded which may be due to toxic effects of metals present in fly-ash. Fly-ash inhibited lateral branching of roots and root biomass. These results are in agreement with earlier reports which suggested that copper and aluminium rich medium reduced the lateral branching of roots and root phytomass (Ouzounidou et al. 1995; Samuels et al. 1997),. Leaf number and total photosynthetic area reflect the general health and vigour of a plant. Fly-ash reduced the leaf number and photosynthetic area in *C. surattensis*. Reduction in leaf number and photosynthetic area of sugar beet (*Beta vulgaris*) grown in fly-ash contaminated soil was reported by Singh et al. (1994).

Total chlorophyll content was not found much affected in fly-ash grown *C. surattensis* plants. δ-aminolevulinic acid dehydratase (ALAD) is a metalloeneyme of chlorophyll biosynthesis (Schoolinglin-Jordan and Cheeing, 1999) and its activity depends on availability of Mg. Since fly-ash contains high amount of essential metals like Mg, Co, Cu etc. therefore, high uptake of Mg may result in induction of ALAD activity. Induction of ALAD activity may maintain the pool of total chlorophyll contents by increased chlorophyll biosynthesis. Besides, species accumulated high amount of Cu and Zn which are reported to induce phytochelatin synthesis in terrestrial and aquatic plants to participate in cellular detoxification (Kneer and Zenk 1992). Reduction in total chlorophyll and carotenoid content after 60 d exposure might be due to toxicity caused by heavy metals present in fly-ash (Khan and Khan 1996).

Plant tissues accumulated varied amount of metals. However, maximum amount of toxic metals was found concentrated in roots than leaves. Roots are the first organ to contact with toxic metals. Therefore, most of the amount of toxic metals was found deposited in the root tissues possibly restricting the movement to the leaves (Mishra and Shukla, 1986). *C. surattensis* plants grown on fly-ash showed less toxicity after 15 d which increased with increasing exposure duration up to 60 d. Leguminous plants e.g., beans at the early growth stages are more resistant to heavy metals. Changes in cell metabolism allow them to cope with the toxicity of metals, thus the negative effects of heavy metals may primarily be observed and diminished at letter stages of plant growth (Krupa and Moniak, 1998). Out of the metals estimated Fe accumulation was highest in comparison to other metals. High accumulation of Fe might be due to the greater requirement of iron as a constituent of many enzymes and the cytochromes of certain porphyrins in early growth stages (Hewitt, 1958).

Fly-ash delayed the nodulation as lesser number of nodules were recorded after 15 d of exposure in comparison to garden soil, however, nodule number was more in fly-ash than garden soil after 30 days. This supports the work of Martensson and Witter (1990) who found that rhizobial isolates were effective in nodulating clover plants from metal rich sludge amended soils. They observed that rhizobia may survive in else elevated metal concentrations in absence of host plant. The reason for delayed nodulation in metal rich soils was explained by Obbard and Jones (1993). They have suggested that effective rhizobial population size in metal contaminated soils was less than that in control. Further, this implies that one or more metals has reduced the effective population in such soil (Obbard et al. 1992). During present study it has been observed that inoculum size affected the growth of *C. surattensis* in fly-ash and an inoculum having larger cell density (8.6×10^5 cells ml^{-1}) has supported more growth of the plant. Giller et al. (1989) also reported that inoculum of an effective strain of *R. leguminosarum* biovar. Trifoli into soils at metal rich site (Woburn) resulted in the loss of N_2 fixation in clover over a 2 month period, unless large densities of cells were inoculated. Nodules formed by plants grown in fly-ash were found effective as they were able to form nodules in saplings grown in Jensen nitrogen free medium. Obbard and Jones (1993) also found that rhizobial populations of metal contaminated sites were able to form an effective symbiosis with *Trifolium repens* when grown on the soil in

the laboratory. During present study *Rhizobium* inoculation enhanced the growth of the *C. surattensis* in N limited fly-ash. Similar growth promotion of cannola and letuce by *R. leguminosarum* under stress conditions was also given by Noel et al. (1996). It could be concluded from present study that *C. surattensis* is an ideal plant for plantation at fly-ash contaminated soils and fly-ash landfills deficient in nitrogen.

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