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Phytoremediation of Flyash by Assessing Growth Responses of the Local Tree Species

Divya Agarwal[1]*, Manoj K. Agarwal[2], Mohammad Yunus [3] and S.K. Gautam[4]
[1]Assosiate Prof., Dept. of Applied Sciences & Humanities, Accurate Institute of Management & Technology, Greater Noida, India.
[b]Professor, D.J. College of Engineering & Technology, Modinagar, India
[3]School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (Central) University, Lucknow, India.
[4]Research Scholar, Department of Chemistry, Institute of Engineering & Management, Mathura, U.P., India.

Flyash is the coal combustion residue generated in bulk quantities from thermal power generation in India. Disposal of flyash is of serious environmental concern due to its hazardous properties, impact on vegetation and crops grown,and long-term risks to ecosystems and human life. Physico-chemical properties of flyash from the Feroz Gandhi Unchahar Thermal Power Plant (FGUTPP), Raebareilly in Uttar Pradesh (India) were compared with that of Garden Soil (GS). Plants of Terminalia arjuna, Bauhinia variegata and Morus alba were grown on flyash substrate to understand the potential of phytoremediation in reclaiming the flyash dumpsites. The plant growth was analysed in different combinations of flyash and garden soil: 100% flyash (T1), 75% flyash and 25% GS (T2), 50% flyash and 50% GS (T3), 25% flyash and 75% GS (T4). Different morphological viz. Shoot & root growth, and physiological parameters of plant growth viz. Chlorophyll, Protein and Nitrate Reductase were analysed. Results of the study are directly useful in developing phytoremediation protocols for flyash disposal sites of application of flyash as a blend to the plantation areas.

Keywords: Flyash, Phytoremediation, *Terminalia arjuna*, *Bauhinia variegata* and *Morus alba.*

Abbreviations: FGUTPP Feroz Gandhi Unchahar Thermal Power Plant, FA Flyash, GS Garden Soil, T1 100% flyash, T2 75% flyash and 25% GS, T3 50% flyash and 50% GS, T4 25% flyash and 75% GS.

1. INTRODUCTION

Flyash (FA) is a toxic solid waste generated in bulk from coal thermal power plants, a preferable source of energy production in India. Increased level of Suspended Particulate Matter in the vicinity of flyash dumps and potentiating of its constituents to contaminate the soil and groundwater are of significant concern due to its risk to life and ecosystems. Photosynthesis is reduced in green plants due to obstruction in stomatal rhythm in the leaf as a result of deposition of flyash particulates. It causes hardening of soil and reduces water infiltration and root penetration [1]. Leaching of heavy metals causes ground water pollution [2]. Hazardous radionuclides were also reported in the emission from coal-fired thermal power plants [3].

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1.1. Applications of Flyash

Technologies for gainful utilization of flyash are being researched. Flyash utilization in synthesis of variety of zeolites, glass-ceramics, porous ceramics and preparing flyash catalysts has been reported. The adsorption capacity increases with an increasing carbon content of flyash. It is used to develop understanding of adsorption process kinetics, to describe the rate of mechanism and to determine the factors controlling the rate of adsorption, as sorbent material for removing various heavy metals from an aqueous solution [4]. Its utilization is only 5% in India due to higher technology cost and expected health hazards from the leaching of heavy metals resulting in low social acceptability.

1.2. Phytoremediation of Flyash Dump-Sites

Phytoremediation is the plant based decontamination strategy for land-reclamation and eco-rehabilitation. Many researchers have carried out crop plantation studies on flyash i.e. utilizing flyash in agriculture [5-14]. Biosafety risks exist due to accumulation of toxic elements in the soil and subsequently their transfer into food-chain. Large scale application of flyash is difficult limiting its bulk utilization in agriculture [15-16]. Use of flyash is not always found beneficial as reported by [12] that *Beta vulgaris* L. Var All Green H1 is sensitive to flyash concentrations.

However, tree species offer adequate potential to develop sustainable phytoremediation strategy, i.e. planting tolerant tree species on flyash landfill dykes. They can improve mutual species interactions; help the wastelands make supportive for other vegetations, and improving socio-economic benefits to the local people. Trees help by arresting the movement of loosely lying flyash and help reduce the air pollution significantly [17-18]. Information on the response of tree species grown on flyash is scanty [19-21] and therefore, an innovative programme to understand the potential of locally growing tree species on flyash sites is undertaken.

2. MATERIALS AND METHODS

2.1. Experimental Setup

Experiments were carried out at the School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow in India. Flyash was collected from Feroz Gandhi Unchahar Thermal Power Plant, Unchahar, Rae Bareli in Uttar Pradesh. Different combinations of flyash and garden soil were filled in the polythene-lined earthen pots for growing the seedlings of selected local tree species. Physico-chemical properties of flyash and garden soil were analysed. Dose-response relationship was analysed and plant growth patterns were analysed to understand the tolerance mechanism and phytoremediation strategy.

Various physico-chemical parameters of flyash and garden soil (GS) analysed were pH [22], (pH meter: Direct Readout Ion Meter, A.P.H.A., 1989), Metal estimation (Perkin Elmer, 2380, Atomic Absorption Spectrophotometer), Electrical Conductivity (dSm^{-1}), Organic Carbon [23], Total Nitrogen (Kjeldahl method), Cation Exchange Capacity and Available Phosphorous [24].

Eighteen plant species were found growing around Feroz Gandhi Unchahar Thermal Power Plant (FGUTPP), namely, *Acacia nilotica*, *Azadirachta indica*, *Albizia lebek*, *Bauhinia variegata*, *Cassia siamea*, *Dalbergia sissoo*, *Delonix regia*, *Embilica officinalis*, *Eucalyptus citridora*, *Eucalyptus officinalis*, *Lagerostroemia parsiflora*, *Leucaena leucocephala*, *Morus alba*, *Pithecolobium dulce*, *Prosopis julifora*, *Syzigium cumini* and *Terminalia arjuna*. Based on the visual observation of tolerance in the flyash polluted area, ten species were selected for assessing their relative tolerance on 100% flyash. These were *Acacia nilotica, Bauhinia variegata*, *Cassia siamea*, *Dalbergia sissoo*, *Delonix regia*, *Leucaena leucocephala*, *Morus alba*, *Pithecolobium dulce*, *Prosopis julifora* and *Terminalia arjuna*. The important features for selecting trees for the experiment are high biomass production, a deep root system, high growth rate, high capacity to grow in soils with low nutrient availability and high capacity to allocate metals in the trunk [25-26].

Ex-situ cuttings of *Morus alba* were obtained from Department of Applied Plant Sciences (Horticulture) of Babasaheb Bhimrao Ambedkar (Central) University, Vidya Vihar, Lucknow. One year old seedlings of other nine tree species namely *Acacia nilotica, Bauhinia variegata*, *Cassia siamea*, *Dalbergia sissoo*, *Delonix regia*, *Leucaena leucocephala*, *Pithecolobium dulce*, *Prosopis julifora* and *Terminalia arjuna* were obtained from the Hi-Tech Nurseries of Forest Department, Lucknow at Indiranagar and Alambagh. The average height, average canopy area and average DSH (diameter at six inch height) was measured. Plant height and DSH were measured manually using the inch tape.

Out of six best growing plant species, *B. varieagata*, *C. Siamea, L. leucocephala*, *Pithecolobium dulce*, *Prosopis juliflora* and *T. arjuna*, one leguminous and one non-leguminous species viz. *B. variegata* and *T. arjuna* respectively, have been selected for the detailed study to understand the mechanism of tolerance from the better grown lot of four plant species viz. *A. Indica*, *D. sissoo, D. regia* and *M. alba, M. alba* has been identified for the relative study.

One year old seedlings of *Bauhinia variegata, Morus alba* and *Terminalia arjuna* were grown on earthen pots (10” diameter), lined with polyethene bags were filled by 100% Garden Soil (C), 100% Flyash (T1), 75% Flyash+ 25% Garden Soil (T2), 50% Flyash+ 50% Garden Soil (T3) and 25% Flyash + 75% Garden Soil (T4). One seedling of each plant was planted in each earthen pot.

2.2. Analysis of Plant Growth

Plants were harvested after 60, 90 and 120 days of plantation. Changes in root & shoot lengths were recorded and total biomass were estimated by oven drying the plant material to 80°C. Biochemical parameters analysed were Chlorophyll [27], Protein [28] and Nitrate Reductase [29]. Leaf area as an index of plant growth was measured by Delta-*T* leaf-area measurement system.

At each harvesting, complete the plant was taken out. Root portion was thoroughly cleaned to get-rid of any adhering soil material. The growth parameters measured at each period were: root length, shoot length, number of nodes & internodes, leaflet number, total leaflet area, fresh weight of stem, roots & leaves (i.e. total fresh weight), respective dry weights and percent moisture content.

For root length, after cleaning, the length was measured manually using a measuring tape. Similarly, shoot length was also measured. For leaflet number, all the leaflets were counted in each plant at each harvesting. For photosynthetic area, three leaves each from the lower, middle and top portion of the plants were taken and the leaflet area was measured on Delta T-Device Area Measurement System (UK), giving read out in cm^2 directly; this area was averaged for the whole plant for each pot. Thus, total leaflet area per plant per pot was calculated. Total dry weight was estimated after oven drying the plant material (both above and below ground biomass) to constant weight at 60°C.

3. RESULTS AND DISCUSSION

3.1. Physico-Chemical Characterization of Flyash and Garden Soil

The garden soil used in the experiment of the present study has pH values near neutral. EC_5 value of garden soil is 1.6 dSm^{-1}. The nitrogen and phosphorous contents are 0.09% and 5.18 μgl^{-1} respectively. The metal content in the garden soil is also within the range to support healthy plant growth. The amended soil with flyash was alkaline with alkalinity proportionally increasing with flyash percentage. Phosphorous is reported to be fixed by Al, Fe and Ca contents of the flyash. Low S content in Indian coals and the presence of Ca, Al, Mg and OH ions along with the presence of other cationic trace elements causes high alkalinity of flyash. Soil pH effects are long-lasting on poorly buffered soils. Nitrogen volatilizes during combustion. So the deficiency of both the elements in flyash retards the plant growth. Organic matter content is also negligible in flyash. However, Fe, Ni, Pb, Cu, Al and Si contents are high to cause toxicity to the plants grown on it The concentration of Ni was very high in flyash (204 ppm) compared with average soil value of 20-30 ppm. Flyash used in the experiment was alkaline. pH was 9.6, containing low nitrogen, phosphorus and high metal (Cu, Zn, Ni, Fe, Mn, B, Al, Cd, Pb, Si etc.) as shown in Table 1. The garden soil has pH 7.7. Organic carbon, total nitrogen and available phosphorous contents are high in garden soil. The presence of high concentration of above reported metals, Nitrogen deficiency and Phosphorous non-bioavailability are the plant growth limiting factors and responsible for retarded plant growth on 100% and 75% flyash substrate, indicating the need for selection of tolerant tree species. Implementation of an efficient revegetation program also provides a cost-effective and reliable means for wasteland reclamation. Concentration of Cu in flyash is 58.6 ppm, which is above the average value of 30 ppm for the most world soils. Lead was also present in higher concentrations in the flyash (40 ppm). Water-holding capacity of flyash is lower than soil. However, many authors reported increase in water-holding capacity of soils by flyash addition, which does not increased the plant’s available water, may be due to the pozzolanic property of wet flyash (soil’s hydraulic conductivity deteriorated).

Table 1: Physico-chemical properties of flyash and garden soil.

S. No.	Parameter	Flyash	Garden Soil
1.	pH	9.6 ± 0.42	7.7 + 0.32
2.	Electrical Conductivity EC (dSm^{-1})	8.6 ± 0.38	1.6 ± 0.08
3.	Total Nitrogen (%)	0.002 ± 0.001	0.09 ± 0.002
4.	Available Phosphorous (μgg^{-1})	0.75 ± 0.006	5.18 ± 0.032
5.	Organic Carbon (%)	0.566 ± 0.059	0.741± 0.072
Elements (μgg^{-1}) concentration			
6.	Zn	82 ± 3.1	22.6 ± 1.10
7.	Fe	4150 ± 207	2850 ± 142
8.	Ni	204 ± 10.2	23.8 ± 1.10
9.	Mn	70 ± 3.4	45.8 ± 2.20
10.	Cu	58.6 ± 2.38	38.4 ± 1.80
11.	Cd	42.3 ± 2.12	N.D.
12.	Pb	40.1 ± 2.00	N.D.
13.	B	29.0 ± 1.26	N.D.
14.	Al	4615 ± 230	N.D.
15.	Si	5600 ± 280	N.D.

The garden soil used in the experiment of the present study has pH values near neutral. EC_5 value of garden soil is 1.6 dSm^{-1}. The nitrogen and phosphorous contents are 0.09% and 5.18 μgl^{-1} respectively. The metal content in the garden soil is also within the range to support healthy plant growth.

3.2. Screening of Tolerant Tree Species

The ten tree species were grown on 100% flyash over a period of three months. Out of ten plants species planted on 100% flyash, *Bauhinia variegata, Cassia siamea, Leucaena leucocephala*, *Pithecolobium dulce*, *Prosopis julifora*, *Syzigium cumini* and *Terminalia arjuna* showed best plant growth. *Azadirachta indica*, *Dalbergia sissoo*, *Delonix regia* and *Morus alba* showed good plant response .

3.3. Morphological Growth Parameters of Selected Tree Species

There were significant differences (DMRT, $p<0.05$) in almost all the parameters evaluated in plants grown in fly-ash (100%) and fly-ash amendments. The total plant biomass remained low in flyash (100%). Decreased leaf numbers, leaf area, shoot length, fresh weight of leaves, shoot & root, dry weight of leaves, shoot & root and dry weight of leaves, shoot & root was observed in T1 grown plants. Brown spots on the leaves of flyash grown plants may be attributed to excessive Fe present in flyash inducing deficiency of other essential elements viz. Mn and K. Plants grown in T2 showed slight increase in overall plant growth compared with the plant growth in T1. The three plants *B. variegata*, *T. arjuna* and *M. alba were* selected to understand the mechanism of tolerance of flyash, showed significant differences in all the growth parameters in the various flyash ameliorants. Increase in shoot length was significantly higher in *T. arjuna*, *B. variegata* and *M. alba* plants grown on 50% flyash amendments (T3) over the control plants.

The shoot length of *B. variegata* and *T. arjuna* grown on 75% flyash (T2) was also better than control. In 100% flyash treatment, *T. arjuna* plants showed shoot length almost comparable to the control plants. Only a slight reduction as compared to control was observed in the *B. variegata* plants grown on flyash alone. Reduction in root growth was observed in all the plants of *M. alba* except in T3 plants. Increase in shoot length was significantly higher in *T. arjuna*, *B. variegata* and *M. alba* plants grown on 50% flyash amendment as compared to respective control plants and other treatments. The results of control of shoot length of the three plant species is comparable to that of T4 treatment indicating the probability of a trend dependent upon concentration of flyash being added and affecting the plant growth accordingly.

The root growth was reduced in all the T1 grown plants. T3 showed highest root length in all the three species. The root length at 100% flyash was slightly lower than control in *T. arjuna.* Fresh & dry weight of shoot and root was highest in the plants grown in T3 amendment in the three consecutive harvestings. T3 supported the growth of plant, increase in photosynthetic area and leaf number was observed. Nodule count was also highest in T3 grown plants of leguminous species *B. variegata* and *M. alba*, reflecting plant's ability to fix atmospheric nitrogen as an adaptation mechanism to survive in N_2-deficient substrate. Water infiltration and root penetration are limited due to pozzolanic property of flyash, which cement the soil particles. The important constituent element of flyash is Al, is also reported to present root elongation.

3.4. Chlorophyll and Protein Synthesis in Leaves

Total chlorophyll concentration increased progressively in the order T1< T2< T4< C< T3 plants of the three species as shown in Table 2.

Table 2: Chlorophyll concentration ($mgg^{-1}fw$) in the plant leaves.

Treatment (s)	Harvesting period of growth (days)		
	60	**90**	**120**
T. arjuna			
C	0.4 ± 0.01	0.48 ± 0.01	0.59 ± 0.01
T1	0.32 ± 0.02	0.41 ± 0.02	0.48 ± 0.02
T2	0.34 ± 0.01	0.46 ± 0.03	0.54 ± 0.01
T3	0.45 ± 0.03	0.57± 0.02	0.68 ± 0.03
T4	0.37 ± 0.02	0.52 ± 0.03	0.56 ± 0.01
B. variegata			
C	0.42 ± 0.004	0.5 ± 0.04	0.64 ± 0.01
T1	0.36 ± 0.01	0.525 ± 0.04	0.57 ± 0.05
T2	0.47 ± 0.005	0.56 ± 0.01	0.62 ± 0.01
T3	0.58 ± 0.003	0.68 ± 0.02	0.67 ± 0.04
T4	0.52 ± 0.005	0.59 ± 0.015	0.62 ± 0.01
M. alba			
C	0.39 ± 1.3	0.65 ± 1.2	0.82 ± 1.0
T1	0.24 ± 1.2	0.36 ± 0.7	0.43 ± 0.6
T2	0.21 ± 0.8	0.4 ± 1.2	0.51 ± 1.2
T3	0.68 ± 1.1	0.82 ± 1.2	0.98 ± 1.3
T4	0.29 ± 1.0	0.42 ± 0.7	0.53 ± 0.5

Values are Mean (n=3) ± SD; All values are significantly different at $p<0.05$ according to two-way ANOVA. Any two means having a common letter (super-script) are not significantly different at the 5% level of significance as per DMRT.

Leaf protein concentration also varied significantly between different treatments. T3 plant leaves of all the three plant species showed maximum protein concentration as in Table 3. Chlorophyll and Protein contents were least in T1 (100% flyash) and highest in T3 (50% flyash) in all the three plant species studies. Decreased chlorophyll concentration in the leaves of plants grown on 100% flyash grown plants may be due to reduced synthesis and accelerated degradation of chlorophyll as metal inhibitory effects. Cu and Pb cause reduction in chlorophyll concentration S-Amino-levulinic Acid Dehydratase (ALAD) is a metal sensitive enzyme, which is associated with the initial steps of chlorophyll synthesis. Reports of the impacts of heavy metals on photosynthesis demonstrated negative effects on pigments, photo-systems, enzymes and gas exchange especially the PSII polypeptide is sensitive to metal toxicity [30].

Table 3: Protein concentration ($mgg^{-1}fw$) in the plant leaves.

Treatment (s)	Harvesting period of growth (days)		
	60	**90**	**120**
T. arjuna			
C	34.0 ± 1.4	38.6 ± 1.3	44.2 ± 1.1
T1	21.6 ± 1.2	27.3 ± 1.1	32 ± 1.3
T2	30.4 ± 1.1	34.1 ± 1.2	38.4 ± 1.2
T3	45.2 ± 1.3	51.6 ± 1.4	59.8 ± 1.1
T4	36.2 ± 1.2	42.3 ± 1.1	48.6 ± 1.3
B. variegata			
C	36.4± 0.4	41.2 ± 1.1	45.3 ± 1.33
T1	28.4 ± 1.2	33.5 ± 1.3	39.1 ± 0.50
T2	31.6 ± 0.6	36.6 ± 1.74	42.7 ± 1.86
T3	49.2 ± 1.4	53.7 ± 1.0	62.8 ± 1.4
T4	39.7 ± 1.3	43.7 ± 1.1	48.4 ± 1.2
M. alba			
C	16.51 ± 3.0	21.6 ± 2.1	26.25 ± 1.7
T1	12.4 ± 1.2	14 ± 2.4	17.5 ± 2.1
T2	21.6 ± 2.8	27.2 ± 3.1	31.2 ± 3.4
T3	36.8 ± 3.6	42.6 ± 1.8	55.5 ± 6.1
T4	23.2 ± 1.4	29.1 ± 3.2	38.75 ± 4.5

Values are Mean (n=3) ± SD; All values are significantly different at $p<0.05$ according to two-way ANOVA. Any two means having a common letter (super-script) are not significantly different at the 5% level of significance as per DMRT.

3.5. Nitrate Reductase Activity

Nitrate Reductase activity was least in T1 treated plants of *T. arjuna* and maximum in T3 plants at the respective sampling days. However, in *B. variegata* which is a leguminous plant, maximum NR activity was observed in C plants and minimum was observed in T3 amendments, with a steady decline in activity at 60, 90 and 120 days as shown in Table 4.

Table 4: Nitrate Reductase concentration ($mgg^{-1}fw$) in the plant leaves.

Treatment (s)	Harvesting period of growth (days)		
	60	90	120
T. arjuna			
C	201.2 ± 8.4	179.9 ± 6.3	166.3 ± 9.1
T1	100.8 ± 5.2	93.6 ± 8.1	87.5 ± 6.9
T2	198.9 ± 7.1	181S.3 ± 5.2	167.7 ± 4.2
T3	258.3 ± 6.3	237.6 ± 7.4	212.3 ± 8.1
T4	220.3 ± 4.2	184.6 ± 1.1	173.4 ± 1.3
B. variegata			
C	283.6± 6.4	274.2 ± 10.1	256.8 ± 5.33
T1	112.4 ± 7.2	102.6 ± 6.3	98.3 ± 4.5
T2	203.4 ± 5.6	183.6 ± 7.7	164.3 ± 5.86
T3	243.2 ± 6.4	221.3 ± 8.0	206.4 ± 6.4
T4	226.3 ± 7.3	192.3 ± 7.1	186.4 ± 5.2
M. alba			
C	399.4 ± 32.9	368.6 ± 32.4	343.4 ± 30.4
T1	214.6 ± 27.7	179.4 ± 15.9	167.7 ± 28.8
T2	194.6 ± 17.2	167.4 ± 14.2	156.7 ± 17.9
T3	170.2 ± 14.4	154.3 ± 12.7	140.4 ± 14.1
T4	158.6 ± 12.9	145.9 ± 14.2	130.2 ± 14.5

Values are Mean (n=3) ± SD; All values are significantly different at $p<0.05$ according to two-way ANOVA. Any two means having a common letter (super-script) are not significantly different at the 5% level of significance as per DMRT.

Prosopis juliflora L., a legume species was reported to show flyash tolerance mechanism indicated by enhancements in plant biomass, photosynthetic pigments, protein content, biomass, in vivo nitrate reductase activity and higher accumulation of metal contents & nodule count [31].

3.6. Efficacy in phytoremediation

The overall plant performance of the three species was found best in T3 flyash amendment indicating the efficacy of T3 flyash amendment in flyash landfill reclamation which is also practically possible. Results of T1 and T2 showed stunted plant growth, comparable to control, indicating the toxic effects of flyash and effective detoxification potential of the plant.

The important parameters analysed for the detoxification mechanism are the nodule count and the nitrate reductase activity. Nodule count in leguminous plant species *B. variegata* and *M. alba* is significantly higher in T3 amendments confirming the efficient nitrogen fixation.

Scope of further study lies in a trial plantation on the landfill dykes with the important aspects towards viability as indicated in the present study i.e. planting tolerant trees (*B. variegata, T. arjuna, M. alba*) along with the locally growing species to ensure positive species interactions towards synergistic ecology. Ultimately, it's a natural phenomenon in which efficacy of phytoremediation is to be facilitated keeping in view the experimentation studies. Biotechnological approach is also an important tool for promoting phytoremediation based eco-rehabilitation.

4. CONCLUSION

Characterization of the tolerance against flyash toxicity is the key feature in selection of locally growing tree species. The essential recommendations by various researchers include fast growth, high biomass and active detoxification mechanism. *B. variegata*, a nodulated leguminous plant, showed efficient potential for bioremediation of flyash. *M. alba* also exhibited detoxification potential. *T. arjuna*, a non-leguminous plant also exhibited sufficient detoxification mechanism as well not retarded growth, thus is also recommended for landfill plantation. Flyash amendments showed favourable impacts. 50% FA + 50% GS (T3) amendment was best suited for the plant growth. 100% flyash hampered plant growth in all the studied species. *B. variegata* species is recommended for plantation on flyash dykes. Keeping in view, the detoxification potential of the three tree species, their plantation is suggested to attain positive interactions at the landfill i.e. one species showing lesser tolerance might help reduce the future growth limiting factor or enhance ecological interactions forming a self-sufficient stable ecosystem.

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