Phytoremediation Studies on Coal Mine Waste and Coal Fly Ash by Leucaena Leucocephala

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***Abstract*—Pot culture experiments were conducted by using various % of Soil, Coal Fly Ash and Coal Mine waste as contaminant to study the relative growth characteristics and yield potentials of *Leucaena leucocephala* .The level of different compositions are Soil (100%), Coal Mine Waste (100%), Coal Fly Ash (100%), Soil + Coal Mine Waste (50:50) %, Soil + Coal Fly Ash (50:50) %.The data on seed germination, growth parameters e.g total length, shoot length, root length, leaf number, total biomass production, chlorophyll, carotenoid and protein was recorded.**

***Index Terms*—Pot culture experiment, Leucaena leucocephala, Coal fly ash, Coal mine waste**

I. Introduction

Coal which used for thermal power generation is known for its high ash content (40-45 %).Due to low grade of coal and use of inferior model of electrostatic precipitator's higher amount of fly ash evolves. Emission of particulate matters and noxious gases cause environmental hazards because the ash comprises of SiO2, Al2O3 and oxides of iron and other toxic metals. These oxides change the chemical and biochemical composition of the plant grown on fly ash[1]. To mitigate pollution due to thermal power plants particularly fly ash, it is essential to select suitable plant species which can easily colonize in fly ash. Fly ash as such is not suitable for plant growth unless it is weathered for some period and organic amendments are added. [2]-[4].One of the most promising use of fly ash is to utilize it for tree plantation purpose which beside land reclamation also helps to lock up the toxic heavy metal present in the fly ash, in the wood biomass for longer periods as compare to crop plants. Present study highlights how *Leucaena leucocephala* is utilized for removal of pollutants from fly ash. Coal mine spoils vary from neutral to acidic in nature. They are sandy and subjected to severe erosion by wind and rain. In order to check the erosion re-vegetation of mine spoil has become mandatory. Keeping the reclamation and re-vegetation of coal mine spoil and coal fly ash in view a pot culture experiment has been conducted to study the effect of garden soil amended coal fly ash and coal mine spoil with *Leucaena leucocephala*.

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II. Materials & Methods

A. Plant Materials and Growth Conditions

Fly ash samples used in research were collected from Talcher thermal power station located at 250 27' – 260 13' N latitude and 850 27' – 86001' E longitude in district of Talcher, Orissa, India and coal mine spoil was collected from south Bolanda Colliery spreads over 2582.90 acres is located within Latitude 200 54' 58" – 200 55' 55" and Longitude850 07' 44" – 850 11' 39" in South West of NCDC'S(National Coal Development Corporation).Talcher Colliery in 200 57' – 850 10' in Angul district of Orissa, India.

B. Soil mixture Nutrient and Physical Parameter Study

Different soil beds were air dried and analyzed for field capacity and for soil texture,[5].Soil pH was determined by using Eutech pH electrode .The estimation of organic carbon and phosphorous was determined by the slight modification of method [6],[7]sample were prepared for elemental analysis and analyzed with Atomic absorption Spectrophotometer [8]

C. Plant Samples and Pot Culture Experiment

Good and viable seeds of *Leucaena* released by RPRC, Bhubaneshwar,Orissa,India were selected for the experiments. Pot culture experiments were conducted at Regional Plant Resource Centre, Bhubaneshwar, survival records up to three months. A total of 50 Plastic black pots of 15 cm long and 30 cm diameter used in the experiment were thoroughly cleaned and filled up with different concentrations of soil, mine waste and coal fly ash i.e., soil(100%), mine waste(100%), coal fly ash(100%), soil :mine waste (50:50)%, soil: coal fly ash(50 :50)%.Seeds of *Leucaena leucocephala* was treated with 0.1% Mercuric Chloride for 5 minutes followed by 70% alcohol for 1 minute. The sterilized seeds were thoroughly washed with distilled water. The seeds of *Leucaena leucocephala* were soaked for 48 hours before sowing.10 seeds were sown in each pot. So 5 set of compositions were taken and each set consists of 10 replica pots. The pots were kept in a growth chamber/temperature controlled Green House under a temperature of 35-40^{0}C approximately and water at least twice daily or as per requirements.

D. Sampling of Plant Parts for Growth Analysis.

Sampling was done at random after 7 days from the day of seed germination.5 plants were collected randomly from each set of study i.e uprooted carefully with their roots intact. The root and shoot portions were separated and

washed with de-ionized water. Samples were now made moisture free and weighed quickly to obtain fresh weight (FW) in grams. After this the plant parts (roots and shoots) were kept in separate labeled paper bags inside an oven at 500C for 48 hours for drying in constant weight in order to determine the dry weight (DW) in g. This will be dry weight of sample or may be termed as above ground or underground biomass yield.

E. Estimation of Photosynthetic Pigments.

On the scheduled sampling dates matured leaves were collected during morning hours from different plants under each set of treatment for the estimation of chlorophyll. Five replicates were taken for each treatment. Chlorophyll-a, Chlorophyll-b, Total chrophyll and Carotenoid content respectively and the quantitative analysis of photosynthetic pigments were calculated on (mg/g) of fresh weight tissue basis [9].

F. Quantitative Estimation of Protein by Bradford Reagent

Protein sample were isolated after ninety days of germination and extracted then estimated [10].500mg of leaf samples were taken in a pre-chilled mortar and pestle. Then they were grinded with 1.5ml cold Tris Buffer (50 mM Tris HCL, PH 7.5) in order to extract Root and Shoot proteins respectively. Then the isolated proteins were transferred to 1.5 ml pre-chilled microfuge tube. Then the tubes were centrifuged at 5000 rpm for 10 minutes at 40C and the supernatant was transferred to another microfuge tubes and stored at 40˚C. Now 5ml of Bradford reagent was added to the microfuge tubes containing 20µl of extracted protein and the absorbance was measured at 595nm in a UV-Vis Spectrophotometer and by taking the values a standards curve was prepared.

III. Result and Discussion

Various physico chemical parameters of the garden soil and individual coal fly ash and coal mine waste were measured in this study and observed that coal fly ash had alkaline pH, followed by garden soil which is less acidic and coal mine waste having still more acidic pH. Field capacity was found to be higher in case of garden soil followed by coal mine waste and coal fly ash. The texture of the soil i.e. the percentage of sand slit and clay also varies and found as tabulated in Table I.

TABLE I: Physico- Chemical Properties of the Soils

Type of Soil	Soil pH	Field capacity	Texture		
			Sand	Silt	Clay
Garden soil	5.8	19%	75	11	14
Coal fly ash	7.5	7%	57.6	12.3	27.6
Coal mine waste	5.5	11%	90	5	4

Nutrient status and heavy metal content of the different soils was found to vary, Al, Cr and Fe content was higher than garden soil in case of coal mine waste but lower in case of coal fly ash. The N, organic carbon, Ca, K, P and Mg quantity was tabulated in Table II. After 7 Days of the experiment the highest no of germinated seed was found on Coal Fly Ash (60%) and Mine Waste (60%) which was 50% in Control and 50%in CFA: Soil (50:50) %.and 40% in mine waste: Soil (50:50) %.(Fig.1)

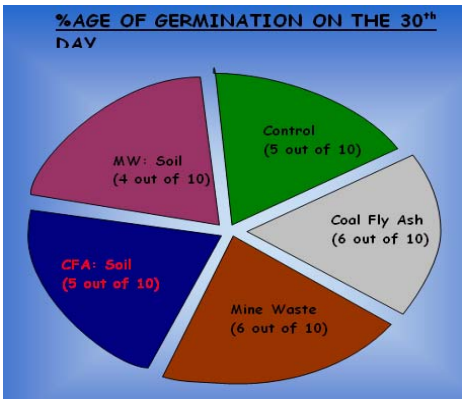

Fig. 1. Effect of different composition on germination of leucaena leucocephala.

TABLE II: Nutrient and Heavy Metal Status of Different Soil Mixture

Types of Soil	Nutrient Status in ppm						Heavy metal status in ppm		
	OC	N	P	K	Ca	Mg	Al	Cr	Fe
Garden soil	0.78	0.39	3.82	290	152	381	1230	25.2	2031.5
Coal fly ash	0.4	0.032	0.19	0.55	1.41	0.32	26.2	8.19	6.31
Coal mine waste	1.53	0.06	1.24	125	128	207	7720	56.4	2935

On the 7th day plant growing on three types of samples (Control, Coal Fly Ash(100%), Mine Waste(100%) have 1 leaf in each, no leaves developed on other two CFA:Soil (50:50)% and Mine Waste (50:50)%.On the 15th day the plant growing on CFA: Soil (50:50) % and Mine Waste (50:50) % has fewer number of leaves i.e., 2. On 21st day Control has maximum number of leaves i.e., 5 where as Mine Waste (100%) has 4 leaves and the other 3 compositions have 3 leaves each .On the 30th day a maximum of six number of leaves were found in 100% Mine waste. Root length was found maximum on Control and it was found to be 15.8 cm on the 30th day of observation. Root Fresh Weight (FW) was found maximum on MW: Soil (50:50) % and it was found to be 0.333 g on 30th day observation. (Fig. 3).

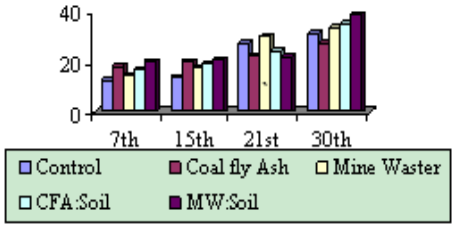

Fig. 2. Effect of different composition on seedling total length of leucaena leucocephala.

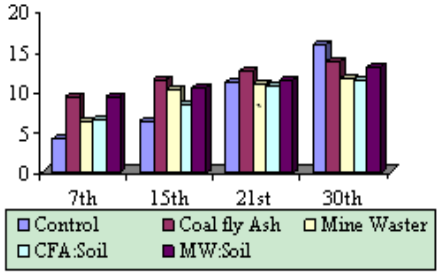

Fig. 3. Effect of different composition on seedling root length of leucaena leucocephala.

On the 7th day of observation maximum shoot length was found on MW: Soil (50:50) % which was 11.1 cm. On the 15th day of observation maximum shoot length was found on Coal Fly Ash (100%) which was 13.7 cm. On the 21st day of observation maximum shoot length was found on MW: Soil (50:50) % which was 19.2 cm. On the 30th day of observation maximum shoot length was found on MW :Soil (50:50)% which was 26.3 cm ,hence rapid growth of tree found on MW : Soil(50:50)%. (Fig.4). Shoot fresh weight was found to be maximum 1.856 g on composition of CFA: Soil (50:50) % on 30th day of observation. Shoot dry weight was found maximum on MW: Soil (50:50) %.It was found to be maximum 3.24 on composition of Mine Waste (100%) on 30th day of observation. The increased biomass yield is mostly found in the mix samples of both Coal Fly ash and Mine Waste with Soil i.e., CFA: Soil (50:50) % and MW: Soil (50:50) % because in vitreous phase of ash sample were slowly released into the soil solution when applied to soil.(Fig.7-12).

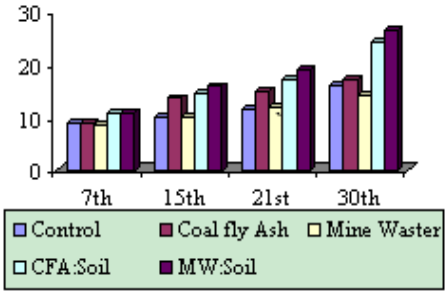

Fig. 4. Effect of different composition on seedling shoot length of leucaena leucocephala

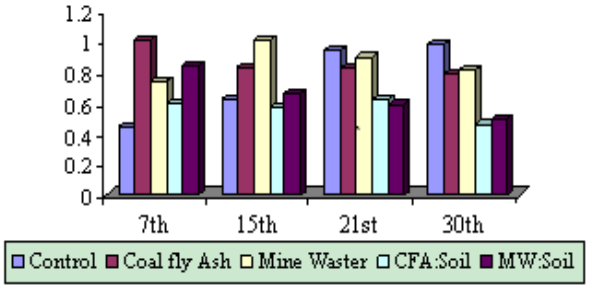

Fig. 5. Effect of different composition on root length/shoot length of the plant leucaena leucocephala

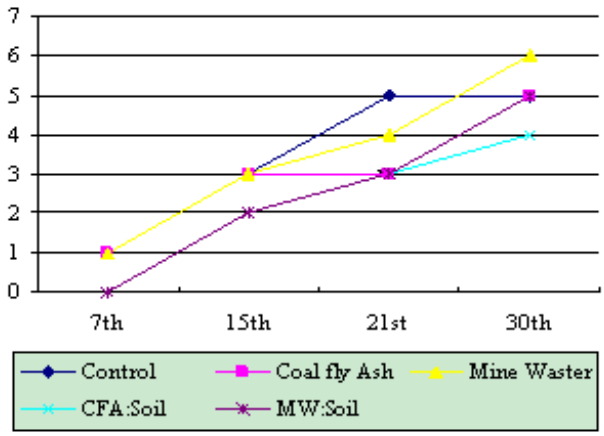

Fig. 6. Effect of different composition on number of leaves of leucaena leucocephala

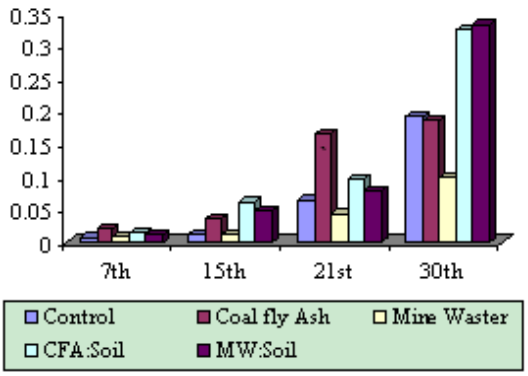

Fig. 7. Effect of different composition on seedling root fresh weight

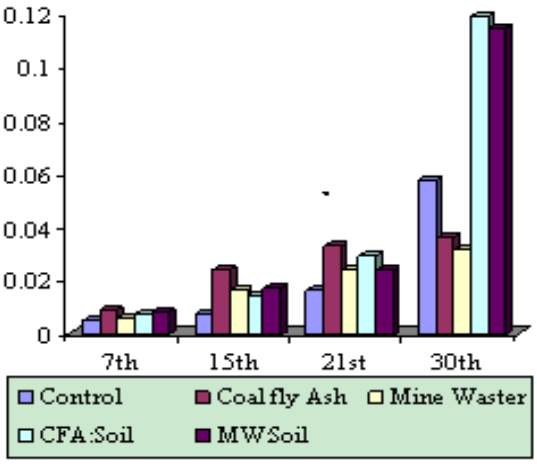

Fig. 8. Effect of different composition on root dry weight of the plant leucaena leucocephala

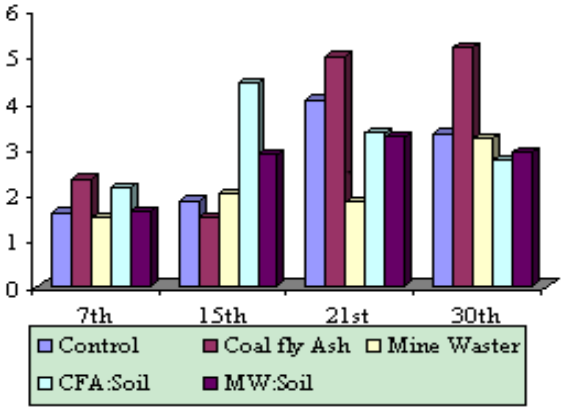

Fig. 9. Effect of different composition on root FW/DW of the plant leucaena leucocephala

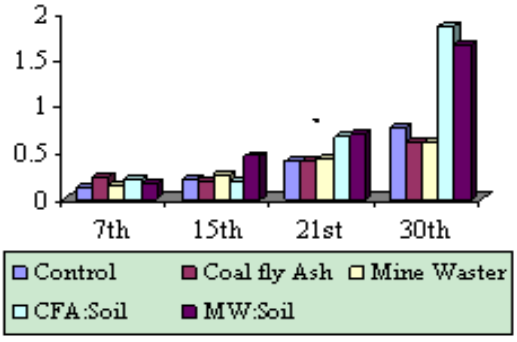

Fig. 10. Effect of different composition on shoot fresh weight of the plant leucaena leucocephala

TABLE III: ESTIMATION OF SHOOT PROTEIN CONTENT OF LEUCAENA LEUCOCEPHALA (90TH DAY)

Sl. No.	Composition	Conc. In µg/µl	Absorbance at 595 nm
1	Coal Fly Ash (100%)	57.0	0.8210
2	MW: Soil(50:50)%	90.0	0.9830
3	Mine Waste (100%)	96.0	1.0501
4	Control	103.0	1.1296
5	CFA: Soil(50:50)%	108.0	1.1831

The total chlorophyll content of the plant grown on various soil beds was found on observed on CFA: Soil (50:50) % which was observed 0.1587 mg/g on 20th day and 0.1446 mg/g on 30th day. The study has been substantiated by the investigation done [11-12].The protein content of shoot of the plant was highest incase of CFA: Soil i.e, 108µg/µl after 90th days of germination. Similar studies have been made by [13-14] on *Acacia nilotica* and *Casia tora* respectively. (Table III).

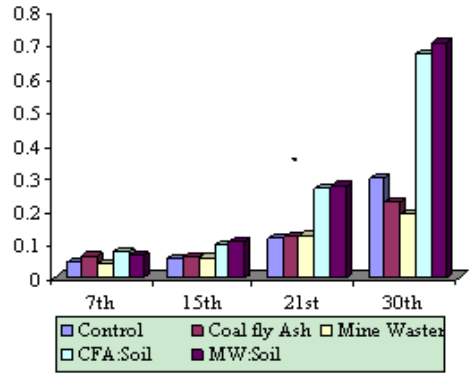

Fig. 11. Effect of different composition on shoot dry weight of the plant leucaena leucocephala

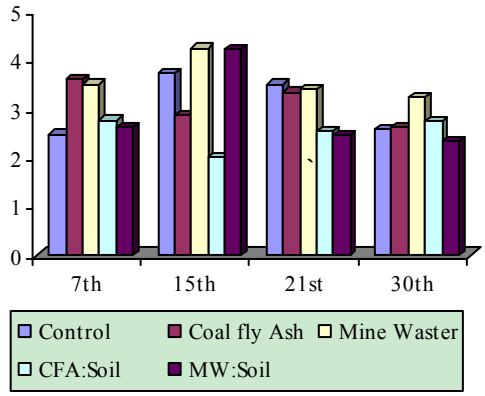

Fig. 12. Effect of different composition on shoot fresh weight/dry weight of the plant leucaena leucocephala

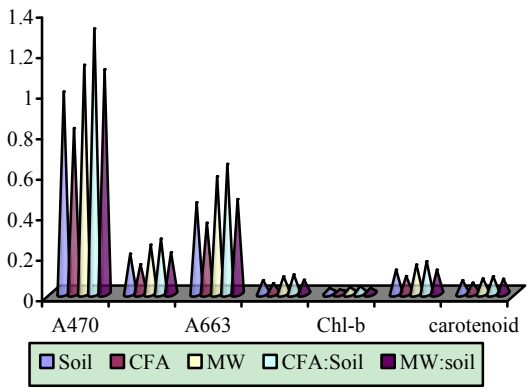

Fig. 13. Estimation of chl-a, b, total chlorophyll and carotenoid content of leaf samples of leucaena leucocephala (Day 20)

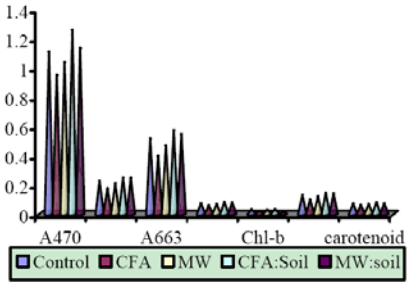

Fig. 14. Estimation of chl-a,b,total chlorophyll and carotenoid content of leaf samples of leucaena leucocephala (Day 30)

IV. CONCLUSION

The phytoremidial studies on coal mine waste and coal fly ash by Leucaena leucocephala revealed that the coal mine sites necessarily require the re-vegetation and plantation strategies based on the regress screening of the plants having the potential to acclimatize and combat the physicochemical parameters in and around the sites. The present investigation suggests that the plantation of *Leucaena leucocephala* is one of the best options for phytoremediation of the coal mine wastes.

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