

Fly ash as an amendment agent for soil fertility

A thesis submitted
in fulfillment of the requirement for
the award of the degree of

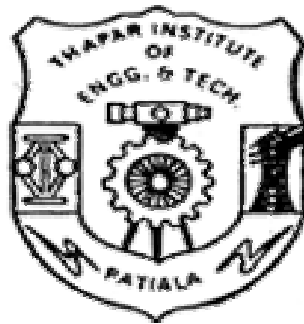
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Certificate

Certified that the thesis "Fly ash as an amendment agent for soil fertility" which is submitted by Ms. Sudha Jala, in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy in the Department of Biotechnology & Environmental Sciences, Thapar Institute of Engineering & Technology, (Deemed University), Patiala, is a record of candidate's own independent and original research work carried out by her under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.



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Synopsis

Coal is an exhaustible energy source, which plays a critical role in meeting the ever-increasing energy demands of countries around the world. Combustion of coal in thermal power stations produces a variety of residues viz. fly ash, bottom ash, flue gas desulfurization waste (scrubber sludge) and fluidized bed boiler waste and coal gasification ash. The physical, chemical and mineralogical characteristics of fly ash depend on a variety of factors such as composition of parent coal, combustion conditions, the efficiency and type of emission control devices and the disposal methods used. Fly ash is an amorphous mixture of ferroaluminosilicate minerals generated from combustion of ground or powdered coal at temperature ranging from 400-1500°C. It is composed predominantly of small, glassy, hollow particles with specific gravities ranging from 2.1 to 2.6. g cm⁻³. Physically, fly ash occurs as very fine particles having an average diameter of <10 µm, low to medium bulk density, high surface area and light texture which are aggregated into micron and sub-micron spherical particles, of size ranging from 0.01 to 100 µm, with smaller particles entrapped within large spheres. 90-99% of fly ash consists of Si, Al, Fe, Ca, Mg, Na and K. Major matrix elements in fly ash are Si and Al together with significant percentage of K, Fe, Ca and Mg. Fly ash contains all naturally occurring elements and is substantially rich in trace elements like lanthanum, terbium, mercury, cobalt and chromium.

Over 225 million tonnes of coal is being produced annually in India. The coal, which is of poor quality, generates on an average about 40% ash. In India over 100 thermal power stations are producing around 108 million tonnes of fly ash every year and it is expected to double by the year 2010. On account of its heterogenous nature fly ash has the potential to be used as a soil-ameliorating agent in agriculture and forestry. Field and greenhouse studies both indicate that many chemical constituents of fly ash may benefit plant growth and can improve agronomic properties of soil. Fly ash addition alters physical properties of soil such as texture, bulk density, water holding capacity and particle size distribution.

The disposal of fly ash by conventional methods leads to degradation of arable land and contamination of the ground water. Therefore, development of proper technology for disposal of this solid waste in an eco-friendly manner becomes mandatory and to derive maximum benefit from its heterogenous nature since it is a storehouse of readily available plant macro and micronutrients. In conjunction with organic manure, microbial inoculants or fertilizers fly ash can be used to design a soil benefaction strategy, which would help in improving the properties of soil and enriching its nutrient status. Presence of almost all essential plant nutrients in ionic form and ameliorating effect on physical, chemical and microbial nature of the soil, makes fly ash an important input for biomass production especially on variously degraded soils and waste lands. Keeping the above factors in mind a comprehensive study was undertaken on “Fly ash as an amendment agent for soil fertility” with following objectives:

Objectives

1. Characterization of fly ash for its physicochemical properties.
2. To study the effect of fly ash amendment in soil on soil microbial activity.
3. To screen fly ash-resistant soil microorganisms such as free-living N₂-fixers and phosphate solubilizers.
4. Molecular tagging of beneficial soil bacteria for ecological monitoring in soil / fly ash-amended soils.
5. To study the effect of fly ash on the growth of nursery seedlings of *Populus*.
6. To study the effect of fly ash on growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in established field plantation.

Approach adopted to meet above objectives

In the present study the effect of fly ash addition at different concentrations in soil was investigated in terms of biomass production, microbial activity and nutrient status of soil. Unweathered electrostatic precipitator fly ash (ESP) from three different sources was used as a soil-amending agent in acidic and alkaline soil of two regions i.e., village Durgaprasad (Dist. Dhenkanal, Orissa) and Patiala (Punjab). Both fly ash and soil were characterized for chemical, physical and microbiological properties. The studies related to effect of fly ash on microbial activity were conducted under laboratory conditions by

mixing fly ash with soil at different concentrations and studied for bacterial enumeration, soil dehydrogenase activity, rate of carbon-dioxide evolution and soil microbial biomass at different time intervals. A number of phosphate-solubilizing bacteria were isolated from the fly ash-amended soil from established field plantations and checked for their efficiency in solubilizing tricalcium phosphate. The phosphate solubilizing bacterial isolate was transformed with the *lacZ* marker and inoculated in soil with and without fly ash to study its proliferation. The effect of fly ash on growth and biomass production was studied in nursery trials of *Populus deltoides* and established field plantations of *Acacia auriculiformis* and *Eucalyptus tereticornis* along with assessment of the nutrient status of soil with the objective to determine optimum dose of ESP fly ash as an agent for improving soil fertility and crop production.

1. Characterization of fly ash for its physicochemical properties

Fly ash from electrostatic precipitators (ESP fly ash) collected from three different sources viz. BILT, Choudwar, Orissa, GGS Thermal Power Plant, Ropar, Punjab and BILT, Yamunanagar, Haryana was directly analyzed for physicochemical properties after air-drying. Acidic soil from village Durgaprasad, Distt. Dhenkanal, (Orissa) and alkaline soil from Thapar Technology Campus, Patiala (Punjab) collected from the 0-30 cm layer, was processed and subjected to physical analysis (bulk density, water holding capacity, particle size distribution, hydraulic conductivity), chemical analysis (pH, electrical conductivity, organic carbon, total and available N, P, K, S, Fe, Zn, Ni, Cr, Pb) and microbiological analysis. The ESP ash from three different sources showed marked heterogeneity in physico-chemical properties. Phosphorous (0.108%) and potassium (0.107 %) were higher in acidic ESP fly ash from Choudwar among all the fly ash samples studied. On the other hand nitrogen (0.30 %) and sulphur (0.07%) was higher in alkaline fly ash from Yamunanagar. Most of the secondary elements (Ca, Mg, Na) and micronutrients (Fe, Zn, Mn) were in higher concentrations in acidic fly ash. Calcium (0.34%) was the dominant element in fly ash from Choudwar followed by sodium (0.04 %) and magnesium (0.03 %). Iron and manganese content was highest in fly ash from Choudwar corresponding to 6760 and 739 mg/kg respectively. The micronutrients were in the order of Fe>Mn>Zn>Cu>Mo for all the fly ash samples studied. In the fly ash from

all sources As (6.2 to 7.0 mg/kg) was higher than Se (3.6 to 4.6 mg/kg) but was within permissible limits. Nickel (13 to 25.3 mg/kg) was less than Pb (28.5 to 67.1 mg/kg) and Cr (33.3 to 330 mg/kg) in the three ESP fly ash samples characterized and overall the order of all nutrients was Fe>Mn>Zn>Cr>Pb>Co, which implies that fly ash is rich in Fe and Mn. All the elements present in fly ash were within range as reported in the literature. The bulk density, hydraulic conductivity and water holding capacity ranged from 0.36 to 0.9 g cm⁻³, 14 to 23.1 cm min⁻¹ and 67 to 73% respectively in the ESP fly ash from different sources. The texture of fly ash in general was loamy sand with sand ranging from 84.6 to 87.5%, silt from 5.5 to 12.0 % and clay from 3.0 to 7.4 %.

All nutrients present in soil used in nursery and field trials were also present within range as reported in the literature. Nitrogen was highest in the acidic (0.11 to 0.14%) and alkaline soil (3.99 to 4.44%) followed by phosphorous and potassium. Calcium was the dominant secondary element in the acidic soil (0.10 to 0.14%) while magnesium was highest in alkaline soil (0.14 to 0.16%). The order of micronutrients was Fe>Mn>Zn in the acidic as well as alkaline soil. Among the heavy metals As (3.5 to 3.9 mg/kg) was greater than Se (2.3 to 2.4 mg/kg). Lead (153 to 163 mg/kg) was much higher than chromium (48 to 54 mg/kg) and nickel (10.7 to 56.0 mg/kg) in the acidic soil. An overall comparison between the acidic and alkaline soil showed greater release of heavy metals and micronutrients in the acidic soil. The bulk density, hydraulic conductivity and water holding capacity ranged from 0.87 to 1.3 g cm⁻³, 14.8 to 21.0 cm min⁻¹ and 33.6 to 45 % respectively in the acidic and alkaline soil respectively. The acidic soil had a clay texture and the alkaline soil had a loamy sand texture.

2. To study the effect of fly ash amendment in soil on soil microbial activity

The rate of carbon-dioxide evolution, microbial biomass, soil dehydrogenase activity and total organic carbon are sensitive indicators of soil quality and sustainability in understanding the complexities of the nutrient profile in soil and therefore were examined to study the microbial activity in fly ash-amended soil. Addition of glucose as an organic carbon source resulted in concentration-dependent increase in soil dehydrogenase activity in soil amended with 5% fly ash. Maximum enzyme activity was observed at 4% glucose, which could be correlated with increased microbial populations and activities resulting

from glucose acting as a carbon source. Fly ash added at an optimum concentration between 10-12% (v/v) to acidic and alkaline soils did not have any adverse effect on microbial activity. No distinct variation was observed in the rate of carbon dioxide evolution in the soil without fly ash and soil plus fly ash mixtures over a given time period and with increasing fly ash percentage. A positive correlation between soil microbial biomass and organic carbon with 10-12% fly ash amendment was observed.

3. To screen fly ash-resistant soil microorganisms such as free-living nitrogen-fixers and phosphate solubilizers

Phosphate solubilizing and free-living nitrogen-fixing bacteria in rhizospheric soil collected from 3-year-old plantation of *Acacia auriculiformis* and *Eucalyptus tereticornis* were screened on Pikovskya and Jensen's media, respectively. The population of free-living nitrogen-fixing bacteria was comparatively much lower than phosphate-solubilizing bacteria in all treatments in the rhizosphere soil of both tree species. Beyond 6 % fly ash addition there was an almost negligible count. No free-living nitrogen-fixing bacteria were observed in soil containing 18-24% fly ash with treatments without fertilizer and with fertilizer. The population of phosphate solubilizing bacteria in fly ash amended soil mixed with chemical fertilizer was higher in the presence of fly ash at level as high as 12%, attributed mainly due to the synergistic effect of fly ash and fertilizers on the soil microenvironment. Further studies were concentrated on phosphate-solubilizing bacterial isolates and their percentage solubilization of tricalcium phosphate (TCP) in Pikovskya media at different time intervals. With increase in time of incubation the concentration of solubilized TCP was found to increase with a corresponding decrease in the pH. The efficiency of percentage solubilization of TCP ranged from 36.5 to 86.1%, the highest being for five bacterial isolates designated as S1, S2, S3, S4 and S5. The percentage solubilization of TCP was 78.5% by strain S1, 80.0% by strain S2, 86.1% by strain S3, 78.7 % by strain S4 and 82.8 % by strain S5.

4. Molecular tagging of beneficial soil bacteria for ecological monitoring in soil / fly ash-amended soils

Molecular tagging of an efficient phosphate solubilizing bacterial isolate was carried out for ecological monitoring in soil and fly ash amended soils. Antibiotic profiling of the selected strains was carried out to check their inherent resistance or sensitivity to standard antibiotics in order to choose a competent strain on media containing either of the antibiotics streptomycin ($50 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), nalidixic acid ($50 \mu\text{g ml}^{-1}$) and chloramphenicol ($10\mu\text{g ml}^{-1}$), respectively. All strains were sensitive to kanamycin and chloramphenicol and were lac Z negative. Among these strains phosphate-solubilizing bacterial isolate S2 was transformed with lac Z and chloramphenicol marker carrying plasmid pMMB277 from E.coli MTCC 2842. The transformant S2: pMMB277 was inoculated in soil under sterile and non-sterile conditions mixed with fly ash at 0, 10 and 30% (on a v/v basis). The ecological monitoring in terms of their population build-up was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient agar plates. Uninoculated control for sterile and non-sterile soil was run as a positive control. The soil was incubated in the dark at 30°C at 40-50% moisture level over two months and was periodically analysed for available phosphorous, organic carbon, soil microbial biomass and bacterial count. The enumeration of inoculated bacteria on selective media indicated that with an increase in fly ash concentration there was a negative effect on soil microbial population and an optimum concentration of 10% is tolerable for microbes indicating that its amendment provides micronutrients for growth. A similar positive correlation was observed for microbial biomass, which increased with increasing fly ash percentage up to 5-10%.

5. To study the effect of fly ash on the growth of nursery seedlings of *Populus*

Unweathered electrostatic precipitator (ESP) fly ash from GGSTP (Guru Gobind Singh Super Thermal Power Plant) Ropar was mixed with alkaline soil of Patiala on v/v basis at 0, 5, 10, 20 and 30% and filled in black half-kg polythene bags. 15 cm long and 0.8 cm thick shoot cuttings of *Populus deltoides* were planted in the bags. The soil without fly ash and soil plus fly ash mixtures before the start of the nursery trial and after the trial were analyzed for physical, chemical and microbiological properties. After the nursery

trial growth and biomass production was studied and the stem and leaves were analysed for metal content. Fly ash used in nursery trials was observed to show a positive effect on soil dehydrogenase activity, bacterial enumeration and microbial biomass at a concentration of 10%. Fly ash had a positive role in plant growth at an optimum concentration of 10-20% and can be used as a secondary source of nutrients for biomass production and for maintaining the fertility of soil. Among the secondary elements the order of preferential uptake of elements by the plants was $\text{Ca} > \text{Mg} > \text{Na}$ and magnesium concentration was higher than calcium concentration in soil. A distinct increase in the concentration of N, P, K, S in soil plus fly ash mixtures was observed after the trial with concomitant increase in fly ash percentage. Micronutrients (Fe, Cu, Zn, Mn and Mo) and heavy metals (Cr, Co) were observed to occur within permissible limits in soil as a result of fly ash addition. No distinct variation in the bulk density and hydraulic conductivity of soil with and without fly ash was observed except the water holding capacity, which increased due to addition of fly ash. The texture of the soil was loamy sand, which remained unchanged with increased fly ash addition before the start of the trial and after the trial.

6. To study the effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in established field plantation

Effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* was monitored on established field plantations on a twenty-acre area in village Durgaprasad, Distt. Dhenkanal (Orissa) in July, 1998 on partially degraded soil belonging to order Ultisols having reddish brown lateritic characteristics with a clay texture. Fly ash having bulk density 0.9 g cm^{-3} and water holding capacity 67% procured from electrostatic precipitator of FBC boiler (source of coal: Talcher coal mines; F grade: 40% coal ash) was mixed with soil and fertilizers in 30 x 45 x 45 cm pits. Five different concentrations of fly ash on v/v basis [F0: 0%, F1: 6%, F2: 12%, F3: 18% and F4: 24%; (v/v)] were used and in each two treatments were designed [control (T1) and chemical fertilizers (T2)] thus creating overall ten treatments including fly ash control (F0T1) and fertilized control (F0T2). Diammonium phosphate (DAP) at 17.5 g/plant and muriate of potash (MOP) at 5g/plant and neem cake at 20 g/plant was added at the time of plantation. Urea

application at 50g/plant was given after three months. The plantations were set up in a block of 7x7 plants with a plant-to-plant and row-to-row distance of 2m using a split block design. Plant growth (biometric) parameters collar diameter, girth at breast height (GBH) and height of the inner block of nine plants in three replications were recorded at yearly intervals and analysed statistically using Graph pad software and soil samples from the rhizospheric zone were collected for physicochemical and microbiological analysis.

Fly ash at 18% (F3T1) resulted in a 15% increase in the growth of *Acacia auriculiformis* and with the co-addition of chemical fertilizers (F3T2) there was significant rise in the collar diameter up to 2.6 cm corresponding to almost a 19% increase. After one year of plantation treatment the effect on plant growth of *Acacia auriculiformis* became statistically insignificant. Similarly, in *Eucalyptus tereticornis* at 12, 18 and 24% fly ash with the co-addition of chemical fertilizers there was increase up to 26% in the collar diameter and the plant growth was significantly increased with increasing fly ash percentage and the difference was maintained over the years. Fly ash had no negative effect on the mean annual increment (MAI) of both the tree species and it was rather higher in the presence of fly ash.

In the rhizosphere soil of *Acacia auriculiformis* and *Eucalyptus tereticornis* the total and available nitrogen, phosphorous and sulphur was higher in soil with fertilizers and phosphorous, potassium and sulphur increased over the years but the effect was non-significant with increasing fly ash percentage. No significant variation was observed in nitrogen content of the soil over the years. Heavy metals lead, nickel and chromium remained within permissible limits over the years with increasing fly ash percentage.

No drastic change in the particle size distribution, bulk density and water holding capacity in rhizosphere soil was observed with increase in fly ash percentage up to 24%. The hydraulic conductivity in the soil increased by 45% in *Eucalyptus* compared to 21% increase in *Acacia* with increasing fly ash percentage. The bacterial count in soil of *Acacia* showed some variation with increasing fly ash percentage and over the years compared to *Eucalyptus*. The population of nitrogen-fixing bacteria was almost negligible over the years. No significant variation was observed in organic carbon content and soil dehydrogenase activity of *Acacia* and *Eucalyptus* with increasing fly ash percentage and

over the years and these were higher in rhizospheric soil of *Acacia* compared to *Eucalyptus*. Fly ash amendment in soil at 12 and 18% was observed to be optimum, which can act in a synergistic manner with fertilizers for maintaining the nutrient balance and soil fertility without any drastic increase in metal content and the nutrient profile remained well within range as reported in the literature for common cropland soil.

Salient findings

1. Fly ash obtained from different sources showed marked heterogeneity and all the elements present in fly ash were within range as reported in the literature. Most of the secondary elements (Ca, Mg, Na) and micronutrients (Fe, Zn, Mn) were greater in the acidic fly ash. Overall the order of all nutrients was Fe>Mn>Zn>Cr>Pb>Co>Ni which implies that fly ash is rich in Fe and Mn.
2. A positive correlation between soil microbial activity in terms of rate of carbon dioxide evolution, soil microbial biomass and soil dehydrogenase activity was observed with 10 to 12% fly ash amendment. Fly ash can promote soil microbial activity and mixing with an organic substrate enhances its benefits, which assumes importance owing to eco-friendly disposal of fly ash.
3. Phosphate-solubilizing bacteria showed good adaptability in fly ash amended soils and better survival compared to free-living nitrogen fixing bacteria exhibiting 36.5 to 86.1% phosphate solubilization. The population of phosphate solubilizing bacteria in fly ash amended soil mixed with chemical fertilizer was higher in the presence of fly ash at levels as high as 12%, attributed mainly to the synergistic effect of fly ash and fertilizers on the soil microenvironment.
4. Phosphate-solubilizing bacterial isolate S2 was tagged with lac Z⁺ *E.coli* strain and its ecological monitoring on selective media indicated that 10 % fly ash is good for the proliferation of microbes since its amendment provides micronutrients for growth. A similar positive correlation was observed for microbial biomass.
5. Unweathered electrostatic precipitator (ESP) fly ash from GGSTP (Guru Gobind Singh Super Thermal Power Plant), Ropar used for establishing nursery trial of *Populus deltoides* was observed to show a positive effect on soil dehydrogenase activity, bacterial enumeration and microbial biomass at a concentration of 10% and on plant growth at an optimum concentration of 10 to 20% acting as a secondary source of nutrients for biomass production and for maintaining the fertility of soil.
6. The overall growth in *Acacia auriculiformis* was enhanced by as much as 6.8 % while it increased significantly to 14.3% in *Eucalyptus tereticornis* and the

increase in GBH ranged from 6.4 to 15.1 % in both the tree species in soil without chemical fertilizers and in soil admixed with fly ash and chemical fertilizers. Fly ash did not have any negative impact on the Mean Annual Increment of the tree species and was higher in the presence of fly ash. The physical and microbial parameters did not show a distinct variation in the rhizosphere soil of both the tree species. Fly ash amendment in soil at 12 and 18% was observed to be optimum, which can act in a synergistic manner with fertilizers for maintaining the nutrient balance and soil fertility without any drastic increase in metal content and the nutrient profile remained well within range as reported in the literature for common cropland soil.

List of Abbreviations

Hr	Hours
Rpm	Revolution per minute
G	Gram
Mg	Milligram
μg	Microgram
L	Litre
$\mu\text{S cm}^{-1}$	Microsiemens per cm
ml	Millilitre
μl	Microlitre
mg ml^{-1}	Milligrams per millilitre
$\mu\text{g ml}^{-1}$	Micrograms per millilitre
%	Percentage
mg/kg (ppm)	Milligrams per kilogram (parts per million)
$\mu\text{g/kg (ppb)}$	Micrograms per kilogram (parts per billion)
cm min^{-1}	Centimeter per minute
$\mu\text{g g}^{-1}$	Microgram per gram
g l^{-1}	Grams per litre
Bq kg^{-1}	Bequeral per kilogram
$(\times 10^6 \text{ cfu g}^{-1} \text{ soil})$	$\times 10^6$ colony forming unit per gram soil
$\mu\text{gTPFg}^{-1} \text{ soil day}^{-1}$	Microgram triphenyl formazan per gram soil per day.
$\text{mg CO}_2 \text{ 100 g}^{-1} \text{ soil day}^{-1}$	Milligram carbon dioxide per hundred gram soil per day
V	Volume of the solution
Mm	Millimeter
Cm	Centimeter
N	Nitrogen
P	Phosphorous
K	Potassium
S	Sulphur
Pb	Lead
Cr	Chromium
Zn	Zinc
Fe	Iron
Ni	Nickel
Mn	Manganese
As	Arsenic
Se	Selenium
Mo	Molybdenum
Ra^{226}	Radium-226
Ac^{228}	Actinium-228
K^{40}	Potassium-40
W	weight in grams
G	glucose
fa	fly ash

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Introduction

Technological progress has put enormous pressure on our valuable natural resources. The global energy need has increased with rapid industrialization, which to a large extent has been met by fossil fuels. Coal is an exhaustible energy source, which plays a crucial role in meeting the ever-increasing energy demands of countries around the world. Combustion of coal in thermal power stations produces a variety of residues – fly ash, bottom ash, flue gas desulfurization waste (scrubber sludge) and fluidized bed boiler waste and coal gasification ash. Over 225 million tonnes of coal is produced annually in India and over 100 thermal power stations generate more than 108 million tonnes of fly ash every year (Jamwal, 2003), which is expected to double within the next 5-6 years.

The physical, chemical and mineralogical characteristics of fly ash depend on a variety of factors such as composition of parent coal, combustion conditions, the efficiency and type of emission control devices and the disposal methods used (Adriano et al., 1980). Generally, two methods for disposal of fly ash are used-the dry or wet method. In the former, fly ash is dumped into dedicated basins or landfills in the vicinity of the plant and in the wet method fly ash mixed with water is pumped in slurry form into lagoons or ponds and is termed pond ash.

Fly ash is an amorphous mixture of ferroaluminosilicate minerals generated from combustion of ground or powdered coal at temperatures ranging from 400-1500°C with 2% excess air (Mattigod et al., 1990). Fly ash is composed predominantly of small, glassy, hollow particles with low to medium bulk density ranging from 2.1 to 2.6 g cm⁻³ (Adriano et al., 1980) with an average diameter of <10 µm, high surface area and light texture which are aggregated into micron and sub-micron spherical particles of sizes ranging from 0.01 to 100 µm (Davison et al., 1974), with smaller particles entrapped within large spheres (Fischer et al., 1976). Approximately 90-99% of fly ash consists of Si, Al, Fe, Ca, Mg, Na and K. Major matrix elements in fly ash are Si and Al together with significant percentage of K, Fe, Ca and Mg. Fly ash contains all naturally- occurring elements and is substantially rich in trace elements like lanthanum, terbium, mercury, cobalt, chromium (Van Hook, 1979; Adriano et al., 1980).

Field and greenhouse studies both indicate that on account of its heterogenous nature fly ash can benefit plant growth and can improve agronomic properties of soil (Aitken and Bell., 1985; Sharma et al., 1990). Fly ash has been found to increase yield of alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), bermudagrass (*Cynodon dactylon*) and white clover (*Trifolium repens*) and improve physical and chemical characteristics of the soil (Martens, 1971; Page et al., 1979; Hill and Lamp, 1980; Elsewi et al., 1980 a, b; Weinstein et al., 1989). A large number of forest species such as *Acacia*, *Eucalyptus*, *Populus*, *Dalbergia*, *Casuarina*, *Sycamore sp.* have been found to show improved growth and biomass production in fly ash amended soils (Riekerk, 1984; Adholeya, et al. 1998; Goyal et al., 2002).

Fly ash has been advocated as a promising material (or amendment) for reclaiming wastelands or mine spoils (Adriano and Weber, 2001). Millions of hectares of land rendered wasteland due to strip mining of coal have been effectively reclaimed and stabilized using fly ash. Fly ash has also been used for the neutralization of acidic mine spoils and restoration of nutrient balance in alkali wastelands. Fly ash positively influences the micro ecology and chemistry of soil in addition to physical properties such as water holding capacity, bulk density and soil structure. Other miscellaneous advantages of fly ash include its use as a safe barnyard paving resource and as an adsorbent for various metals (Singh and Rawat, 1993; Stout et al., 1999; Gupta and Sharma, 2003).

The disposal of fly ash by conventional methods leads to degradation of arable land and contamination of groundwater. Therefore, development of proper technologies for disposal of this solid waste in an eco-friendly manner becomes essential to derive maximum benefit from its heterogenous nature, since it is a storehouse of readily available plant macro and micronutrients. In conjunction with organic manure, microbial inoculants or fertilizers, fly ash can be used to design a soil benefaction strategy, which can help in improving soil properties and enriching its nutrient status. The presence of almost all essential plant nutrients in ionic form and the ameliorating effect on the physical, chemical and microbial nature of soil makes fly ash an important input for biomass production, especially on variously degraded soils and wastelands.

Keeping the above factors in mind a comprehensive study was undertaken with following objectives:

Objectives

1. Characterization of fly ash for its physicochemical properties.
2. To study the effect of fly ash amendment in soil on soil microbial activity.
3. To screen fly ash-resistant soil microorganisms such as free-living N₂-fixers and phosphate solubilizers.
4. Molecular tagging of beneficial soil bacteria for ecological monitoring in soil / fly ash-amended soils.
5. To study the effect of fly ash on the growth of nursery seedlings of *Populus*.
6. To study the effect of fly ash on growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in established field plantation.

In the present study the effect of fly ash addition at different concentrations in soil was investigated in terms of biomass production, microbial activity and nutrient status of soil. Unweathered electrostatic precipitator fly ash (ESP) from three different sources was used as a soil amendment in acidic and alkaline soil of two regions, village Durgaprasad (Dist. Dhenkanal, Orissa) and Patiala (Punjab). Both fly ash and soil were characterized for chemical, physical and microbiological properties. The studies related to effect of fly ash on microbial activity were conducted under laboratory conditions by mixing fly ash with soil at different concentrations and studied for bacterial enumeration, soil dehydrogenase activity, rate of carbon-dioxide evolution and soil microbial biomass at regular intervals. A number of phosphate-solubilizing bacteria were isolated from the fly ash-amended soil from established field plantations and checked for their efficiency in solubilizing tricalcium phosphate. The phosphate solubilizing bacterial isolate was transformed with *lacZ* marker and inoculated in soil with and without fly ash to study its proliferation. The effect of fly ash on growth and biomass production was studied in a nursery trial of *Populus deltoides* and established field plantations of *Acacia auriculiformis* and *Eucalyptus tereticornis* along with nutrient status of soil with the objective of determining the optimum dose of ESP fly ash as an agent for improving soil fertility and crop production.

Review of Literature

1. Background

Environmental pollution due to release of smoke, gases, effluents and solid wastes is one of the major issues of global concern. The power sector is largely based on the use of coal as the source of energy. Combustion of bituminous and sub-bituminous coal as well as lignite generates fly ash, bottom ash, boiler slag and flue gas desulfurization (FGD) materials, which are commonly known as coal combustion by-products (CCPs) (Mattigod, 1990; Vom Berg, 1998). Fly ash is the mineral residue consisting of small particles that are carried up and out of the boiler in the flow of exhaust gases and are collected from the stack gases using electrostatic precipitators (ESP), flue gas desulfurization (FGD) systems and baghouses. About 70 % of the by-product is ESP fly ash which is the most difficult to handle. Nearly 10-12 % of the by-product is bottom ash, which is coarse and solid mineral residue. The individual particles are much larger than fly ash particles and fall down through the airflow to the bottom of the boiler. The material is mechanically removed from the bottom of the boilers. Boiler slag constitutes 4-6 percent of bottom ash produced by older boilers, where the material is actually melted in the boiler, quenched in the bottom hoppers and becomes hard and glassy. FGD material (10-12%) is the solid material resulting from the removal of sulfur dioxide gas from the utility boiler stack gases in the flue gas desulfurization process. The material is produced in the flue gas scrubbers by reacting slurried limestone or lime with the gaseous sulfur dioxide to produce calcium sulfite.

In United States alone over 118 million tons (mt) of coal combustion products are generated each year and in India the figure is more than 108 mt per annum (Kumar and Sharma, 1998; ACAA, 1997; Jamwal, 2003) of which nearly 4 mt is released into the atmosphere. Coal combustion by-products were largely treated as waste materials; however, in the recent past many applications have been identified due to the presence of basic mineral elements resembling earth's crust, which makes them an excellent replacement for natural materials. They can be used as a substitute for Portland cement in manufacturing roofing tiles and as structural fills, sheetrock, agricultural fertilizer and

soil amendments. Per cent ash utilization of the total ash generated in different countries amounts to more than 85 % in West Germany, 73 % in Denmark, 60 % in France and UK, 50 % in Poland, 32 % in United States, 25 % in China and 15% in India (Sinha and Basu, 1998). According to the report published by American Coal Ash Association (1997), 32% of the fly ash, 30% of the bottom ash, 94% of the boiler slags and 9% of flue gas desulfurization sludge were used for agriculture, wasteland reclamation and civil engineering purposes.

Fly ash is disposed off either by a dry or wet method. In dry disposal, the fly ash is dumped in landfills and fly ash basins. In wet method, the fly ash is washed out with water into artificial lagoons and is called pond ash. Both methods ultimately lead to dumping of fly ash on open land, which degrades the soil and endangers human health and the environment. Establishment of vegetation and raising of forests on fly ash basins and landfills serves a variety of functions like stabilizing the ash against wind and water erosion, providing shelter and habitat for wildlife and transforming the area into an aesthetically pleasing landscape. The coal industry in the United States spends millions of dollars on lining fly ash dumping grounds. Fly ash particles are small enough to escape emission control devices and easily get suspended in the air. Repeated exposure to fly ash can cause irritation in eyes, skin, nose, throat and respiratory tract and result in arsenic poisoning (Davison et al., 1974; Natusch and Wallace, 1974; Carlson and Adriano, 1993; Belkin et al., 1999; Finkelman et al., 2000).

Fly ash is chemically heterogenous in nature on account of being composed of large number of trace and heavy metals in variable proportions. Field and greenhouse studies have shown that fly ash can help in growing agricultural crops and forestry species. The presence of relatively large concentrations of elements like K, Mg, Fe, Zn and Ca in available form can alleviate deficiencies of these elements (Ciravolo and Adriano, 1979). Fly ash addition generally has shown positive impact on plant biomass production and nutrient uptake (Ciravolo and Adriano, 1979; Elfving et al., 1981). It can be used for wasteland reclamation, agriculture and forestry as it plays a vital role in altering physical, chemical and microbiological status of soil. Judicious application of fly ash can bring about favorable alteration in the nutrient status of soil, provided all aspects are constantly monitored for overall benefit. Fly ash helps in restoring the ion balance in wastelands

thereby rendering them suitable for raising plants and an absorption medium for industrial effluents. The use of fly ash in agriculture and wasteland reclamation can be important from the point of management of this solid waste. It gains additional importance since soils have a tremendous buffering and diluting effect on these materials. Therefore, disposal and utilization of fly ash needs careful assessment in order to prevent conversion of arable land into landfills and use it as an ameliorant for problem soils (Jala and Goyal, 2004). Restoration and utilization of fly ash dumps for biomass production will be an adjunct to these efforts. The following review highlights the various attributes of fly ash for its application in agriculture and for deriving agronomic benefits.

2. Physico-chemical properties of fly ash

The mineralogical, physical and chemical properties of fly ash (Fisher et al., 1978; Page et al., 1979; Adriano et al., 1980; Carlson and Adriano, 1993) depends on the nature of parent coal, conditions of combustion, type of emission control devices and storage and handling methods. Therefore, ash produced by burning of anthracite, bituminous and lignite coal has different compositions. Fly ash is an amorphous mixture of ferroaluminosilicate minerals generated from the combustion of ground or powdered coal at 400-1500°C (Mattigod et al., 1990). Physically, fly ash occurs as very fine particles having an average diameter of <10 µm and has low to medium bulk density, high surface area and light texture. The fine particles are aggregated into micron and sub-micron spherical particles of 0.01 to 100 µm size (Davison et al., 1974). The sub-micron particles are entrapped into large spheres (Paulson and Ramsdon, 1970; Fischer et al., 1976). Fly ash particles are hollow, empty spheres (cenospheres) filled with smaller amorphous particles and crystals (plerospheres). The cenosphere fraction constitutes as much as 1% of the total mass and becomes airborne easily (Hodgson and Holliday, 1966).

The specific gravity of fly ash ranges from 2.1 to 2.6 g cm⁻³. Mean particle density for non-magnetic and magnetic particles is 2.7 and 3.4 g cm⁻³ respectively (Natusch and Wallace, 1974). Bulk density of fly ash varies from 1 to 1.8 g cm⁻³ while moisture retention ranges from 6.1% at 15 bar to 13.4% at 1/3 bar. Fly ash addition alters physical properties of soil such as texture, bulk density, water holding capacity and particle size distribution (Chang et al., 1977; Fail and Wochok, 1977; Capp, 1978; Page et al., 1979; Sharma, 1989). Addition

of 10 % ash increased the water holding capacity (WHC) by factors of 7.2 and 13.5 for fine and coarse sands, respectively (Campbell et al., 1983). Certain other physical properties of fly ash like shear strength, densification and permeability are also improved which hold importance in civil engineering. Because of the dominance of silt-sized particles in fly ash, this material is often substituted for topsoil in surface mined lands, thereby improving the physical condition of soil, especially water holding capacity.

Chemically, 90-99% of fly ash is composed of Si, Al, Fe, Ca, Mg, Na and K with Si and Al forming the major matrix (Rees and Sidrak, 1956; Adriano et al., 1980). There are mainly two types of ash, i.e: Class F (low lime) and Class C (high lime) based on total amounts of silica, alumina and iron oxide. Al in fly ash is mostly bound in insoluble aluminosilicate structures, which considerably limits its biological toxicity (Page et al., 1979). It is substantially rich in trace elements like lanthanum, terbium, mercury, cobalt and chromium (Van Hook, 1979; Adriano et al., 1980). Many trace elements in fly ash like As, B, Ca, Mo, S, Se and Sr are concentrated in the smaller ash particles (Page et al., 1979; Adriano et al., 1980). The Fe-oxide contents of spheres influences their color which ranges from water-white to yellow orange to deep red or brown to opaque.

Ca was found to be the dominant cation in ESP ash and fly ash collected from dump sites, followed by Mg, Na and K (Maiti et al., 1990). Minerals like quartz, mullite, haematite, magnetite, calcite and borax were also identified in fly ash (Hogdson and Holliday, 1966). The authors opined that oxidation of C and N during combustion drastically reduces their quantities in ash. The pH of fly ash varies from 4.5-12.0 depending largely on the sulphur content of the parent coal (Plank and Martens, 1974) and the type of coal used for combustion affects the sulphur content of fly ash (Page et al., 1979). Eastern U.S. coals that include anthracite are generally high in S and produce acidic ash, while western U.S. coals, which includes lignites, tends to be lower in S and higher in Ca and produce alkaline ash (Natusch et al., 1975; Bern, 1976; Furr et al., 1977; Page et al., 1979). The coal produced in India is poor in S but high in ash content (40%) whereas the coal produced in U.S. is rich in S (2%) and contains only 5-10% ash. Studies on chemical constituents of fly ash and soil showed that both of them had comparatively much lower content of major and trace elements (Table 1a).

The solubility of fly ash depends directly on the physicochemical disintegration of the particles, for example indicating that a major portion of total K is localized in the interior glassy matrix while the external glass is enriched with Mg. When the solubility of alkaline fly ash was studied by selective dissolution in mineral acids, it was found that significant quantity of K occurred in the highly refractory magnetic Fe fraction and that the solubility of Mg in acids was much higher (Green and Manahan, 1978). The relative leachability of elements from fly ash was related to the physical location of the element in the fly ash matrix (Natusch et al., 1975).

The melting and agglomeration of the mineral inclusions during the combustion of coal particles results in breakup and formation of a number of usually spherical fly ash particles. During the combustion of pulverised coal at high temperature, a considerable portion of the inorganic compounds vaporizes in the cooler parts of the installation and condenses on fly ash particles. This volatilization-condensation theory which correlated mineral concentration with particle size (Davison et al., 1974), established three groups of elements; Group I with pronounced concentration (As, Cd, Ni, Pb, S, Sb, Se, Ti, Zn), Group II with limited concentration (Be, C, Fe, Mg, Mn, Si, V) and Group III with no concentration (Ca, Co, Bi, Cu, Sn, Ti).

Group I elements are classified as 'lithophiles' (Al, Ca, Fe, K, Mg, Na, Ti) showing little or no enrichment in smaller fly ash particles, Group II elements as 'chalcophiles' (As, Cd, Cu, Pb, Sb, Se) show increased concentration with decreasing particle size and Group III elements (Be, Cu, Ni, V, Co) have intermediate behaviour in being enriched in smaller particles but to a lesser extent than those of Group II. The variation in the concentration of elements, depending on particle size, is related to combustion conditions and physical and chemical properties of the coal.

Table 1a. Chemical composition of ESP fly ash and common cropland soil.

Element	Soil	Soil ¹	Fly ash (ESP)	Fly ash ¹
	Lit. values*		Lit. values*	
Major Elements in %				
Al	4-30	4.4-10.89	0.1-17.3	31.2
Ca	0.7-50	0.2-0.97	0.11-22.2	3.4
Fe	0.7-55	21-38.9	1-29	6.8
Mg	0.06-0.6	0.34-0.53	0.04-7.6	0.14
Na	0.04-3.0	0.1-0.13	0.01-2.03	0.42
K	0.04-3.0	0.31-0.39	0.15-3.5	1.08
S	0.01-2.0	0.002-0.004	0.1-1.5	0.002
P	0.005-0.2	0.07-0.18	0.04-0.8	1.08
N	0.01-1.0	0.11-0.14	-	0.2
Trace elements in mg/kg				
As (20-50)	0.1-40	3.5	2.3-6300	6.2
B	2-100	-	10-618	-
Cd	0.01-7.0	0.4-2.4	0.7-130	1.9
Co	1-40	40-178	7-520	58
Cr (10)	5-3000	21-54	10-1000	330
Cu	2-100	4.5-10	14-2800	20
Hg	-	-	0.02-1.0	-
Mn	100-4000	119-1090	58-3000	739
Mo (2-40)	0.2-5.0	2.6-3.0	7-160	4.0
Ni (50)	10-1000	6.5-94	6.3-4300	13
Pb (100)	2-100	153-163	3.1-5000	35
Se (5-10)	0.1-2.0	2.2-2.5	0.2-134	3.6
Zn (300)	10-300	59-96	10-3500	79
Radioactivity in Bq kg ⁻¹				
Ra ²²⁶ (370)	-	31	-	100
Ac ²²⁸ (2-40)		75		141
K ⁴⁰ (810-925)	-	260	-	376

(*Page et al. 1979; ¹Goyal et al., 2002. Values in parentheses indicate critical levels in soil; Pendas and Pendas, 1984)

3. Fly ash as a soil-ameliorating agent

3.1 Fly ash for improving soil fertility

Fly ash has immense potential as a soil-ameliorating agent in agriculture, forestry and wasteland reclamation because of its heterogenous nature. Previous work (Reynolds et al., 1999) to determine the feasibility of converting waste disposal problem into a soil benefaction strategy has proven true. Fly ash has been studied as a useful soil-amending agent with agronomic and environmental benefits (Zhang et al., 2004). Studies have been carried out to report the efficacy of fluidized bed combustion (FBC) and flue gas desulfurization (FGD) byproducts, when amended with dairy, swine and broiler litter manures, in reducing phosphorus (P) solubility and potential impact on water quality (Zhang et al., 2004). Pilot scale studies have been conducted on use of fly ash at rates of 100 to 650 tonnes per hectare of land as soil modifier and microfertilizer under field conditions for vegetable crops in Orissa, Madhya Pradesh and Uttar Pradesh, with a positive influence reported on the soil nutrient status as well as on plant growth (Saxena et al., 2005). Fly ash upon amelioration at 10, 20, 30 and 40% (w/w) in clay, sandy-clay loam, sandy and sandy-loam soil has been reported to increase its pH, electrical conductivity and modify water retention capacity (Kalra et al., 2000).

Influence of fly ash on soil properties has been studied by several workers (Aitken et al., 1984; Sikka and Kansal, 1994; Desmukh et al., 2000; Grewal et al., 2001). Fly ash, which can be acidic or alkaline depending on the source, can be used to buffer the soil pH (Elsewi et al., 1978 a, b; Phung et al., 1978). The electrical conductivity of soil increases with fly ash application and so does the metal content. Lime in fly ash readily reacts with acidic components in soil and releases nutrients such as S, B and Mo in forms and amounts beneficial to crop plants. Application of fly ash for increasing the pH of acidic soils (Phung et al., 1979) and improving soil texture (Chang et al., 1977) was investigated for agronomic benefits (Plank et al., 1975; Adriano et al., 1980; Elsewi et al., 1981 and El-Mogazi et al., 1988) and improving the nutrient status of soil (Doran and Martens, 1972; Schnappinger et al., 1975; Hill and Lamp, 1980; Wallace et al., 1980; Elsewi and Page, 1984). ESP ash collected directly from a thermal power station in Bathinda, India, was found to be finer in texture, lower in pH and generally richer in nutrients than the ash collected from the

dumping sites (Sikka and Kansal, 1994). Major matrix elements were found to be Al and Si, together with significant percentages of K, Fe, Ca and Mg. The saturation moisture percentage of both the fly ash samples was higher, but the bulk density was lower than that for normal cultivated soils. Calcium was present as the dominant cation of the exchange complex followed by Mg^{2+} , Na^+ and K^+ in addition to high sulphur content. A number of studies have shown that addition of alkaline ash can increase the pH of acidic soils (Plank et al., 1975; Martens and Beahm, 1976; Phung et al., 1978; Elseewi et al., 1980a; Molliner and Street, 1982; Elseewi and Page, 1984; Riekerk, 1984; Petruzzelli et al., 1987).

Phung et al. (1978) compared the neutralizing ability of fresh fly ash (pH 12.5) from the Mojave generating station in Nevada to that of reagent grade $CaCO_3$, the main constituent of agricultural limestone. Plank et al. (1975) noted that two years after addition of weathered fly ash with a pH of 7.8 at a rate of 144 mt/ha, the pH of Groseclose soil increased from 5.8 to 6.8. In soil solutions the average Ca and Mg concentrations increased from 3.2 to 5.0 meq/L and B increased from 0.3 to 0.5 $\mu g/ml$. Soil salinity increased five-to six-fold by application of 8% fly ash to soil. The increase was accompanied by substantial gains in water-soluble Ca and Mg. Na increased slightly and K was not significantly affected (Plank et al., 1975). Mulford and Martens (1971) indicated that applications of about 5% fly ash to Tatum silt loam soil increased the EC of the soil saturation extract from about one to about four $mmhos\ cm^{-1}$. Townsend and Hodgson (1973) indicated that the soluble salt content of fresh ash is considerably reduced during the ash lagooning process. They also indicated that 2-3 year weathering in the field usually reduces the soluble salt content of the ash to harmless levels, i.e., to conductivity values less than four $mmho\ cm^{-1}$. Page et al. (1977) have found that amount of water required to reduce the salinity of leachates from two soils amended with 5% fly ash in the top 3 cm layer to background levels varied from 60 cm for an acid soil to 116 cm for a desert soil.

Studies conducted by Martens and Beahm (1976), Elseewi et al (1980a), Molliner and Street (1982), Elseewi and Page (1984) demonstrated that addition of unweathered fly ash can substantially increase soil salinity, alkalinity, concentration of macronutrients and trace elements. The amount of ash needed to raise soil pH to levels conducive to maximum plant growth varies with the composition of ash and the properties of soil. Generally lower application rates are required for fresh ash than for weathered ash to reach the same pH in a

given soil (Martens and Beahm, 1976). In addition pH effects are more pronounced in poorly buffered, acidic soils (Elsewi et al., 1980a; Elsewi and Page, 1984; Petruzzelli et al., 1987). Approximately 13 million tonnes of fly ash produced by power stations in Australia is currently being explored for its ameliorating properties in soils with inherent structural and nutritional limitations and for biomass production of plants such as clover (*Trifolium subterraneum*) and turfgrass (*Cynodon dactylon*). Fly ash has been observed to have a positive effect on water holding capacity, hydraulic conductivity and pH apart from acting as source of nutrients (Yunusa et al., 2005).

Addition of unweathered fly ash up to 8% to calcareous or acidic soils resulted in higher crop yield due to increased availability of S from fly ash (Page et al., 1979). Fly ash applied on acidic strip mine spoils at different places increased the yield of many crops which was attributed to increased availability of Al^{3+} and Mn^{2+} and other metallic ions at the resultant higher pH (Fail and Wochok, 1977). Fly ash does not seem to be a good source of phosphorus as it was found inferior to monocalcium phosphate (Martens, 1971). It, however, accelerated Ca^{2+} and Mg^{2+} uptake by legumes (Adriano et al., 1978; Page et al., 1979). Although, higher B availability limits the use of fly ash in crop production (Page et al., 1979; Aitken and Bell, 1985), the problem can be overcome by proper weathering of the fly ash, which reduces the availability to below toxic level (Cope, 1962; Hodgson and Townsend, 1973; Townsend and Gilham, 1975). Increased selenium accumulation in plant tissues with increased fly ash dose warrants close monitoring and use of appropriate quantity of weathered fly ash depending upon the end use of the produced biomass (Furr et al., 1977; Straughan et al., 1978).

Fly ash is predominantly composed of silt-size particles so its addition in soils very high in either sand or clay can improve soil texture (Chang et al., 1977; 1978; 1989) by reducing soil bulk density and increasing aeration. Fly ash addition at 70 t/ha was reported to alter the texture of sandy and clay soils to loamy soils (Fail and Wochok, 1977 and Capp, 1978) and improve the water-holding capacity of sandy soils (Salter and Williams, 1967; Salter et al., 1971; Chang et al., 1977; Aitken et al., 1984; Aitken and Bell, 1985; Sharma et al., 1990; Chang et al., 1989). It was generally observed that both sandy and clayey soils tend to become loamy in texture (Capp, 1978) with fly ash amendment. The water holding capacity of sandy/loamy soils increased by 8% due to fly ash amendment (Chang et al.,

1977) and the accompanied increase in hydraulic conductivity helped in reducing surface encrustation. Fly ash has been found to improve soil porosity and workability and enhanced water-retention capacity (Page et al., 1979). Fly ash mixed with municipal sewage sludge and water hyacinth was used for restoration of degraded soils under the Land Management project with Indo-Canadian collaboration (Powell and Tripathi, www.irdc.ca/en/ev-5358-201-1-1-DO-TOPIC.html).

3.2 Use of fly ash with organic manure and its effect on soil microbes

The strong correlation between pH correction and nutrient availability in the soil suggests that in fly ash, most elements are associated with the mineral phase. One can therefore expect that interaction between the predominantly inorganic fly ash and organic matter may further enhance its beneficial effect on plant growth in problem soils (Page et al., 1979; Molliner and Street, 1982). Boron toxicity is the major limiting factor in agricultural use of fly ash. Boron induced inhibition of microbial respiration can be prevented by the co-application of a readily oxidizable organic substrate (Page et al., 1979). Adriano et al., (1980) recommended mixing alkaline fly ash with highly carbonaceous acidic material to make compost for soil treatment. Use of swine manure with fly ash increased the availability of Ca and Mg balancing the ratio between monovalent and bivalent cations ($\text{Na}^+ + \text{K}^+ / \text{Ca}^{2+} + \text{Mg}^{2+}$), which otherwise proves detrimental to the soil (Giardini, 1991). The ability of calcium to enhance flocculation/aggregation of soil particles, particularly clay, keeps the soils friable, enhances water penetration and allows roots to penetrate hard/compact soil layers. Calcium readily replaces Na at clay exchange sites to enhance soil flocculation and stability.

An appreciable change in the soil physicochemical properties, rising of pH and increased rice crop yield was obtained by mixed application of fly ash and paper factory sludge and farmyard manure (Hill and Lamp, 1980; Molliner and Street, 1982). Menon (1993) studied the effect of mixed application of fly ash and organic compost on soil and availability and uptake of elements by various plant species. Co-utilization of 'slash,' a mixture of fly ash, sewage sludge and lime in the ratio of 60:30:10 had beneficial soil ameliorating effect (Reynolds et al., 1999). Incorporation of 'slash' in soil had positive effect on soil pH and Ca, Mg and P content and reduction in the translocation of Ni and Cd (Rethman et al.,

2001) and enhanced growth of corn, potatoes and beans in pot trials. The mine spoils could be revegetated by enhancing the activities of various enzymes such as dehydrogenase, phosphatase and nitrogenase upon inoculation with arbuscular mycorrhizal fungi *Glomus mosseae* (Rao and Tak, 2001). Very little is known regarding the effect of fly ash amendment on soil biological properties (Schutter and Fuhrmann, 2001). Several short-term laboratory incubation studies found that addition of unweathered fly ash to sandy soils severely inhibited microbial respiration, enzyme activity and soil nitrogen cycling processes such as nitrification and N mineralization (Cervelli et al., 1986; Wong and Wong, 1986; Pichtel, 1990; Pichtel and Hayes, 1990; Garau et al., 1991). Fly ash was mixed with organic matter in the form of cow dung at 1:3, 1:1 and 3:1 ratio and incubated with and without epigeic earthworm (*Eisenia fetida*) for 50 days which resulted in a significant increase in the population of phosphate-solubilizing bacteria and increased bioavailability of phosphorus and nutrients by vermicomposting (Bhattacharya and Chattopadhyay, 2002). Studies on CO₂ evolution and enzyme activities (dehydrogenase, protease and amylase) of fly ash amended soil in the presence and absence of earthworms (*Drawida willsi* Michaelsen) under laboratory conditions for two months at 50 % water holding capacity, showed stimulation of soil respiration and microbial activities in the presence of fly ash up to 5% (Pati and Sahu, 2003).

Fly ash composted with wheat straw and 2% rock phosphate (w/w) for 90 days had an encouraging effect on chemical and microbiological parameters of the compost and fly ash up to 40-60% did not exert any detrimental effect on either C:N ratio or microbial population (Gaind and Gaur, 2003). Increased microbial activity was reported for ash-amended soils containing sewage sludge (Pichtel, 1990; Pichtel and Hayes, 1990). Moreover, the microorganisms invariably adapt to the stressed conditions and show a gradual increase in respiration after an initial lag. Presence of organic matter had additive effect as it reduces the concentration of toxic metals through sorption, lowers the C/N ratio and provides organic compounds, which promote microbial proliferation and diversity (Wong and Wong, 1986; Pichtel and Hayes, 1990).

Available data indicate that microbial incidence and diversity generally increases as ash weathers and nutrients accumulate (Rippon and Wood, 1975). The supply of simple organic compounds and N are crucial for sustaining rich microbial population at these sites (Klubek

et al., 1992). Fly ash-sludge mixtures containing 10% ash had positive effect on soil microorganisms in terms of enzyme activity for urease, dehydrogenase and phosphatase, N and P cycling and reduction in the availability of heavy metals (Lai et al., 1999). Fatty acid methyl ester (FAME) profiles obtained directly in situ have been used to estimate microbial community structure in wastes like coal fly ash, coal mine spoil and non-metallurgical slag (Kozdroj, 2000) where Cytophaga-Flavobacterium group was found to be dominant in coal fly ash.

Soil biochemical activity using fly ash as soil conditioner and nutrient supplement was studied for *Oryza sativa* alongwith amylase, invertase, dehydrogenase and protease activity and rate of carbon dioxide evolution (Sarangi et al., 2001). The soil conductivity, available P, and organic matter increased significantly by 32, 48 and 29% respectively. (Sarangi et al., 2001). Alkaline coal fly ash and lime were tested for their effectiveness in pathogen removal from biosolids (Wong et al., 2001). They opined that 10 % ash-biosolids mixture should be amended with a minimum of 8.5% lime on dry weight basis for at least two hours to achieve acceptable levels of *Salmonella* and total coliforms. Alkaline fly ash was added to swine manure at 10% and 20% (w/v) and production of CO₂ studied over 12-day period (Vincini et al., 1994). The observed reduction in CO₂ production was attributed to high pH value caused by fly ash addition, rather than inhibition of microbial activity and marked mobilization of inorganic phosphorus occurred in the fly ash amended manure, possibly as a consequence of microbial activity and not acidification.

Application of fly ash at 40 t/ ha in conjunction with phosphate solubilizer, *Pseudomonas striata* improved the bean yield and phosphorus uptake by grain and fly ash did not exert any detrimental effect on the population of *P. striata* in soil (Gand and Gaur, 2002). Amendment of Class F bituminous fly ash to soil at a rate of 505 Mg/ha did not show any detrimental effect on soil microbial communities. Analysis of community fatty acids indicated elevated populations of fungi, including arbuscular mycorrhizal fungi and Gram-negative bacteria (Schutter and Fuhrmann, 2001).

3.3. *Effect of fly ash on soil quality*

Soil quality, which determines the soil health, manifests into soil productivity and has far reaching effect on the soil ecosystem. While the health of soil depends on inorganic and organic matter content, processes like erosion, salinization and chemical contamination has a direct bearing on groundwater contamination, land use and management practices (Acton and Gregorich, 1995). Fly ash contains trace and heavy metals, which readily percolate down from conventionally used earthlined lagoons and pollute groundwater. The solubility of these elements is < 10% (Rohriman, 1971). Natusch and Wallace (1974) observed that 5 to 30% of toxic elements especially Cd, Cu and Pb are leachable. There are several reports on the presence of radionuclides, especially of uranium and thorium series in fly ash, but studies on their impact have been sporadic (Eisenbud and Petrow, 1964). While thorium concentrations may be less than 10 %, the concentration of natural uranium may vary from 14-100 mg/kg and in exceptional cases it may be as high as 1500 mg/kg. Besides gaseous and trace metal oxides, fly ash also contains radioactive elements like Ru^{222} and Ru^{220} (Sharma, 1989). The activity levels of some natural radionuclides and Cs^{137} in the surface soil layer (0-5 cm) within and outside three recultivated coal fly ash disposal sites have been determined by γ -ray spectrometry with an REGe detector. The activity of natural radionuclides from the U^{238} and Th^{232} series in the soil samples did not show significant difference in the concentration within and outside the two disposal sites (Bem et al., 1998).

The radioactivity analyses showed that the activity levels of gamma emitting radionuclides K^{40} , Ra^{226} , Ac^{228} were within the permissible limits when fly ash was mixed at a rate of 24% (v/v) (Goyal et al., 2002). Adriano et al. (1978) reported that at higher levels of fly ash, some heavy metals might become more active and hinder microbial activity. These metals form complexes, which undergo transformation, influenced by various factors like pH, moisture, cation exchange and microbial activity (Milovsky and Kononov, 1992). Ritchey et al. (1996) reported that surface layers or caps of fluidized-bed combustion residue-fly ash mixture/calcium sulfate spread on soil surface were found to help in reduction of evaporation, mitigation of sub-soil acidity and improvement of plant growth of Sudangrass (*Sorghum bicolor* L. Moench).

Effect of fly ash on groundwater is a function of physical and chemical characteristics of ash and hydro-geologic and climatic conditions of the disposal site (Theis et al., 1978, Kopsick and Angino, 1981; Goetz, 1983). Weathered fly ash deposits cause more groundwater contamination because of the presence of higher levels of soluble salts. In unweathered ash, although there is generally a higher release of soluble salts initially, it declines rapidly with time (Jones and Lewis, 1960; Kopsick and Angino, 1981; Theis et al., 1989, Hjelmar, 1990; Mattigod et al., 1990). In contrast, when water-saturated, weathered ash from a settling pond is deposited in a landfill, there is a rapid release of leachate containing much lower concentration of soluble salts. On the other hand, it may take a year or longer for dry unweathered ash to absorb sufficient moisture to release leachates (Theis et al., 1989). Remedial measures for clean up of sites contaminated with heavy metals are often complex. In a study conducted on soils from Italian mine site contaminated severely with heavy metals, mixed with fly ash showed decreased levels of heavy content in percolating water indicating that fly ash in such soils can lead to immobilization of heavy metal ions (Ciccu et al., 2001). Limited measurements of dissolved uranium and radium in water leachates of fly ash and in natural water from some ash disposal sites indicate that dissolved concentrations of these radioactive elements are below levels [(Ra: 5 pCi/l (picocuries per litre); U: 20 µg/kg (parts per billion)] of human health concern (Cothorn and Smith, 1987; Zielinski and Finkelman, 1997).

Chemical analysis of soil and leachates from experimental wetland mesocosms lined with flue gas desulphurization by-products and fly ash in a two-year study revealed no unwanted increase in metal concentration compared to the unlined mesocosms thereby indicating their possible use in agriculture without harming soil quality (Changwoo and Mitsch, 2001). Effect on the soil quality and crop establishment after incorporation of flue gas desulfurization sludge in soils was assessed on mesocosm studies @ 0.2, 5.0, 7.5 and 10% FGD residues (Punshon et al., 2001). The FGD residue elevated surface soil pH from 5.5 (control) to 8.1 (at 10% FGD). Leachate pH was unaffected by FGD, but salinity rose sharply with increasing application rates of FGD. Leachates contained higher concentrations of B, with small increase in As and Se and 2.5% was found to be optimum without producing any detrimental effect on soil leachate (Punshon et al., 2001).

Fly ash added at 0, 5, 10 and 20% (w/w) to field lysimeters containing sandy soils and planted with rhizomes of *Cynodon dactylon* (L.) Pers. Cv or wintergreen was observed to reduce leaching of nitrate, ammonium and phosphorus alongwith an increase in extractable phosphorus by 2.5 to 4.5 fold thereby indicating its potential as a tool for improved management of sandy soils (Pathan et al., 2003). Four cultivars of sunflower (*Helianthus annus*) were grown on sandy loam soil amended with graded fly ash from 0-100% (v/v) and it resulted in an improvement in nutrient status and physical properties of soil. In the seeds except Fe, Pb, Mn, Zn and other heavy metals remain untraced upto 40% fly ash above which their level increased but within permissible limits (Siddiqui et al., 2004).

5. Application of fly ash for biomass production

The presence of almost all essential plant nutrients in ionic form and ameliorating effect on physical, chemical and microbiological nature of the soil makes fly ash an important input for biomass production especially on variously degraded soils and waste lands.

5.1 Agriculture

Agricultural utilization of fly ash has been proposed because of its considerable content of K, Ca, Mg, S and P (Page et al., 1979; Adriano et al., 1980; Cerevelli et al., 1987; Sikka and Kansal, 1995; Kalra et al., 1997; Singh et al., 1997). Fly ash addition generally results in consistently favourable impact on plant growth and nutrient uptake (Furr et al., 1977; Ciravalo and Adriano, 1979; Elfving et al., 1981; Aitken et al., 1984). Research in England dealing with the establishment of successful vegetative covers on fly ash deposits have indicated that plant growth is conditioned by the amount of total soluble salts, pH and available B in the ash and by the physical characteristics of the ash deposits (Hodgson and Townsend, 1973). An extensive list of natural and crop species grown on fly ash has been presented by Hodgson and Holliday (1973). Of the natural species observed, *Funaria hygrometrica* and *Atriplex hastata* were among the first species to grow on fly ash (Hodgson and Holliday, 1973). Crop species were classified into tolerant, semi-tolerant and sensitive species with respect to their ability to withstand fly ash (Hodgson and Holiday, 1966; Hodgson and Townsend, 1973).

Fly ash procured from thermal power plant, Dadri was used in field experiments up to 50t/ha to study its effect on the soil properties and yield of wheat (*Triticum aestivum* L.),

mustard (*Brassica juncea* L.), rice (*Oryza sativa* L.) and maize (*Zea mays*, L.). The yield of wheat increased for 20t/ha fly ash while paddy and mustard were observed to survive well in soil amended with 10t/ha of fly ash, all three crop plants showed improved growth over control (Kalra et al., 1998, 2003).

Bituminous fly ash provided by Central Illinois Public Service power plant, Illinois was mixed with Terra lite Metro-mix growing medium (Hummert Seed Co. St. Louis, MO) at rates of 0 to 500 tonnes per hectare to study its effect on maize seedlings where a significant effect on total nuclear DNA content and cell cycle parameters was observed. These effects were small and did not affect normal development. Coal fly ash exposure at agronomic levels did not result in triploidy in Maize (Taets and Rayburn, 1996). The most tolerant crops belong to the families Leguminosa, Chenopodiaceae and Graminae. Application of 144 metric tons of weathered Glen Lyn fly ash @ 144 mt/ha (6.4%) increased the yield of corn on Woodstown soil by approximately 27% (Plank et al., 1975). The increase in yield was attributed to increased water availability to plants. Application of 2.6-5.2% Muskingham River fly ash to Fredrick silt loam nearly doubled the yield of corn as a result of increased availability of Zn (Schnappinger et al., 1975). Increased rates of application of Kanawha River and Crawford Edison fly ash to Groseclose silt loam increased the availability of Mo to alfalfa and markedly increased its yield (Doran and Martens, 1972). Greenhouse experiments conducted with fly ash from a western United States coal source (Mohave fly ash) added to a desert calcareous and three acid soils at rates ranging up to 80% by weight have been demonstrated to improve yields of dry matter of a number of crop species (Page et al., 1977). The increase in yield was attributed to an increased availability of S from fly ash to plants (Elseewi et al., 1978 b). A typical effect of fly ash incorporation into soils on the mineral composition of plants was studied for alfalfa grown on Arizona calcareous and Redding acid soils amended with up to 8% of fly ash (Page et al., 1977). The P and Zn content of plants were found to decrease while those of Ca, Na and B were generally increased. Reductions in P and Zn concentrations in the plant tissue were not, however, sufficient to induce deficiencies of these elements in the plants.

Reduced availability of Zn with application of alkaline fly ash to soil was also observed by Schnappinger et al. (1975) on corn grown on a slightly acid soil (Frederick silt loam) amended with fly ash at rates ranging from 0.8 to 13% by weight. Addition of acidic fly ash

to the same soil increased Zn uptake and corrected the deficiency in plants. The increase in S availability in plants was associated with significant yield improvement in a number of plant species. Furthermore, the availability of fly ash-S compared well with that of H₂SO₄, gypsum and sewage sludge as revealed by a series of greenhouse studies with alfalfa, bermudagrass, white clover and turnips (Elsewi et al. 1978 b). Application of these materials at the rate of 25, 50 and 100 mg/kg of soil increased the yield and S content of turnips grown on S-deficient Josephine soil. Increased levels of Na and B in plants grown on fly ash amended soils although with no adverse effects on the growth of alfalfa were partially responsible for yield reductions in lettuce (Elsewi et al., 1978 a).

The availability of B in fly ash to alfalfa was shown by Mulford and Martens (1971) and Plank and Martens (1974) to be essentially equal to that of sodium borate-B. Doran and Martens (1972) observed that the availability of fly ash Mo to alfalfa was approximately equal to that of sodium-molybdate. Mesocosm units were designed using FGD residue at different rates of 0, 25, 50, 75 and 100 tons per acre⁻¹ and germination and biomass production of corn (*Zea mays* L. var. Dekalb DK-683), soyabean [*Glycine max* (L.) Merr. Var. Haskell Pupa 94], radish (*Raphanus sativus* L. var. Sparkler), and cotton (*Gossypium hirsutum* L. var. Deltapine 51) was found to show a significant increase (Punshon et al., 2001). Many workers (Martens, 1971; Page et al., 1979; Hill and Lamp, 1980; Elsewi et al., 1980 a, b; Weinstein et al., 1989; Tarkalson, et al., 2005) have demonstrated beneficial role of fly ash in increasing crop yield of alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), bermudagrass (*Cynodon dactylon*), white clover (*Trifolium repens*), field corn (*Zea mays*) and improvement in the physical and chemical characteristics of the soil. Yellow sweet clover (*Melilotus officinalis*) and white sweet clover (*Melilotus alba*) abounded in several fly ash landfills in New York (Gutenmann et al., 1976). Winter wheat (*Triticum aestivum*) grown on a deep bed of fly ash produced grains containing 5.7 mg/kg Se (dry wt.) as compared to 0.02 mg/kg in control (Stoewsand et al., 1978). Furr et al. (1978 a) demonstrated that alfalfa, sorghum (*Sorghum bicolor*), field corn (*Zea mays*), millet (*Echinochloa crusgall*), carrots (*Daucas carota*), onion (*Allium cepa*), beans (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), potatoes (*Solanum tuberosum*) and tomatoes (*Lycopersicon esculentum*) could be grown on a slightly acidic soil (pH 6.0) treated with 125 mt/ha of unweathered fly ash.

Greenhouse experiments conducted by Sikka and Kansal (1995), showed that application of 2-4 % fly ash significantly increased N, S, Ca, Na and Fe content of rice (*Oryza sativa*) plants. They did not find any residual effect of fly ash application on the following wheat crop except for a slight increase in Fe content of the soil. The post harvest soil samples from rice and wheat also did not show any change in the nutrient content and pH. The iron content of the soil, however, increased to 18 % from 12 %. Straughan et al., (1978) also reported that addition of fly ash significantly increased Fe content of soil. The Ca-rich alkaline fly ash has been frequently found to be useful for crop production (Mishra and Shukla, 1986) and neutralizing acidic soils (Beresneiwicz and Nowosielsky, 1987; Taylor and Schumann, 1988). Application of 5-20 % fly ash on w/w basis in the plough layer (0-15 cm) increased both grain and straw yield of pearl millet (*Sorghum vulgare*) followed by wheat (Grewal et al., 2001).

Use of fly ash as a soil-amending agent has been investigated for a variety of other crops (Elsewi et al., 1978 a, b; Adriano et al., 1980; Tolle et al., 1983; Wallace and Wallace, 1986). The crop response appeared to depend on a combination of factors such as method of application, physicochemical properties of soil, precipitation and plant species. Inconsistencies have been reported in the uptake of K, Ca, Mg due to the interaction among these element species in the root-soil solution-interface and within the plant system. Ca and Mg relieved K uptake by plants grown in fly ash treated soils (Martens et al., 1970 a, b; Adriano et al., 1978). An increase in the yield of alfalfa and Bermudagrass was reported on fly ash application (Elsewi et al., a, b). The S content of Swiss chard (*Beta vulgaris*), corn (*Zea mays*) and beans also increased in presence of fly ash irrespective of the soil type and fly ash source (Elsewi et al., 1978 a, b; Adriano et al., 1978). The amino acid content in soybean (*Glycine max*) was found to show an increase when grown in fly ash-amended soils in pot cultures (Goyal et al., 2002). The effect of fly ash collected from a 530 MW capacity thermal power plant, Kasimpur was mixed with field soil at rates of 0-100% on v/v basis and used to study the effect of growth of *Lycopersicon esculentum* Mill cv. Pusa Ruby. The fly ash added at a rate of 40 % was most beneficial for soil properties and improved the yield and market value of tomato fruit (mean weight) by 81 and 30%, respectively (Khan and Khan, 1996). An increase in the yield of *Agropyron elongatum* was

observed in loam soil amended with 0-50% sludge (w/w) in the ratio 1:1 and 1:5 (v/v), with 35% sludge amendment as the optimum for artificial soil mix (Wong and Su, 1997).

High yield of aromatic grasses particularly palmarosa (*Cymbopogon martini*) and citronella (*Cymbopogon nardus*), in presence of different fly ash-soil combinations, was attributed to increased availability of major plant nutrients (Asokan et al., 1995; Neelima et al., 1995). Fly ash applied at 25% showed higher yield of brinjal (*Solanum melongena*), tomato and cabbage. Oil seed crops like sunflower (*Helianthus* sp.) and groundnut (*Arachis hypogaea*) also responded favourably to fly ash amendment. Medicinal plants such as cornmint (*Mentha arvensis*) and vetiver (*Vetiver zizanoides*) were successfully planted in fly ash used in conjunction with 20 % farmyard manure (FYM) and mycorrhiza (Adholeya et al., 1997; Sharma et al., 2001 a, b).

In another study conducted by Adriano and Weber, (2001), unweathered ESP fly ash was applied at unusually high rates (280-1120 Mg/ha) and was spread evenly over each plot area in Latin Square plot design. Fly ash amendment in soil substantially improved water holding capacity and plant available water without adversely affecting the growth and marketability of the turf species, centipedegrass [*Eremochloa ophiuroides* (munro) Hack]. Fly ash at 40 % had nematicidal cum fertilizer effect and was recommended for the management of root knot disease caused by *Meloidogyne* species in tomato (Khan et al., 1997). Tomato cultivars grown on fly ash amended soils had higher tolerance to wilt fungus *Fusarium oxysporum* (Khan and Singh, 2001). Growth and nutrient uptake of two arbuscular mycorrhizal fungi, *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe and *Glomus versiforme* (Karsten) Berch, grown on soil admixed with fly ash showed good growth and better nutrient uptake by maize (Bi et al., 2003). Effect of fly ash and *Helminthosporium oryzae* on growth and yield of three cultivars of rice Pusa Basmati, Pant dhan-4 and Pant dhan-10 was observed in a 120-day old greenhouse experiment where fly ash amendment up to 40% (v/v) showed an increase in growth and yield respectively (Singh and Siddiqui, 2003).

An integrated plant nutrition system was developed utilizing fly ash, paper factory sludge, farmyard manure, crop residues and chemical fertilizers for rice-peanut cropping system (Mitra et al., 2003). Fly ash applied at 10 t ha⁻¹ in combination with organic sources was

found to increase the grain yield of rice, pod yield of peanut and equivalent yield of both crops by 31, 24 and 26% respectively as compared to chemical fertilizers alone (Mittra et al., 2003). Studies were conducted on sandy loam acid lateritic soils to observe direct effect of fly ash, organic wastes and chemical fertilizers on rice (*Oryza sativa*) and their residual effects on mustard (*Brassica napus* var *glauca*) grown in sequence. The integrated use of all the three amendments was observed to show an increase in rice-mustard yield by 14%, compared to use with fertilizers 10% and fly ash alone at 3%, respectively (Rautaray et al., 2003).

Fly ash was used as a liming material for cotton (*Gossypium hirsutum*) on Bosket fine sandy loam (fine-loamy, mixed, thermic Mollic Hapludalf) soil with an initial pH of 4.8 (Steven and Dunn, 2004). Field studies have evaluated environmental and technical parameters associated with coal combustion byproduct (CCBP)-organic waste utilization as growth media in bermudagrass sod production (Schlossberg et al., 2004). Studies were carried out to determine feasibility of CCBP, organic waste and a sand compost control for use as growth media of selected horticultural ornamentals and turfgrass sod. In the sod production component, experimental mixes were uniformly spread to heights of 2,3 and 4 cm on compacted subsoil at rates from 200-400 m³ha⁻¹ followed by spreading over with hybrid bermudagrass [*Cynodon dactylon* (L.) Pers.x*C. traansvalensis* Burtt-Davy]. At the end of production cycle sod biomass was greatest in media containing biosolids and fly ash (Schlossberg et al., 2004).

Fly ash from thermal power plant Kasimpur, India was found to have a positive influence on plant growth parameters of bottle gourd by helping in reducing infection by root rot fungus, *Rhizoctonia solani* by 25% at concentration of 10% (Shamim et al., 2004). Alkaline fly ash with pH higher than 8 from Illinois has been reported to be beneficial for growth of turfgrass (*Cynodon dactylon*) and (*Lycopersicon esculentum*) at 12.5% with the nutrient content within permissible limits in soil and plants (Chou et al., 2005).

5.2 Forestry

A number of forestry species like *Dalbergia sisso*, *Albizia lebbek*, *Eucalyptus hybrid*, *Acacia* and *Tamarindus* have been grown on fly ash amended soils for rehabilitation of fly ash dump sites. Tree species, *Populus deltoides*, *Dalbergia sisso*, *Eucalyptus* sp. meethi

neem (*Melia azadirac*) and flowering and aromatic plants, marigold (*Calendula sp.*), carnation (*Dianthus sp.*), sunflower, lemon grass (*Cymbopogon citratus*), tuberose (*Polianthus tuberosa*), corn flag (*Gladiolus sp.*) and lily (*Lilium sp.*), were found to grow and establish well on fly ash overburdens (Adholeya et al., 1998). Riekerk (1984) reported that addition of fly ash to a poorly drained sandy soil (ultic haplohumods) in Florida increased pH of the runoff from 4.7 to 6.0 and doubled the growth rate of planted Australian Pine (*Casuarina cunninghamiana* Miq.). Scanlon and Duggan (1979) planted eight tree species on a sluiced ash disposal site in Tennessee to check their tolerance and found elevated levels of B, Ni, Se, Cd, Cu and Zn in the trees. Carlson and Adriano (1991) compared the growth of sycamore (*Platanus occidentalis* L.) and sweet gum (*Liquidambar styraciflua* L.) on two abandoned ash basins, one with pH 5.6 and the other with pH 8.3 in South Carolina. Sycamore trees grew better on the alkaline basin while sweetgum grew better on the acidic basin, reflecting pH preferences of the two tree species. In field trials conducted on degraded soils using ESP fly ash at 18-24 % v/v, growth of *Eucalyptus tereticornis*, *Acacia auriculiformis* and *Casuarina equisetifolia* was observed to increase by 10 % during the initial six months (Goyal et al., 2002). Levels of As, Se and Mo in fly ash were 6.2, 3.6 and 4.0 mg/kg respectively. Fly ash at 10-20 % level (v/v) was also found to be a good potting mix material in nurseries of *Eucalyptus tereticornis* in terms of improving biomass production by 20 %.

Experiments have been conducted to evaluate the effects of inorganic fertilizer, boiler ash, biosolids, and the co-application of ash and biosolids on tree growth and soil properties by measuring hybrid poplar clone NM-6 (*Populus nigra* L.x *P. maximowiczii*) yield, nutrient uptake and select post harvest soil properties after 15 weeks of greenhouse growth (Cavaleri et al., 2004). Treatments included a control of no amendment; agricultural lime; inorganic N, P, K; three types of boiler ash; biosolid application rates equivalent to 70, 140, 210 and 280 kg available N ha⁻¹ and boiler ash co-applied with biosolids. All the amendments of biosolids, boiler ash and co-application of biosolids and boiler ash resulted in an increase in biomass production without any adverse effects (Cavaleri et al., 2004).

5.3 Wasteland reclamation

Unweathered thousands of acres of land have been converted to ash ponds. India has approximately 65,000 acres of ash ponds (Jamwal, 2003; Kumar and Sharma, 1998). New usage options that can utilize greater percentage of fly ash are being explored and large-scale application on land has been advocated as a promising utilization option (Adriano and Weber, 2001). Millions of hectares of land worldwide have also become wastelands largely due to strip mining of coal where fly ash was effectively used in reclaiming and stabilizing such areas (Capp and Spencer, 1970; Berg and Vogel, 1973; Capp and Gillmore, 1973; Fail and Wochok, 1977). Fly ash has also been used for the neutralization of acidic mine spoils and restoration of nutrient balance in alkaline wastelands. The quantity of fly ash required to reclaim such areas depends upon the pH of fly ash, state of weathering and pH of the land to be reclaimed. Fly ash acts as alternative to lime for reclaiming the acidic mine spoils (Singh et al., 1982; Haering and Daniels, 1991; Carlson and Adriano, 1993 and Stehouwer et al., 1995 a, b). Efficacy of fly ash for treating acidic coal mine spoils was evidenced by the results of a pot culture experiment using Sudan grass (*Sorghum sudanens*) and Oats (*Avena sativa*) as indicator crops (Srivastava and Chhonkar, 2000). They observed that all levels of application, fly ash and lime were comparable and significantly increased the pH and availability and uptake of phosphorus, sulphur and exchangeable potassium.

Laboratory studies have shown that alkaline fly ash was chemically equivalent to approximately 20 % of reagent grade CaCO_3 in reducing soil pH and supplying Ca to the plants (Phung et al., 1979). Although, addition of large quantity of slightly acidic fly ash may not alter the pH appreciably but can still increase the available Ca^{2+} and Mg^{2+} (Plank et al., 1975; Adriano et al., 1978). The neutralizing ability of fly ash also depends on its source and extent to which it is weathered. Addition of unweathered fly ash is not advisable as it may substantially increase soil salinity. Leaching, lagooning and stockpiling appreciably solve this problem, minimize boron toxicity and other ill effects of unweathered fly ash (Page et al., 1979; Phung et al., 1978; Mulford and Martens, 1971). Large quantities of neutral coal fly ash (NFA) can be profitably used (Abbott et al., 2001) by co-application of a lime-stabilized biosolid (LSB) for the reclamation of acid mine spoil (AMS).

Studies conducted to investigate the long term effects of amending sulfide-rich lignite mine spoil with fly ash and agricultural limestone, showed a sustained increase in pH which can be attributed to gradual weathering of aluminosilicates (Seoane and Leiros, 2001). The eroded soil of Palouse region of eastern Washington and Northwest Idaho, USA, were restored by treatment with compost, coal ash and straw amendments since the ridge tops in the dryland farming region suffered from low productivity and reduction in soil quality from years of soil erosion. The first phase of application involved use of two rates of compost and a control and the second phase involved compost, coal ash, wheat straw, three rates of inorganic N, and a control (Seoane and Leiros, 2001).

Addition of alkaline fly ash to wasteland and mine spoils increases pH, decreases bulk density, increases water-holding capacity and reduces compaction (Fail and Wochok, 1977; Capp, 1978; Jastrow et al., 1981; Fail, 1987; Taylor and Schumann, 1988). Fly ash has also been recommended as an amendment for pyritic mine tailings in the western USA (Sonderegger and Donovan, 1984) where it facilitated the establishment of vegetation at barren sites by bringing about changes in soil pH (Capp, 1978) and acting as source of plant nutrients (Jastrow et al., 1979). Pyrite (FeS_2) in wastes and mine spoils is oxidized to form sulphuric acid resulting in highly acidic ($\text{pH} \leq 3.5$) conditions (Adams et al., 1971; Fail and Wochok, 1977; Capp, 1978; Jastrow et al., 1981). This necessitated the use of as much as 1792 t/ha of fly ash for reclamation. Planting of halo-tolerant plant species has been recommended to partially reduce this quantity (Capp et al., 1975; Capp, 1978). The U.S. Bureau of Mines studies in West Virginia and Pennsylvania indicated that the best initial planting mixture was a combination of grasses and legumes, particularly Kentucky 31 fescue (*Festuca arundinacea*), redtop (*Agrostis alba* L.), ryegrass (*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.) and birdsfoot trefoil (*Lotus corniculatus* L.). After several years, trees and shrubs like crab apple (*Malus* sp.), European alder, Scotch pine (*Pinus sylvestris* L.) and Norway spruce [*Picea abies* (L.) Karst.] could be planted (Capp, 1978). Fly ash was also reported to suppress pyrite oxidation (Keefer, 1993) by acting as a blanket to prevent oxygen diffusion. Spoil areas having pH of 4.4 to 5.0 were reclaimed by fly ash at 70 mt/ha (Fail and Wochok, 1977) while highly acidic spoils with pH of 2.0 to 3.5, required 335-1790 t/ha of fly ash (Adams et al., 1972).

A mixture of fly ash and municipal sludge was used for restoration of 2000 acres of land on Blue Mountain, Palmerton, Pennsylvania which had been rendered barren due to the zinc smelter emissions of New Jersey Zinc Company from 1898 to almost 1980 (Sopper, 1988). The sludge fly ash mixture provided macronutrients, micronutrients and microbial activity for the plants (Oyler, 1993). The highest percentage of vegetation cover was from a mixture of orchardgrass, tall fescue and crownvetch. In 1986, this seed mixture had an average cover of 90% and ranged from 82-95% among the three fly ash-sludge amendments. Of the tree species planted, larch had the greatest growth in height of the conifers and blackcherry had the greatest of all hardwood species (Sopper, 1988).

In a 3-year field study restorative effects of coal fly ash applied at 0, 22, 280, 560 and 1120 Mg (tonne) ha⁻¹ and poultry biosolid at 5 and 10 Mg ha⁻¹ was conducted at a 12 ha soil-borrow area in South Carolina (Punshon et al., 2002). The area was seeded with erosion control species Atlantic Coastal Panic grass (*Panicum amarum* var *amarum* L.), Sericea (*Lespedeza cuneata* var. *appalow* [Dumont]G. Don.) and weeping lovegrass (*Eragrostis curvula* Wolf). The area was successfully revegetated with a significant increase in plant biomass production by a maximum of 26% using 1120 Mg ha⁻¹ of fly ash and 10 Mg ha⁻¹ of poultry biosolid along with increase in pH, P, K, Ca, Mg, organic matter content and water holding capacity. Cassia surattensis Burm, an ornamental plant of the Leguminosae family, was used for revegetation of fly ash landfills and impact of fly ash on growth, chlorophyll content and nodule number along with metal uptake capacity of the plant was evaluated (Vajpayee et al., 2000).

6. Other applications of fly ash

One of the major problems in feedlot operations of dairy farms is directly linked to pen maintenance and manure management. Odour and dust problems, animal health and performance, water runoff, protection of ground and surface water are interwoven feeding operations. Animal performance is reduced by as much as 25% under unfavourable conditions. A major problem faced by dairy farmers is the turning of soils into knee-deep mud owing to spring rains and severe winter conditions, which make the cattle prone to diseases and sap them of energy to produce milk. Research conducted by the Agricultural Research Service and industry scientists has shown that paving with hydrated form of fly

ash gives a solid foundation to feedlot areas. Not only the fly ash is cheaper than concrete, it poses little danger to animals and environment. ARS soil scientist Stout and his group (1999), conducted pilot studies at the experimental dairy farm north of Harrisburg, Pennsylvania to examine the environmental impact of spreading 33 tons of fly ash on a 900 square-foot feedlot. Using suction lysimeters, the team monitored the concentrations of various elements and heavy metals seeping into groundwater from the fly ash pads, which was compared with the data collected from an unpaved feedlot. Laboratory analysis revealed minute traces of calcium and nickel much lower than the unacceptable levels. Based on the results from the Harrisburg study and earlier ARS projects, the New York Department of Environmental Conservation subsequently approved farmer use of fly ash as a safe barnyard paving resource. Evidence collected so far indicates a sharp drop in the incidence of hairy foot wort, viral hoof infection and mastitis.

Fly ash has been reported to help in removal of COD from textile mill effluents under ambient pH and temperature conditions (Patnaik et al., 1995). Fly ash has been reported to adsorb mercury (Srivastava et al., 2000; Bhattacharya and Sharma, 1993), copper (Pandey et al., 1985; Kapadia et al., 2000) and zinc (Singh and Rawat, 1993; Gupta and Sharma, 2003) from aqueous solutions. Decolorization of effluents by fly ash has been reported earlier by a number of workers (Viraraghavan and Ramakrishna, 1999; Robinson et al., 2001; Khan et al., 2003) and a mixture of fly ash and coal in the ratio of 1:1 can be substituted for activated carbon owing to increase in surface area available for absorption (Gupta et al., 1990).

Materials and Methods

1. Nursery trial

ESP fly ash from GGSTP (Guru Gobind Singh Super Thermal Power Plant), Ropar was mixed with alkaline soil of Patiala taken from topmost soil profile 0-30 cm, on a v/v basis at concentrations of 0, 5, 10, 20 and 30%. A portion of the soil-fly ash mixtures was separated for physical, chemical and microbiological analysis prior to beginning of trial. The different fly ash-soil mixtures were placed in half-kg polythene bags perforated at the bottom to allow air passage. Shoot cuttings for the nursery trial were taken from full grown trees of *Populus deltoides* with 3 to 5" alternate simple medium green leaves with a modest fall colour. These were further segregated for uniform diameter (0.8 cm) and length (15 cm), washed and treated with IBA (4000 ppm) before being planted into the bags with 40 replications per treatment. The planted shoots were watered with normal tap water and weeding was carried out regularly and the bags were kept in a screen house. The soil-fly ash mixtures before and after the trial were processed by sieving and separating into different fractions for analysis of physico-chemical and microbiological properties as described in Section 5.

2. Field trial

An established field plantation of *Acacia auriculiformis* and *Eucalyptus tereticornis* located at Village Durgaprasad, Distt. Dhenkanal (Orissa) was selected to study the effect of fly ash on biomass production and nutrient status of soil. The plantation was established on a twenty-acre area, 55 kms from the Ballarpur paper mills in July 1998. Around 3750 seedlings each of two multipurpose tree species namely *Eucalyptus tereticornis* and *Acacia auriculiformis* procured from the Ballarpur nursery were selected for the experiment. The seedlings were 3-4 months old and about 25-30 cm long at the time of planting, which was done just prior to the monsoons. The experimental design is a split block design. The area receives an average annual rainfall of 1400 mm with 80-85% being received during June to September. The land is partially degraded with soil belonging to order Ultisols having reddish brown lateritic characteristics. The soil texture ranges from sandy clay loam to sandy loam. The plantation involved five different

concentrations of fly ash on v/v basis [F0: 0%, F1: 6%, F2: 12%, F3: 18% and F4: 24%], and in each there were two treatments [control (T1) and chemical fertilizers (T2)]. T2 involved chemical fertilizer creating an overall of ten mixtures including fly ash control and fertilized control. The fly ash was applied only in pits (30x45x45 cm) after mixing with soil and chemical fertilizers as per the experimental plan. Fly ash was obtained from the electrostatic precipitator of FBC boiler (source of coal: Talcher coal mines; F grade: 40% coal ash) in June 1998 and transported to the site. Bulk density of fly ash used was 0.9 gm/cc and the water holding capacity was 67%. Diammonium phosphate (DAP) at 17.5 g/plant and muriate of potash (MOP) at 5g/plant and neem cake at 20 g/plant were added at the time of plantation. Urea application at 50g/plant was given after three months. The plantation was set up in a block of 7x7 plants with plant-to-plant and row-to-row distance of 2 m using randomized block design. Soil samples from the rhizospheric zones were collected at yearly intervals for physicochemical and microbiological analysis as described in Section 5.

3. Laboratory trials

To study the effect of fly ash amendment on soil microbial activity, alkaline soil of Patiala was mixed with ESP fly ash from Yamunanagar at different concentrations and glucose was added at varying concentrations as a carbon source in a different set of experiment. Soil was analysed for dehydrogenase activity, rate of carbon dioxide evolution, microbial biomass and enumeration of bacteria under laboratory conditions in one litre Erlenmeyer flasks at room temperature by incubating under dark conditions as described in Section 5.3.

Experiment I

Fly ash was mixed with soil on v/v basis at 0, 5, 10, 20 and 30 %.

Experiment II

Fly ash was mixed with soil on v/v basis at 0, 25, 50 and 75 %.

Experiment III

Soil was mixed with 5% fly ash on v/v basis and glucose was added at 1, 2, 3 and 4 % (w/w) rates separately.

Experiment IV

Soil of Patiala was mixed with varying concentrations of fly ash at 1, 3, 5 and 10% on a (v/v) basis and 4% glucose was added.

Experiment V

Soil of Patiala was mixed with varying concentrations of glucose at 1, 2, 3 and 4% on (v/v basis).

4. Collection and processing of fly ash and soil

Fly ash from the electrostatic precipitator (ESP fly ash) was collected from three different sources, viz. BILT, Choudwar, Orissa, GGS Thermal Power Plant, Ropar, Punjab and BILT, Yamunanagar, Haryana and was directly analyzed for physicochemical properties after air-drying. The acidic soil from Village Durgaprasad (Orissa) and alkaline soil from Thapar Technology Campus, Patiala, Punjab was collected from a 0-30 cm depth and processed and analyzed for physical, chemical and microbiological characteristics as described in Section 5. The soil after collection was divided into two portions, one of which was immediately refrigerated for later microbiological analysis. The remaining part was gently broken up for clods and macro aggregates using pestle and mortar followed by removal of plant residues, gravel and other debris. The soil was spread on polythene sheets, air-dried and sieved through a 2 mm mesh sieve and used for physical analysis. The 2 mm-sieved soil was crushed to pass through 0.2 mm sieve for chemical analysis. The 2 mm sieved soil was used for various laboratory and nursery trials.

5. Soil analysis

5.1 Chemical analysis

pH

pH was determined as per the method given by Jackson (1967) in a soil-water/fly ash-water suspension of 1:2 ratio. Ten g of soil/fly ash was placed in a 100 ml beaker and 20 ml of distilled water was added and the soil was stirred well for five minutes and kept undisturbed for some time followed by stirring again. pH was measured using a Thermo Orion Model 290 pH meter after calibration with buffers of pH 4.0, 7.0 and 9.2.

Electrical conductivity

Electrical conductivity was measured in $\mu\text{S cm}^{-1}$ as per the method given by Jackson (1967). Ten g of soil /fly ash was placed in a 100 ml beaker and 20 ml distilled water was added. The soil-water mixture was allowed to stand undisturbed until the soil settled completely. The conductivity meter (Orion Model 125) was calibrated with 0.01 M potassium chloride ($1413 \mu\text{S cm}^{-1}$).

Organic carbon

Total organic carbon was estimated as per the method given by Walkley and Black (1934).

Reagents

1. 1 N $\text{K}_2\text{Cr}_2\text{O}_7$: 49.04 g of potassium dichromate per litre of solution.
2. 0.5 N ferrous ammonium sulphate: 198 g salt per litre of solution.
3. Diphenylamine indicator: 0.5 g of diphenylamine in a mixture of 20 ml water and 100 ml concentrated sulphuric acid.
4. Concentrated sulphuric acid.
5. Orthophosphoric acid (85%) and sodium fluoride (NaF).

Procedure

1. 1 g of soil/fly ash was taken in a 500 ml conical flask followed by the addition of 10 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$. The flasks were swirled for mixing the soil and reagent.
2. 20 ml of H_2SO_4 was added and the flask was allowed to stand undisturbed for 30 minutes after which 200 ml of distilled water was added.
3. To the mixture, 10 ml of Orthophosphoric acid, 0.5 g of NaF and 1 ml diphenylamine indicator was added.
4. The contents were ultimately titrated with freshly prepared 0.5 N ferrous ammonium sulphate till the end-point is observed from blue-violet to green. A blank was also run without soil.

Calculation

$$\text{Organic carbon (\%)} = \frac{10 (B-T) \times 0.003 \times 100}{B \times \text{Wt.of soil (g)}}$$

where: B is the volume of ferrous ammonium sulphate solution required for blank titration.

T is the volume of ferrous ammonium sulphate solution required for soil sample titration.

Available phosphorus

Available phosphorus in the alkaline soil/fly ash was estimated as per the method given by Olsen et al. (1954).

Reagents

1. 0.5 M NaHCO₃ extracting solution: 84 g of sodium bicarbonate was added in distilled water and the volume was made up to 2 l. The pH was adjusted to 8.5 with 1M or 1N NaOH.
2. Reagent A: 12.0g of ammonium molybdate in 250 ml distilled water and 0.2908g of antimony potassium tartarate in 100 ml distilled water was added to 1000 ml of 2.5 M H₂SO₄, mixed thoroughly and volume made upto 2l with distilled water.
3. Reagent B (freshly prepared): 1.058g of ascorbic acid in 200 ml of reagent A and mixed.
4. Sulphuric acid (2.5 M): 140 ml of concentrated H₂SO₄ diluted to 1l.
5. Stock Standard P solution (50 ppm P): 0.2917 g KH₂PO₄ dissolved in water to a final volume of 1 l.
6. Working Standard P solution (1 ppm): 20 ml of (50 ppm P) solution diluted to 1l.

Procedure

1. 2.5 g soil/fly ash was placed in a 100 ml Erlenmeyer flask followed by the addition of 50 ml extracting solution.
2. The solution was kept on a shaker for 30 minutes and filtered through Whatman No. 42 filter paper.
3. 10 ml aliquot of the filtrate was transferred to a 100 ml beaker followed by addition of 1 ml of 2.5 M H_2SO_4 , 15.5 ml of distilled water, 8 ml of Reagent B and another 15.5 ml of distilled water.
4. A blank was prepared as above. For the standard curve: 0, 2, 5, 10, 15 and 20 ml of standard solution was placed in 50 ml volumetric flasks separately. Ten ml of extracting solution, 1.0 ml of 2.5 M H_2SO_4 , 8 ml Reagent B was added and the final volume was made up to 50 ml. The P concentrations of these solutions were 0.04, 0.1, 0.2, 0.3 and 0.4 ppm respectively. After 10 minutes, the P concentration was read at 882 nm.

Calculation

P in soil (ppm) = P in extract (ppm) x 20 (the standard soil to solution ratio).

Available phosphorus

Available phosphorus in acidic soil/fly ash was estimated as per the method given by Bray and Kurtz (1945).

Reagents

1. Ammonium fluoride (extracting solution) : 22.2 g of NH_4F dissolved in 41.6 ml HCl and volume made up to 2l.
2. Reagent A: 12.0g of ammonium molybdate in 250 ml distilled water and 0.2908 g antimony potassium tartarate in 100 ml distilled water. These two solutions were

- mixed, 1000 ml of 2.5 M H_2SO_4 was added and volume made upto 2l with distilled water.
3. Reagent B (freshly prepared): 1.058g of ascorbic acid dissolved in 200 ml of reagent A and mixed.
 4. Sulphuric acid (2.5 M): 140 ml of concentrated H_2SO_4 diluted to 1l.
 5. Stock Standard P solution (50 ppm P): Dissolved 0.2917g of KH_2PO_4 in water to a final volume of 1l.
 6. Working Standard P solution (1 ppm): Diluted 20 ml of (50 ppm P) solution to 1l.
 7. 40% NaOH.

Procedure

1. 2.5 g soil/fly ash was taken in a 100 ml flask and 25 ml extracting solution was added.
2. The solution was kept on a shaker for 30 minutes and filtered through Whatman filter paper No. 42.
3. The 2 ml aliquot of the filtrate was transferred to a 100 ml beaker followed by addition of 20 ml of distilled water, 8 ml of reagent B and 20 ml of distilled water.
4. Blank prepared as above. For the standard curve: 0, 2, 5, 10,15 and 20 ml of standard solution were measured in 50 ml volumetric flasks. Two ml of extracting solution and 8 ml Reagent B was added and the final volume was made upto 50 ml. The P concentrations of these solutions were 0.0, 0.04, 0.1, 0.2, 0.3 and 0.4 ppm respectively. After 10 minutes, the P concentration was read at 882 nm.
5. For total phosphorus 1 g soil was digested with HNO_3 and HClO_4 in the ratio 3:1 on hot plate at 100°C until a whitish brown mass was obtained.
6. The sample was treated with a HCl and water mixture and filtered through Whatman No. 42 filter paper and the filtrate was stored in bottles.

7. Ten ml of filtrate was taken and pH adjusted to 5.0 using 40% NaOH and volume was made up to 50 ml. A 2 ml aliquot was taken and analysis carried out as in step 3 and 4.

Calculation

P in soil (mg kg^{-1}) = P in extract (mg l^{-1}) x 10 (the standard soil to solution ratio).

Available Nitrogen

Available nitrogen was estimated as per the method given by Subbiah and Asija (1956).

Reagents

1. 0.32% Potassium permanganate: 3.2 g of KMnO_4 dissolved in water and final volume made up to 1l.
2. 2.5% NaOH: 25 g of sodium hydroxide pellets dissolved in water and volume made up to 1l.
3. 2% boric acid: 20 g of boric acid powder dissolved in warm H_2O by stirring and diluted to 1l.
4. Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green dissolved in 100 ml of ethyl alcohol. 20 ml of the mixed indicator added to each litre of 2% boric acid solution and final pH adjusted to 4.5 with dilute HCl or dilute NaOH.
5. 0.1N potassium hydrogen phthalate: Dissolve 20.422 g of the salt in water and dilute to 1l.
6. 0.1 N NaOH: 4g of NaOH dissolved in water and diluted to 1l, standardized against 0.1 N potassium hydrogen phthalate solution.
7. 0.02 N H_2SO_4 : 0.1 N H_2SO_4 prepared by adding 2.8 ml of concentrated H_2SO_4 to about 990 ml of distilled water. From this 0.02 N H_2SO_4 made by diluting a suitable volume five times with distilled water and standardized against 0.1 N NaOH solution.

Procedure

1. 5 g of soil/fly ash was weighed and placed in a 800 ml Kjeldahl flask.
2. The soil was moistened with 10 ml distilled water and any adhering soil on the neck was washed down followed by addition of 100 ml of 0.32% KMnO_4 . Glass beads measuring 0.4 mm were added to prevent bumping.
3. 20 ml of 2% boric acid containing mixed indicator was measured in a 250 ml conical flask and placed under the receiver tube. The receiver tube end was dipped in the boric acid.
4. Tap water was allowed to run into the condenser for cooling.
5. 100 ml of 2.5 % NaOH solution was added and the rubber stopper was quickly fitted in the alkali trap.
6. The heaters were switched on to continue distillation until about 100 ml of distillate was collected.
7. The conical flask containing distillate was removed before switching off the heater to avoid back suction.
8. The distillate was titrated against 0.02 N H_2SO_4 in a burette until a pink colour started appearing. A blank was run without soil.

Calculation

Available N in soil in ppm = $(X) \times 0.00028 \times 100/5$ where X stands for the titre value of 0.02 N H_2SO_4 consumed.

Total Nitrogen

Total nitrogen was estimated as per the Kjeldahl method given by Piper (1960).

Reagents

1. Concentrated H_2SO_4 .
2. 0.02 N H_2SO_4 .
3. Sulphuric-Salicylic acid: 1 g salicylic acid mixed with 30 ml sulphuric acid.
4. Sodium thiosulphate.
5. 4% boric acid.
6. Mixed indicator. 0.066 g of methyl red and 0.099 g of bromocresol green dissolved in 100 ml of ethyl alcohol.
5. 50% NaOH.
6. Digestion mixture: 10g HgO , 5g CuSO_4 and 100 g K_2SO_4 (2:1:20).

Procedure

1. 5 g soil/fly ash was mixed thoroughly with sulphuric-salicylic acid followed by 5g of sodium thiosulphate. Heating was carried out for 5 minutes followed by cooling and addition of 10g digestion mixture. The contents were mixed well in a Kjeldahl flask.
2. The flask was kept in the digestion chamber at 100°C for two hours.
3. The color change was monitored from dark brown to greenish white after which the contents were cooled and 300 ml distilled water was added.
4. 20 ml of the digested sample, 15-20 ml NaOH and glass beads were added to the distillation flasks through the open end of the condenser attachment and stoppered. Water flow was maintained through the condenser.
5. The distillate was collected through a receiver tube in a beaker containing 15 ml boric acid and 2 drops of mixed indicator till the end-point color changes from pink to green.
6. The distillate was titrated against 0.02 N H_2SO_4 until the endpoint colour changed from green to pink.

Calculation

$$\text{Total N \%} = \frac{(T-B) \times \text{Normality of H}_2\text{SO}_4 \times 1.4 \times 300}{\text{Weight of sample}}$$

Weight of sample

where T is the titre value for sample and B is for blank.

Available sulphur

Estimation of available sulphur was done by the method given by Chesnin and Yien (1950).

Reagents

1. 0.15% calcium chloride.
2. 30-60 mesh barium chloride.
3. 0.25% gum acacia.
4. K_2SO_4 solution: 0.5434 g of reagent grade potassium sulphate in distilled water and diluted to 1l.
5. Whatman No.42 filter paper.

Procedure

1. 50 ml of 0.15 % calcium chloride was added to 10 g soil sample taken in a 150 ml conical flask.
2. The sample was agitated at 130 r.p.m. for 30 minutes followed by filtration through Whatman No. 42 filter paper.
3. 20 ml of filtrate was taken in a 25 ml volumetric flask and 1 g of 30-60 mesh barium chloride was added, swirled followed by 1 ml of gum acacia. The volume was made upto 25 ml with distilled water and absorbance read at 420 nm.
4. Standard curve: Different volumes 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm standard K_2SO_4 solution were taken in 25 ml volumetric flasks. Ten ml of 0.15% calcium chloride, 1.0 g barium chloride, 1.0 ml gum acacia were added and volume made up with distilled water. The absorbance was read at 420 nm.

5. 1 g soil was digested with HNO₃ and HClO₄ in the ratio 3:1 on hot plate at 100°C until a whitish brown mass was obtained.
6. The sample was treated with HCl and water mixture and filtered through Whatman filter paper No. 42 and filtrate stored in bottles.
7. 10 ml of filtrate was taken and pH adjusted to 5.0 using 40% NaOH and volume was made up to 50 ml. Then a 20 ml aliquot was taken and further analysis was carried out as in available sulphur.

Total Fe, Mn, Ni, Cr, Pb, Zn, Ca, Mg, Na

Estimation of total metals was done as per the method given by Page et al. (1982). As, Se and Mo and radioactivity for Ra²²⁶, Ac²²⁸ K⁴⁰ was analyzed at The Indian Institute of Physics, Bhubaneswar, Orissa.

Reagents

1. Concentrated perchloric acid (HClO₄) and nitric acid (HNO₃).
2. Acid water solution containing HCl and water in a 1:1 ratio.

Procedure

1. 1g of soil/fly ash/plant sample was placed in a 150 ml beaker.
2. HNO₃ and HClO₄ in a 3:1 ratio was added to the sample.
3. The sample was digested on a hot plate at 100°C for 3-4 hours until a whitish brown dry mass was obtained.
4. The samples after digestion were treated with acid water mixture and filtered through Whatman No.42 filter paper.
5. The filtrate was analyzed for total Fe, Mn, Ni, Cr, Pb and Zn in both soil and plant samples in addition to Ca, Mg and Na in stem and leaves of *Populus deltoides* using an atomic absorption spectrophotometer (GBC 932AA). The various metals along with their sensitivity limits are as follows.

Element	Sensitivity ($\mu\text{g ml}^{-1}$)
Cr	0.05
Fe	0.05
Ni	0.04
Pb	0.06
Zn	0.008
Mn	0.02
Ca	0.02
Mg	0.003
Na	0.004

Analysis of radioactivity (Ra^{226} , Ac^{228} and K^{40})

A gamma ray spectrometer with a 60 % efficiency High Purity Germanium (HPGe) detector (resolution of 2.1 keV at 1.33 MeV) and having a volume of 270 cm³ was used for gamma ray measurements (Vijayan, et al., 1999; Vijayan and Rautray, 2003). Emitted gamma rays from the samples were collected by an HPGe detector with lead shielding lined with 1mm aluminium. The pulses from the detector were fed through an amplifier and recorded by using a PC based Multi Channel Analyser. The gamma ray spectra were analysed and radionuclides such as Ra^{226} , Ac^{228} and K^{40} were estimated.

Analysis of As, Se and Mo

An energy dispersive X-ray fluorescence (EDXRF) system, being used for analysis of heavy elements, essentially consists of a low power air-cooled X-ray tube as an excitation source with tri-axial geometry. The operating voltage and the current of the X-ray tube were 25 kV and 0.6 mA respectively (Vijayan et al., 1997, 1999; Ray et al., 2004). The X-rays from the tube were exposed on a molybdenum or tin secondary exciter and the generated K-characteristic X-rays of the secondary exciter were used to excite the elements present in the studied samples. The characteristic X-rays emitted from the elements present in each sample were collected using a Si(Li) detector and a PC-based Multi Channel Analyser. The photo-peak areas in each spectrum were

evaluated using the computer program AXIL supplied by the International Atomic Energy Agency (IAEA) and elemental concentrations were obtained.

Ammonium-acetate extractable K

Estimation was done as per the method given by Merwin and Peech (1951).

Reagents

1. Double distilled water.
2. Neutral ammonium acetate solution: 77.10 g of ammonium acetate was weighed and dissolved in double distilled water and the final volume was made upto 11 after adjusting the pH to 7.0.
3. Whatman No.42 filter paper.

Equipment

1. Atomic Absorption Spectrophotometer (GBC 932AA).
2. Analytical balance.
3. Horizontal mechanical shaker.
4. 100 ml Erlenmeyer flask.

Procedure

1. 10 g of air-dried soil was weighed and added to 100 ml Erlenmeyer flask followed by 50 ml of ammonium acetate solution.
2. The flask was placed on mechanical shaker for 10 minutes at 25°C, and 130 oscillations per minute.
3. After ten minutes, the soil was immediately filtered using Whatman No.42 filter paper.
4. The filtrate was analyzed for metal content using an atomic absorption spectrophotometer (GBC 932AA).

DTPA-extractable Fe, Mn, Ni, Cr, Pb and Zn

Estimation was done as per the method given by Lindsay and Norwell (1978).

Reagents

- a. Deionized water.
- b. DTPA extracting solution: 0.005M DTPA (diethylenetriaminepentaacetic acid), 0.01 M CaCl_2 and 0.10 M triethanolamine (TEA) adjusted to pH 7.3. For 10 l solution 19.67 g DTPA and 149.2 g of TEA in 5 l deionised water. 14.69 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ added to 5l of deionized water followed by DTPA-TEA mixture and pH adjusted to 7.3 ± 0.05 using 1.0 N HCl.
- c. Whatman No.42 filter paper.

Equipment

1. Atomic absorption spectrophotometer (GBC 932AA).
2. Analytical balance.
3. Horizontal mechanical shaker.
4. Extraction vessel.

Procedure

1. 10 g of air-dried soil was weighed and added to extraction vessel followed by 50 ml of DTPA extracting solution.
2. The extraction vessel was placed on mechanical shaker for 2 hours at 25°C and 180 oscillations per minute.
3. After two hours, the soil was immediately filtered using Whatman No.42 filter paper.
4. The filtrate was analyzed for metal content using an atomic absorption spectrophotometer (GBC 932AA).

5.2 Physical analysis

Water holding capacity

Water holding capacity was measured as per the method given by Black (1965).

Apparatus

Circular brass boxes (keen boxes) of 5.6 cm internal diameter and 1.6 cm depth were taken which had 0.75 mm holes spaced 4 mm apart at the bottom. Each box is fitted with a brass lid.

Procedure

1. A filter paper strip of the size of the base of the keen box was cut.
2. The filter disc was weighed and placed in a petridish containing water for measuring the moisture absorbed by the filter paper.
3. The disc was placed at the bottom of the keen box and weighed followed by filling of the box with soil/fly ash. Each time the box was tapped to make a uniform soil/fly ash column.
4. The box containing soil/fly ash was weighed and kept in a petridish containing water for overnight saturation.
5. The box was removed the next day, wiped and weighed followed by overnight drying at 80° C in the oven in order to obtain constant weight.
6. The box containing oven-dry soil/fly ash was weighed finally at room temperature.

Calculation

Weight of box+ filter paper = W1

Weight of the box +oven dry soil = W2

Weight of the box+ soil after moistening = W3

Weight of dry soil = W2-W1

Weight of moisture absorbed	= W3-W2
<i>Moisture absorbed by filter paper</i>	= W4
<i>Moisture held by soil alone</i>	= W3-W2-W4
<i>Water holding capacity of the soil</i>	= W3-W2-W4/W2-W1x100

Bulk density

Bulk density was measured as per method given by Black (1965).

Apparatus

Specific gravity bottle.

Procedure

- 1. The specific gravity bottle was weighed and the volume of water, which could fill it up to the brim, was measured.*
- 2. The bottle was filled with soil/fly ash and weighed.*

Calculation

<i>Weight of empty bottle</i>	= W1
<i>Weight of bottle and soil</i>	= W2
<i>Weight of soil</i>	= W2-W1
<i>Volume of the soil or volume of water needed to fill the bottle</i>	= V ml
<i>Bulk density of the soil/fly ash</i>	= $\frac{W2- W1}{V} \text{ g cm}^{-3}$

Particle size distribution

Particle size distribution was measured as per method given by Buoycous, 1962.

Apparatus

1. Mechanical stirrer.

2. Soil hydrometer (Buoycous hydrometer).
3. Stopwatch.
4. Measuring cylinders (1000 ml).
5. 10% sodium oxalate solution.

Procedure

1. 100 g of soil sample was weighed and placed in a 500 ml beaker followed by addition of 200 ml distilled H₂O and 100 ml sodium oxalate solution.
2. The contents were stirred well in a mechanical stirrer and given 4-5 washings with distilled H₂O , making the volume to 500 ml.
3. The sample was transferred to a 1000 ml suspension cylinder and the volume was made up.
4. The cylinder was stoppered and vigorously shaken for complete dispersion of particles.
5. The cylinder was placed on a table and the hydrometer was placed inside.
6. The first recording was noted exactly 40 seconds after placing the hydrometer.
7. The sample was kept undisturbed for two hours and reading was taken in a similar way with the hydrometer.

Calculation

Explained through example

Correction factor (CF)= (Actual room temperature in °F- 68) x0.2

Time	Temperature	Hydrometer reading	Correction	Correct reading	% suspension
40 secs	78°F	33.5	2.2	35.7	35.7

2 hrs.	77°F	15.0	2.0	17.0	17.0
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Sand per cent = 64.0

Reading at 40 secs = 35.7

Clay per cent = 17.0

Reading at 2 hours = 17.0

Silt per cent = 18.7

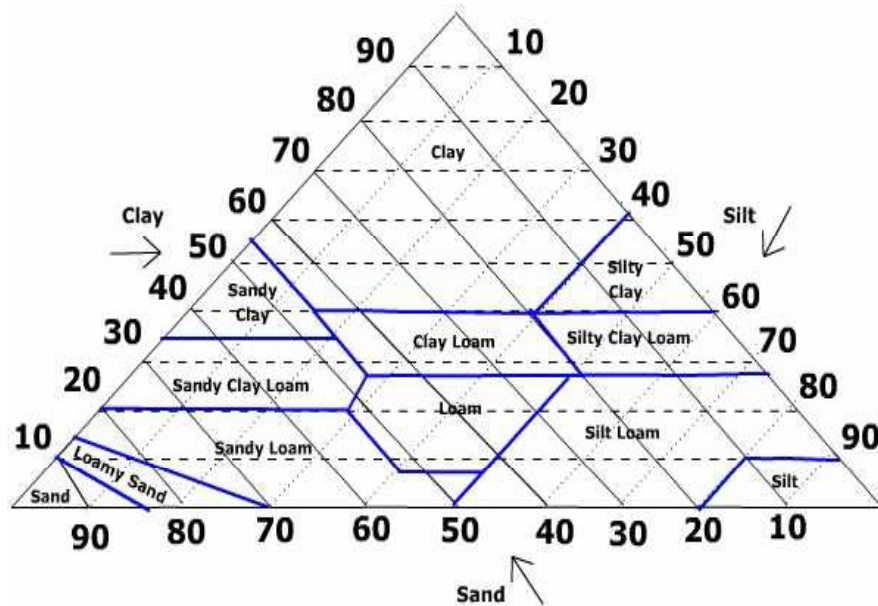
Difference = 18.7

1. The clay percentage is given by reading the hydrometer taken at 2h, which is 17.0.
2. The silt percentage is obtained by subtracting the reading at 2h from the reading at 40 seconds, which is 18.7.
3. The sand percentage is obtained by subtracting the reading at 40 seconds (35.7) from 100, which equals to 64.0.

Soil texture

Soil texture was determined using soil texture triangle (Chopra and Kanwar, 1991)

(www.usp.edu/geo/faculty/ritter/glossary/s_u/soil_texture_triangle.html).



Procedure

In the soil textural diagram, the points corresponding to percentage of silt and clay in soil were located on silt and clay lines respectively. Lines were then projected inward, parallel in the first case to the clay side of triangle and in the second case parallel to the side. The name of the compartment in which the two lines intersect is name of class of soil in question.

Hydraulic conductivity

Hydraulic conductivity was measured as per method given by Black (1965).

Apparatus

1. Brass permeameters of 7 cm diameter and perforated base.
2. A wooden stand for supporting the permeameter.
3. Iron water head cylinder with inlet and outlet pipes connected to permeameters.

4. Funnels supporting permeameter.

Procedure

1. Filter discs of the size of the perforated base were cut and placed on the base.
2. The permeameter cylinders were filled with soil /fly ash with constant tapping until an even column was obtained.
3. The water flow was regulated through the inlet connections from the water head cylinder with the help of screw clamps attached to the tubings.
4. Beakers were placed under each permeameter for the collection of percolates.
5. A constant saturation of soil was maintained and percolate water was collected for 15 minutes.
6. The volume of percolates was measured with a measuring cylinder and screw clamps closed to stop the flow of water.

Calculation

Hydraulic conductivity (K) cm min^{-1} : $qL/t A\Delta H$, where $\Delta H = L+h$.

q is the volume of the percolate collected.

L is the length of the sample through which water flows.

h is the height of water head above the sample column.

A is the cross sectional area of given hydraulic-head drop.

t is the time in which percolate is collected.

5.3. Microbiological analysis

Soil dehydrogenase activity

Soil dehydrogenase activity was measured as per the method given by Cassida (1977).

Reagents

1. Triphenyl tetrazolium formazan (1 mg ml^{-1} TPF).
2. 1,3,5 Triphenyl tetrazolium formazan ($100 \mu\text{g ml}^{-1}$).

3. Methanol.
4. Calcium carbonate.
5. 0.1% and 0.2% yeast extract.
6. Sterile water.
7. Sterile glassware.
8. Whatman No 1 Filter Paper.

Procedure

1. 10 g of soil sample was weighed and mixed with CaCO_3 in the ratio 100:1.
2. 3g of each sample was dispensed in screw cap glass vials and 0.5 ml sterile water was added followed by 1 hour incubation at 28°C .
3. 0.25 ml single strength substrate solution was added (0.1% yeast extract) followed by 0.25 ml sterile water. The vials were then incubated for 8 hours at 28°C .
4. 0.5 ml of 3% aqueous TTC (2,3,5 triphenyl tetrazolium chloride) and 0.25 ml double strength (0.2 %) yeast extract was added and mixed thoroughly with sterile glass rod.
5. This was followed by 6-hour incubation at 37°C and immediate extraction with 25 ml methanol and subsequent filtration through Whatman No. 1 Filter Paper.
6. The methanol extract containing red coloured formazan was read at 480 nm.
7. In 100 ml volumetric flasks 5, 10, 15 and 20 ml of working solution of $100 \mu\text{g ml}^{-1}$ 1,3,5 triphenyl tetrazolium formazan (diluted from stock solution of 1 mg ml^{-1} TPF) was added and final volume adjusted with methanol. The absorbance was read at 480 nm.

Rate of carbon dioxide evolution.

The rate of carbon dioxide evolution was measured as per method given by Stotzsky (1965).

Requirements Alkaline soil, glucose.

Reagents

1. 0.5 N standard NaOH.
2. 0.5 N hydrochloric acid.
3. 0.1% phenolphthalein indicator.
4. Saturated barium carbonate solution.

Procedure

1. 100 g of soil samples were placed in a 500 ml flask and 15 ml water was added in each.
2. A test tubes containing 15 ml 0.5 N NaOH was suspended in the flasks and stoppered. The flasks were incubated for 24 hours.
3. The next day the contents of the tube were transferred to 10 ml barium carbonate saturated solution.
4. 1-2 drops of 0.1 % phenolphthalein were added.
5. This was followed by titration with 0.5 N HCl and fresh tube containing 15 ml NaOH immersed in the soil-containing flask for further observation.

Calculation

$\text{CO}_2 \text{ evolution (mg CO}_2 \text{ 100g}^{-1} \text{ soil day}^{-1}) = \text{Volume of NaOH taken} - (\text{B}-\text{S}) \times 5.5$

B is the volume of 0.5N HCl required to neutralize NaOH in blank
S is the volume of 0.5N HCl required to neutralize NaOH in sample.

2 Moles of NaOH = 1 Mole of CO₂.; 0.5 N NaOH = 5.5 mg of CO₂.

Microbial biomass

The microbial biomass estimation was done as per method given by Vance et al. (1987).

Apparatus

Vaccum dessicator, vaccum pump, separatory funnel.

Reagents

1. Distilled chloroform.

2. Concentrated sulphuric acid.
3. 0.5 M K_2SO_4 : 43.563 g of K_2SO_4 dissolved in distilled water and diluted to 500 ml.
4. Whatman No.1 filter paper.
5. 0.2 N $K_2Cr_2O_7$: 0.9808 g of $K_2Cr_2O_7$ in distilled water and dilute to 100 ml.
6. Orthophosphoric acid.
7. 0.005 N ferrous ammonium sulphate (FAS) : 3.92 grams of FAS and 0.15 ml of H_2SO_4 in distilled water and dilute to 2 l.
8. Ferroin indicator.

Procedure

1. The soil samples were placed in a plastic bag to prevent drying.
2. Sets of 10g soil samples were made and soil water content was measured in one portion of the sets.
3. Out of remaining portion, half were kept in beakers for fumigation and the remainder packed in a refrigerator at 4°C for extraction the next day.
4. 20 ml of chloroform was taken for each 10 g of soil in a separatory funnel and it was washed twice with concentrated sulphuric acid (half the volume of chloroform), discarding the acid (bottom phase) after phase separation.
5. Washing was repeated done using distilled water twice (same volume) to make the chloroform free from ethanol and the bottom whitish phase was collected.
6. Glass beads were placed in the beakers containing chloroform to prevent bumping and these along with soil samples were placed in a vacuum dessicator.
7. The dessicator was connected to a vacuum pump and switched on until the chloroform boiled for about five minutes.
8. The outlet was closed and dessicator incubated in the dark for 24 hours.
9. After 24 hours the vacuum was released carefully with a 5-6 times back suction to remove any excess/adhered chloroform vapour.

10. The unfumigated sample was thawed.
11. The fumigated and unfumigated soils were transferred to 250 ml conical flasks followed by addition of 25 ml of 0.5 M K₂SO₄.
12. The samples were kept on a shaker for 30 minutes and the suspension was filtered through Whatman No.1 filter paper.
13. 10 ml of filtrate was transferred to a 500 ml conical flask and 2 ml of 0.2 N K₂Cr₂O₇ was added.
14. 10 ml concentrated H₂SO₄ and 5 ml of orthophosphoric acid was added to each flask.
15. The flasks were kept on hot plate at 100°C for half an hour under refluxing conditions followed by addition of 250 ml of distilled water immediately.
16. 2-3 drops of ferroin indicator were added and the sample titrated against 0.005 N FAS to obtain a brick-red end point.

Calculation

1. Soil water content (WS):

$$WS (\%) = \frac{\text{Weight of wet soil (g)} - \text{Weight of oven-dry soil (g)}}{\text{Weight of oven-dry soil (g)}} \times 100$$

2. Weight of soil sample (oven-dry weight equivalent) taken for microbial biomass measurement (MS):

$$MS (\text{g}) = \frac{\text{Weight of wet soil (g)}}{[100 + WS (\%)]} \times 100$$

3. Total volume of solution in extracted soil (VS):

$$VS (\text{ml}) = \text{wet soil weight (g)} - \text{oven-dry soil weight (g)} + \text{extractant volume (ml)}.$$

4. Determination of extractable carbon (EC in mg ml⁻¹)

- 4.1. Standardisation of FAS solution:

$$\text{Normality of FAS (x N)} =$$

$$\text{Vol. of K}_2\text{Cr}_2\text{O}_7 \text{ (1 ml)} \times \text{Strength of K}_2\text{Cr}_2\text{O}_7 \quad (0.2 \text{ N})$$

$$\text{Average titre value for the blank (ml)}$$

4.2. Volume of $\text{K}_2\text{Cr}_2\text{O}_7$ solution consumed by FAS in any sample (Y ml):

$$\frac{\text{Normality of FAS (xN)} \times \text{Titre value (ml)}}{\text{Normality of K}_2\text{Cr}_2\text{O}_7 \text{ (0.2 N)}}$$

4.3. Volume of $\text{K}_2\text{Cr}_2\text{O}_7$ consumed for oxidizing easily mineralisable C in 10 ml of extractant = 2-Y ml.

4.4 Extractable C (EC) in $\mu\text{g ml}^{-1}$:

(2-Y) ml of 0.2 N $\text{K}_2\text{Cr}_2\text{O}_7$ oxidises $600 \times (2-Y) \mu\text{g}$ of C

Amount of extractable C (EC):

$$\text{EC } (\mu\text{g ml}^{-1}) = \frac{600 \times (2-Y)}{10}$$

5. Total weight of extractable C in the fumigated (EC_F) and unfumigated (EC_{UF}) soil samples:

$$\text{EC}_F \text{ or } \text{EC}_{UF} (\mu\text{g g}^{-1} \text{ soil}) = \text{EC } (\mu\text{g ml}^{-1}) \times \text{VS (ml)} / \text{MS (g)}$$

6. Microbial biomass carbon in soil (MB-C):

$$\text{B-C } (\mu\text{g g}^{-1} \text{ soil}) = (\text{EC}_F - \text{EC}_{UF}) / K_{EC}$$

$K_{EC} = 0.25$, the efficiency of extraction of microbial biomass carbon.

Enumeration of bacteria

Bacterial enumeration on nutrient agar

Bacterial counts were carried out according to the standard plate count method on Nutrient agar (Cappuccino, 1987).

Nutrient Agar (g l⁻¹)

Peptone

5.0g

Sodium chloride	5.0g
Beef extract	1.5g
Yeast extract	1.5g
pH	7.0

Procedure

1. 1g of soil was added to 10 ml water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml water making a dilution corresponding to 10^{-1} .
3. Further dilutions were prepared in a similar way up to 10^{-6} .
4. 100 μ l inoculum was taken from dilution 10^{-5} and 10^{-6} and added to the petri plate and the inoculum was spread with the help of a sterilised spreader.
5. The plates containing inoculum were incubated at 30°C for 24 hours and colony-forming units were counted.

Enumeration on free-living nitrogen fixing bacteria

Free-living nitrogen fixers were enumerated on Jensen's medium (Jensen, 1954).

Jensens Medium (g l^{-1})

Sucrose	20.0g
K_2HPO_4	1.0g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5g
NaCl	0.2g
CaCO_3	2.0g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1g
pH	7.5
Agar	15.0 g

Procedure

1. 1g of soil was added to a 10 ml water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml water making a dilution corresponding to 10^{-1} .
3. Further dilutions were prepared in a similar way upto 10^{-6} .
4. 100 μ l inoculum was taken from dilution 10^{-2} and 10^{-3} and added to the petri plate and the inoculum was spread with the help of a spreader.
5. The plates containing inoculum were incubated at 30°C for 24 hours and colony-forming units were counted.

Enumeration and isolation of phosphate solubilizing bacteria

The phosphate-solubilizing bacteria were screened and isolated on Pikovskya media (Pikovskya, 1948).

Pikovskya medium (g l^{-1})

Glucose	10.0g
Tri calcium phosphate	5.0g
$(\text{NH}_4)_2 \text{SO}_4$	0.5g
NaCl	0.2g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1g
KCl	0.2g
Yeast extract	0.5g
MnSO_4	0.025g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.020g
Agar	15g
pH	7 ± 0.2

Procedure

1. 1g of soil was added to a 10 ml water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml water making a dilution corresponding to 10^{-1} .
3. Further dilutions were prepared in a similar way upto 10^{-6} .
4. 100 μ l inoculum was taken from dilution 10^{-3} and 10^{-4} and added to nutrient agar plates and the inoculum was spread with the help of a spreader.
5. The plates containing inoculum were incubated at 30°C for 24 hours and observations recorded.
6. The single colonies were streaked repeatedly on nutrient agar and Pikovskya medium for isolation of phosphate-solubilizing bacteria.
7. Bromophenol blue (0.01%) was added to the Pikovskya medium and the colonies were streaked on petri plates for obtaining zones of P-solubilization after a 24 hour incubation at 30°C.

Enumeration of E.coli S-17 and S2 transformant

Bacterial counts in soil with and without fly ash was carried out as per the standard plate count agar method for *E.coli S-17 lac Z⁺* and transformant S2: pMMB277 on TY media containing (50 μ g ml⁻¹) kanamycin and nutrient agar containing 10 μ g ml⁻¹ chloramphenicol (Cappuccino, 1987).

TY media (g l⁻¹)

Bactotryptone	2.5g
Yeast extract	1.5g
Calcium chloride	0.33g
Agar	9g
pH	7.0

Requirements: Kanamycin containing TY media, (v/v) fly ash amended soil (0, 10 & 30%), IPTG+X-gal + kanamycin containing TY plates, chloramphenicol + IPTG+X-gal containing nutrient agar plates.

Preparation of stock solutions

IPTG (Isopropyl-β-D-thiogalactopyranoside) :

100 mg ml⁻¹ of sterile water and filter sterilized by 0.22μ disposable filter.

X-gal (5-Bromo-4-chloro-3 indolyl-β-D-thiogalactopyranoside) :

20 mg ml⁻¹ of dimethylformamide wrapped in aluminium foil and stored at - 20°C.

Kanamycin Stock solution (10 mg ml⁻¹) and working solution (50μg ml⁻¹)

Chloramphenicol Stock solution (10 mg ml⁻¹) and working solution (10μg ml⁻¹)

Procedure

1. Soil amended with 0, 10 and 30% fly ash (after nursery trial) were divided into two sets of 50 g and 400 g each, the former used in studies with E.coli S-17 and the latter for S2: pMMB277.
2. One set was sterilized by autoclaving for four hours and the other set was kept under non-sterile conditions.
3. E.coli S-17 and S2: pMMB277 were grown in 5 ml of kanamycin and chloramphenicol containing TY and nutrient broth.
4. The absorbance of the cultures was measured the following day at 600 nm followed by centrifugation at 7000 r.p.m. for 15 minutes.
5. The supernatant was removed and the pellet was washed with 0.85 N saline followed by vortexing.
6. The suspension was washed thrice with saline repeatedly and finally the pellet was suspended in sterile water and inoculated in the soil.
7. E.coli S-17 and S2: pMMB277 was inoculated in both sterile and non-sterile soil and uninoculated soil was run as a control with each set.
8. IPTG (8 μl) and X-gal (30μl) was spread on to the TY and nutrient agar plates and left undisturbed for half an hour.

- 100- μ l inoculum from the sterile and non-sterile soils was added to the plates and enumeration studies carried out over a number of days.

Estimation of phosphate solubilization activity by the bacterial isolates

The phosphate solubilizing bacterial isolates were studied for the efficiency of percentage solubilization of tricalcium phosphate (Gaur, 1990).

Reagents

- Chloromolybdic acid: 15.0 g of ammonium molybdate dissolved in about 400 ml of warm distilled water followed by addition of 400 ml of 10N HCl or 342 ml of 12N HCl. The solution was stirred, cooled and volume made up to 1l with water.
- Chlorostannous acid: 10.0g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ crystals dissolved in 25 ml of concentrated HCl and kept in an airtight bottle. The solution was freshly prepared by taking 1 ml of the solution and 132 ml of distilled water.
- Standard P solution (100 ppm): 0.439 g of dried KH_2PO_4 was dissolved in 400 ml of distilled water and 25 ml of 7N H_2SO_4 was made up to 1l.
- Working P solution (10 ppm): 10 ml of standard P solution was diluted to 100 ml with distilled water.

Procedure

- 200 μ l of bacterial filtrate (obtained from each of the phosphate-solubilizing bacterial isolate) growing under shaker conditions at 28°C was transferred to 50 ml volumetric flask.
- 10 ml of chloromolybdic acid was added along the sides of the flask and the contents were diluted to 40 ml.
- Chlorostannous acid was added to the flask (1 ml) and the volume was immediately made up to 50 ml. The blue colour intensity was measured at 690_{nm}.
- To prepare a standard curve, 0.0, 0.5, 2.5, 5.0, 7.5 and 10 ml of 10 ppm solution was taken in 50 ml volumetric flasks and steps 2 to 4 were followed.

Calculation

TCP solubilized by bacterial strain = x ppm

TCP solubilized in control = y ppm

The percent solubilization of tricalcium phosphate (TCP): $\frac{x-y}{x} \times 100$

Gram staining

Gram staining of phosphate solubilizing bacterial isolates S2, S3 and S5 was done and the strains were analysed microscopically (Gram, 1884).

Requirements

1. Aqueous Crystal Violet (1%) : 1 g crystal violet in 100 ml distilled water.
2. Gram's Iodine : 2 g potassium iodide in 100 ml distilled water.
3. Decolorizer : acetone (50%) and alcohol (50%).
4. Aqueous safranin (2%) : 2 g safranin in 100 ml distilled water.

Procedure

1. Preparation of a fixed bacterial smear: A drop of bacterial culture was placed on a slide with the help of an inoculation needle spread evenly in the center of the slide. The smear was dried and heat-fixed.
2. The slide was placed on a staining rack and flooded with crystal violet for about 1 min.
3. The stain was washed gently with iodine solution and stained with fresh iodine solution for 1 min followed by washing in tap water or by dipping in a beaker containing water.
4. Few drops of decolorizer were added and continued until colour ceased to come out of the preparation. This took 5 seconds to 1 minute.
5. Washing repeated gently with water as in step 4.
6. Counter-stained with dilute carbol fuchsin or safranin for 10-30 seconds.
7. *Again the slide was washed with water and dried with absorbent paper and left for drying by evaporation.*

8. The dry slide is a permanent preparation, which was examined under the microscope directly without a cover slip first under low power and then under higher magnification.

Growth curve

The growth curve of phosphate solubilizing bacterial isolates S2, S3 and S5 was studied by plotting the absorbances of cultures at hourly intervals as a function of time (Cappuccino, 1987).

Requirement

1. Overnight grown bacterial cultures S2, S3 and S5.
2. Test tubes containing nutrient broth.
3. Mechanical shaker
4. Cuvettes.
5. Autopipettes.
6. Spectrophotometer.

Procedure

1. The overnight grown bacterial culture each of S2, S3 & S5 (in triplicates) was checked for absorbance at 600_{nm} and a zero hour reading was taken.
2. The culture was inoculated in the test tubes containing approximately 10 ml nutrient broth and the tubes were placed on the shaker.
3. The absorbance was measured after every 1 hour and the process continued upto 30 hours.
4. The growth of isolates was measured as a function of time by plotting the absorbance against each hour.

Antibiotic profiling of bacterial isolates

Antibiotic profiling of bacterial isolates S1, S2, S3, S4 and S5 was carried out by using standard antibiotics (Cappuccino, 1987).

Requirements

Phosphate-solubilizing bacteria S1, S2, S3, S4 and S5, kanamycin, streptomycin, nalidixic acid and chloramphenicol.

Stock solutions

Antibiotics: 10 mg ml⁻¹

Working solution

Streptomycin	50 µg ml ⁻¹
Kanamycin	50 µg ml ⁻¹
Nalidixic acid	50 µg ml ⁻¹

Chloramphenicol $10 \mu\text{g ml}^{-1}$

TY and Nutrient Agar medium

Procedure

1. 50 ml media was dispensed in 10 ml flasks (250 ml) and broth in test tubes (10 ml each).
2. Working solutions of antibiotics were made and filter-sterilized in the laminar flow cabin.
3. Two plates each of control, Str 50, Kan 50, Nal 50 were prepared.
4. The five selected cultures S1, S2, S3, S4 and S5 were inoculated in 5ml broth and kept for overnight growth at 30°C .
5. The absorbance of the cultures was checked at 600_{nm} the following day after which each culture was spotted on antibiotic-containing medium by dividing each petri plate into five sections.
6. The plates were left undisturbed for an hour followed by incubation at 30°C for 24-48 hours.

Plasmid isolation

Plasmid pMMB277 was isolated from E.coli 2842 by the alkali lysis method (Brinboim and Doly, 1979).

Alkali lysis

Solutions used

Solution I	50mM glucose, 25 mM Tris-Cl, 10 mM EDTA, pH 8.0.
Solution II	0.2 N NaOH, 1% w/v SDS.
Solution III	60 ml 5M potassium acetate, 11.5 ml glacial acetic acid, 28.5 ml water and stored at 4°C .
Lysozyme	10 mg ml^{-1} .
1X TE Buffer	10mM Tris-Cl, 1mM EDTA.

Procedure

1. Cells were harvested from 1.5 ml of exponentially grown bacterial culture by centrifugation at 12000 r.p.m. for 2 minutes after measuring the absorbance (O.D. $600_{nm}=1.0$). The supernatant was discarded and the bacterial pellet air-dried.
2. 200 μ l of ice-cold solution I was added immediately to the dried pellet followed by 50 μ l lysozyme.
3. The culture was kept on an ice bath for five minutes and 400 μ l of freshly prepared solution II was added and mixed gently.
4. 300 μ l of ice-cold solution III was added followed by reincubation in ice for 20-60 minutes with intermittent mixing.
5. Centrifugation was conducted further at 12000 r.p.m. for 10-15 minutes and 800 μ l of supernatant was collected in a fresh eppendorf tube. Equal volumes of isopropanol and supernatant were mixed.
6. The mixture was incubated at 4°C for 10-15 minutes followed by centrifugation at 10,000 r.p.m. for 15 minutes.
7. The supernatant was discarded and the pellet air-dried followed by addition of 30 μ l TE buffer and stored at 4°C until further use.

Solvent extraction

1. RNase was activated for 8-10 minutes at 100°C and slowly cooled to room temperature after which 1-2 μ l was added to the eppendorf tube and mixed by vortexing. The culture was centrifuged at the rate of 30 seconds /100 r.p.m. followed by incubation at 37 °C for one hour.
2. Equal volumes of phenol and chloroform (250 μ l: 250 μ l) were added to the eppendorf tube and mixed well. Centrifugation was carried out at 10000 g for 10 minutes.
3. The aqueous layer was separated in a fresh eppendorf tube and equal volume of chloroform was added followed by centrifugation at 10000 r.p.m. for 10 minutes.

4. The aqueous layer was separated and 50 μ l of 0.3 M CH_3COONa (1/10 of the volume) was added followed by mixing of an equal volume of isopropanol. The culture was stored at 4°C for 20-25 minutes.
5. Centrifugation was carried out at 10000 r.p.m. for 10 minutes and the supernatant was removed. The pellet was rinsed with 1 ml of 70% ethanol at 4°C and air-dried.
6. The pellet was redissolved in 50 μ l of TE (pH 8.0) and stored at -20°C followed by gel electrophoresis using 0.7% agarose.

Transformation

Transformation of phosphate-solubilizing bacterial isolates with plasmid pMMB277 of *E.coli* 2842 was carried out by the calcium chloride method (Mendel and Higa, 1970).

1. Overnight grown bacteria were reinoculated in 25 ml of fresh nutrient broth and incubated at 37°C for two and half hours followed by incubation in ice.
2. The cells were harvested by centrifugation at 7000 r.p.m. for 8 minutes at -4°C and resuspended in 100mM CaCl_2 and kept for 10-20 minutes in ice.
3. The step was repeated and cells were resuspended in ice chilled CaCl_2 and kept at -4°C for two and half hours to make the competent cells.
4. 100 μ l of competent cells were transferred to a sterile eppendorf tube and 10 μ l of plasmid DNA was added, mixed and kept on ice for 30 minutes.
5. Heat shock treatment was given next in the water bath at 42°C for 2 minutes and 1.0ml of fresh nutrient broth was added followed by incubation for one hour at 37°C.
6. The cells were harvested by centrifugation at 6000 r.p.m. for 6 minutes.
7. 100 μ l of concentrated cell suspension was plated on chloramphenicol (10 μ g ml^{-1}) plus IPTG and X-gal containing nutrient agar plates and the transformant checked for their β -galactosidase expression.

Kado lysis

The transformation process yielded positive results only for the phosphate-solubilizing bacterial isolate S2 from which the plasmid was further isolated by Kado lysis (Kado and Liu, 1981) followed by plasmid purification.

Reagents

TE buffer 40Mm Tris-acetate, 2mM EDTA, pH 7.9 adjusted with GAA.

Lysis buffer 3% SDS, 50 mM Tris, pH 12.5 adjusted with 2 N NaOH.

Procedure

1. Exponentially grown 1.5 ml bacterial culture (O.D $600_{nm}=1.0$) was harvested and the pellet washed with 1 ml TE buffer.
2. The pellet was resuspended in 100 μ l of TE buffer and 200 μ l of lysis buffer.
3. The cell suspension was incubated at 65°C for 30 minutes to obtain a clear solution.
4. Double volumes of phenol, chloroform and iso-amyl alcohol solution (24:24:1) were added and mixed gently by inverting the tubes for a few minutes.
5. The mixture was centrifuged at 10000 r.p.m. for 15 minutes at 4°C and the upper aqueous layer was transferred carefully to a fresh eppendorf tube.
6. 35 μ l of the aqueous layer along with 10 μ l of loading dye was loaded in a 0.7% agarose gel and run in an electrophoresis unit at 40V at low temperature up to two-thirds of gel length.
7. The bands were observed under UV light after staining the gel with ethidium bromide (2 μ g ml⁻¹) after which plasmid purification was carried out using low melting agarose gel.

8. Slices of gel containing DNA bands were melted at 65°C and mixed with equal volume of phenol, followed by centrifugation at 8000 r.p.m. for 8 minutes.
9. The upper aqueous layer (200 µl) was collected in a fresh eppendorf tube and the residue re-extracted with 300 µl STE buffer (by centrifugation at 8000 r.p.m. for 8 minutes).
10. Equal volumes of iso-propanol and supernatant were mixed followed by incubation at 4°C followed by centrifugation at 10000 r.p.m. for 20 minutes.
11. The pellet was dissolved in 30 µl of 1X TE buffer (10Mm Tris-Cl, 1 mM EDTA) and stored at- 20°C for further use.

6. Biometric data collection

Nursery trial

At the end of the nursery trial of *Populus deltoides* the plants were gently uprooted and washed in normal tap water to remove any adhering soil particles. Thereafter the plants were dried with blotting sheets and measured for fresh weight and dry weight. For dry weight the plants were kept overnight in an oven at 80°C and the weight was taken the following day. In order to study metal uptake by the plants, they were oven-dried overnight at 80°C. Leaves and stem were separated and crushed in a mixer grinder after which they were sieved through 0.2 mm sieve to obtain a fine powder form. The powder was digested using concentrated nitric and perchloric acid for metal analysis using atomic absorption spectrophotometry as discussed in section 5.1.

Field trial

Survival and growth parameters, i.e., height and collar diameter of the inner block of nine plants in three replications for *Acacia auriculiformis* and *Eucalyptus tereticornis* were recorded and analysed statistically using Graph pad software.

7. Statistical analysis

The various statistical parameters were analysed as per the methods given by Rao, 1996 using Graph pad Prism Software 2.01 and Microsoft Excel.

Coefficient of dispersion or variation

To compare the variability of two series, which differ widely in their averages, a relative measure of dispersion is used which is known as coefficient of variation or dispersion. The ratio of a measure of dispersion to an average will give the coefficient of dispersion.

Coefficient of dispersion is defined as:

- | | | | |
|------|--|-----|--|
| i. | $\frac{\text{Mean deviation}}{\text{Median}} \times 100$ | ii. | $\frac{\text{Mean deviation}}{\text{Mean}} \times 100$ |
| iii. | $\frac{\text{Quartile deviation}}{\text{Median}} \times 100$ | iv. | $\frac{\text{Standard deviation}}{\text{Mean}} \times 100$ |

where the fourth definition is the well-known coefficient of variation (CV). When the variability of two series is compared, the series having greater CV is said to have greater variation than the other and the series having lower CV is said to be more homogenous than the other.

Variance

The variance is measured as the square of the units in which the variable X is measured. For example, if X is the height in centimeters (cm), the variance will be measured in cm² (square centimeters). The formula for variance is:

$$\text{Variance} = \frac{\sum (X_i - \bar{X})^2}{n} = \frac{\sum X_i^2 - n\bar{X}^2}{n}$$

= Sum of the squares of the deviations of individual values from the mean \bar{x} ÷ sample size

where n is the number of observations; \bar{x} is the arithmetic mean of the observations of X₂s are the individual observations – x₁, x₂, x_i, x_n.

Standard Deviation

It is convenient to have a measure of variation expressed in the original units of X and this can be done by taking the square root of the variance. This quantity is known as the standard deviation and is,

$$SD = \sqrt{\text{Variance.}}$$

Standard Error

The standard error (SE) is a measure of the variation or dispersion of the means of a set of measurements. It is, therefore, smaller than the standard deviation of a single series of measurements from the same of population. It is used to compare means with one another.

$$\begin{aligned} \text{Formula for } S^2 &= \text{variance} = \sum (X_i - \bar{X})^2 / (n-1) \\ &= (\sum X_i^2 - n\bar{X}^2) / (n-1) = (\text{SS-CF}) \div (n-1) \end{aligned}$$

$$\text{Standard deviation} = \text{square root of variance} = \sqrt{S^2}$$

$$\text{Standard error} = \sqrt{(\text{variance/sample size})} = \sqrt{(S^2/n)}$$

Standard error is the standard deviation of the means of measurements. It is an indication of the magnitude of variation between sample mean values. Standard error is also called the standard deviation of the mean.

Two-way classification – two way ANOVA

Two-way analysis of variance is utilized when there is a need to study the impact of two factors on variations in a specific variable. When the effect of two factors on any variable is to be studied, two way analysis of variance is utilized. The assumptions made in this type of ANOVA are that (i) the subject must be chosen at random; (ii) the variable under study must have normality characteristics (i.e., coefficient of skewness is equal to zero and coefficient of kurtosis is equal to three); (iii) variances between comparable groups are mostly same or homogenous and (iv) there is no interaction between the two factors.

Two-way ANOVA is utilized for the experimental designs like the randomized complete block design. Data from an experiment utilizing the randomized complete block design is displayed in the following tables.

X_{ij} is the observation with i th block and j th treatment.

There are k treatments and n blocks

Total number of observations are = $Kn = N$

The total of the i th block = $T_i = \sum_{j=1}^k x_{ij}$

The total of the j th treatment $T_j = \sum_{i=1}^n x_{ij}$

The grand total = $T = T_{..} = \sum_{i=1}^n T_i = \sum_{j=1}^k T_j$, which indicates that the grand total can be obtained either by adding row totals or by adding column totals.

	Sample values					Sample size	Total	mean
	Treatments							
Blocks	1	2	3	...	k			
$i.$	x_{i1}	x_{i2}	x_{i3}	...	x_{ik}	k	$T_{i.}$	$\overline{x_{i.}}$
$ii.$	x_{21}	x_{22}	x_{23}	...	x_{2k}	k	$T_{2.}$	$\overline{x_{2.}}$
$iii.$	x_{31}	x_{32}	x_{33}	...	x_{3k}	k	$T_{3.}$	$\overline{x_{3.}}$
\cdot	\cdot	\cdot	\cdot	...	\cdot	\cdot	\cdot	\cdot
\cdot	\cdot	\cdot	\cdot	...	\cdot	\cdot	\cdot	\cdot
n	x_{n1}	x_{n2}	x_{n3}	...	x_{nk}	k	$T_{n.}$	$\overline{x_{n.}}$
Sample size	n	n	n	...	n		$nk=N$	
Total	$T_{.1}$	$T_{.2}$	$T_{.3}$...	$T_{.k}$		$T_{..}$	
Mean	$\overline{x_{.1}}$	$\overline{x_{.2}}$	$\overline{x_{.3}}$...	$\overline{x_{.k}}$			$\overline{x_{..}}$

$$\sum_{i=1}^n T_i = T_{.1} + T_{.2} + \dots + T_{.n} = T_{..}$$

$$\sum_{j=1}^k T_j = T_{.1} + T_{.2} + \dots + T_{.n} = T_{..}$$

The mean of the i th block = $\frac{\text{total of the } i\text{th block}}{\text{sample size}} = \frac{T_{i.}}{k} = \frac{\sum_j x_{ij}}{k}$

The mean of the j th treatment = $\frac{\text{total of the } j\text{th block}}{\text{sample size}} = \frac{T_{.j}}{k} = \frac{\sum_i x_{ij}}{k}$

Calculations needed for the two way ANOVA or for the randomized complete block design are the following :

Total sum of square (SS_{total}) = sum of squares of blocks (SS_{blocks})
 + sum of squares of treatment ($SS_{\text{treatments}}$)
 + sum of squares
 of residual (SS_{residual})

i.e., $(SS_{\text{total}}) = (SS_{\text{blocks}}) + (SS_{\text{treatments}}) + (SS_{\text{residual}})$

$$\sum_{i=1}^n \sum_{j=1}^k (x_{ij} - \bar{x}_{..})^2 = \text{total SS}; \sum_{i=1}^n \sum_{j=1}^k (\bar{x}_{.j} - \bar{x}_{..})^2 = \text{treatments SS}$$

$$\sum_{i=1}^n \sum_{j=1}^k (\bar{x}_{i.} - \bar{x}_{..})^2 = \text{block SS};$$

$$\sum_{i=1}^n \sum_{j=1}^k ((x_{ij} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{x}_{..})^2 = \text{residual SS.}$$

The computation formula when simplified are :

$$(SS_{\text{total}}) = \sum_{i=1}^n \sum_{j=1}^k (x_{ij} - \bar{x}_{..})^2 = \sum_{i=1}^n \sum_{j=1}^k x^2_{ij} - C$$

$$(SS_{\text{treatments}}) = \sum_{i=1}^n \sum_{j=1}^k (\bar{x}_{.j} - \bar{x}_{..})^2 = \sum_{i=1}^n \sum_{j=1}^k x^2_{.j} - C = \sum_{j=1}^k \frac{T_j^2}{n} - C$$

$$(SS_{\text{blocks}}) = \sum_{i=1}^n \sum_{j=1}^k (\bar{x}_{i.} - \bar{x}_{..})^2 = \sum_{i=1}^n \sum_{j=1}^k x^2_{i.} - C = \sum_{i=1}^n \frac{T_i^2}{k} - C$$

$$(SS_{\text{residual}}) = SS_{\text{total}} - SS_{\text{blocks}} - SS_{\text{treatments}}$$

$$C = \left(\sum_{i=1}^n \sum_{j=1}^k x_{ij} \right) \div N = \frac{\left(\sum_{j=1}^k T_j \right)^2}{N} = \frac{\left(\sum_{i=1}^n T_i \right)^2}{N} = \frac{T^2}{N} = \frac{T^2}{kn}$$

The appropriate degrees of freedom for each of the sums of squares are :

$$\text{Total} = N - 1 = kn - 1$$

$$\text{Blocks} = \text{number of blocks} - 1 = n - 1$$

Treatments = number of treatments - 1 = k-1
Residual = kn-1- (n-1) - (k-1) = kn-1-n+1-k+1=kn-n-k+1
= n(k-1) - 1(k-1) = (n-1) (k-1).

Degrees of freedom of total = degrees of freedom of [blocks + treatments + residual].

TWO WAY ANOVA TABLE

S.No.	Source	Sum of squares	Degrees of freedom	Mean sum of squares (MSS)	Variance ratio "F"
i.	Blocks	SS _{blocks}	(n-1)	MS _{blocks} = $\frac{SS_{blocks}}{n-1}$	$F_1 = \frac{MS_{blocks}}{MS_{residual}}$
ii.	Treatments	SS _{treatments}	(k-1)	MS _{treatments} = $\frac{SS_{treatments}}{k-1}$	$F_1 = \frac{MS_{treatments}}{MS_{residual}}$
iii.	Residual or	SS _{residual}	(n-1) (k-1)	MS _{residual} = $\frac{SS_{residual}}{(n-1)(k-1)}$	
iv.	Total	SS _{total}	(nk-1) = (N-1)		

F₁ = Variance ratio for blocks with df of (n-1) Vs (n-1) (k-1)

F₂ = Variance ratio for treatments with df of (k-1) vs (n-1) (k-1)

The values can be compared with F values for their degrees of freedom at 5 percent or 1 percent levels of significance.

If the calculated values are higher than their critical values at the 5 percent or 1 percent level, it is an indication for significance.

If the calculated values are lower than their critical values for their degrees of freedom, it is an indication for significance differences.

i.e., if $F_1 > F_{0.05}$ then probability of significance is $P < 0.05$.

if $F_1 > F_{0.01}$ then probability of significance is $P < 0.01$.

if $F_1 > F_{0.05}$ then probability of significance is $P < 0.05$ (not significant).

if $F_2 > F_{0.05}$ then probability of significance is $P < 0.05$.

if $F_2 > F_{0.05}$ then probability of significance is $P < 0.05$ (not significant).

Results

1. Characterization of fly ash for its physicochemical properties.

Chemical properties of fly ash

The main source of chemical elements in fly ash is coal, which contains almost all the naturally occurring elements; thus the characterization of fly ash provides useful information for its economic utilization. The ESP fly ash from various sources used in the study was either acidic or alkaline in nature, had low bulk density and was rich in nutrients (Table 1b).

pH and Electrical conductivity

The pH of ESP fly ash from BILT, Choudwar and GGSTPP, Ropar was in acidic range (5.28 to 5.30) while the ESP ash from Yamunanagar was alkaline with pH 7.53 (Table 1b). The electrical conductivity was $1140 \mu\text{S cm}^{-1}$ of the ESP fly ash from Choudwar and 50 to $52 \mu\text{S cm}^{-1}$ of ESP fly ash from Ropar and Yamunanagar.

Table 1b. Chemical characterization of ESP fly ash procured from different sources used in nursery, field and laboratory trials.

Element	Fly ash (Literature values) *	ESP ash (Electrostatic precipitator ash)		
		BILT ¹ , Choudwar	GGSTPP, Ropar	BILT, Yamunanagar
pH		5.30	5.28	7.53
EC ($\mu\text{S cm}^{-1}$)		1140	50	52
Major Elements in %				
N	-	0.19	0.20	0.30
P	0.04-0.8	0.108	0.224	0.292
K	0.15-3.5	0.107	0.0043	0.068
S	0.1-1.5	0.001	0.069	0.071
Ca	0.11-22.2	0.34	0.024	0.063
Mg	0.04-7.6	0.038	0.187	0.018
Na	0.01-2.03	0.041	0.0018	0.094
Fe	1-29	0.676	0.219	0.236
Trace elements in mg/kg				
Cu	14-2800	20	19.9	38.6
Zn (300)	10-3500	79	210	92.0
Mn	58-3000	739	530	105
Mo (2-40)	7-160	4.0	4.4	4.9
As (20-50)	2.3-6300	6.2	6.7	7.0
Se (5-10)	0.2-134	3.6	4.2	4.6
Pb (100)	3.1-5000	35	28.5	67.1
Ni (50)	6.3-4300	13	25.3	15.9
Cd	0.7-130	1.9	<0.009	6.73
Cr (10)	10-1000	330	49.4	33.3
Co	7-520	58	15.1	10.9
Radioactivity in Bq kg ⁻¹				
Ra ²²⁶ (370)	N.A.	100	-	-
Ac ²²⁸ (2-40)	N.A.	141	-	-

K ⁴⁰ (810-925)	N.A.	376	-	-
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(*Page et al., 1979, ¹Goyal et al., 2002).

(Values in parenthesis are permissible limits: Pendias and Pendias, 1984).

(N.A.: Not Available ; - : Not done)

Table 1c. Physical and microbiological properties of ESP fly ash procured from different sources.

	Source of ESP (Electrostatic precipitator) ash		
Physical properties	BILT, Choudwar	GGSTPP, Ropar	BILT, Yamunanagar
Water holding capacity (%)	67	73	67.6
Bulk density (g cm ⁻³)	0.9	0.36	0.41
Hydraulic conductivity (cm min ⁻¹)	23.1	16	14
Particle size distribution (Hydrometer method)			
Sand %	85	87.5	84.6
Silt %	12	5.5	8.0
Clay %	3.0	7.0	7.4
Texture	Loamy sand	Loamy sand	Loamy sand
Microbiological properties			
Bacterial count (x10 ⁶ cfu g ⁻¹ fly ash)	0.0	0.0	0.0
Soil dehydrogenase activity (µgTPPg ⁻¹ fly ash day ⁻¹)	0.0	0.0	0.0
Organic carbon (%)	1.0	<i>n.d</i>	<i>n.d</i>

n.d: not detected

Essential elements (N, P, K)

Nitrogen content measured 0.19 to 0.30 % in the ESP ash from the three different sources (Table 1b). Phosphorus measured 0.108 % in ESP fly ash from Choudwar and ranged from 0.224 to 0.292 % in fly ash from Ropar and Yamunanagar. Potassium measured 0.0043% in the ESP ash from Ropar and ranged from 0.068 to 0.107 % in the ESP ash

from Choudwar and Yamunanagar. Phosphorus and potassium ranged from 0.107 to 0.108 % in the ESP ash of Choudwar, where phosphorus was within and potassium was less than the range reported in the literature (Table 1b). The concentration of these two essential elements corresponded to 0.108 and 0.107% and was highest among the three fly ash studied. Nitrogen measured 0.30% in ESP fly ash from Yamunanagar and phosphorus and potassium were 0.108 and 0.107 % respectively in fly ash from Choudwar, the highest recorded among the three fly ash samples studied. The overall concentration of the essential elements varied as N>P>K in the fly ash from Choudwar and Yamunanagar (Table 1b).

Secondary elements (S, Mg, Ca, Na)

Sulphur measured 0.001% in the ESP ash from Choudwar and was the lowest among all the fly ash samples studied. Sulphur content varied from 0.069 to 0.071% in the fly ash from Ropar and Yamunanagar (Table 1b). Calcium was present at a concentration equal to 0.34 % in the ESP fly ash from Choudwar and ranged from 0.024 to 0.063 % in fly ash from Ropar and Yamunanagar. Magnesium measured 0.187% in the ESP ash from Ropar and ranged from 0.018 to 0.038 % in the ESP fly ash from Yamunanagar and Choudwar. Sodium measured 0.0018% and 0.094% in the ESP ash from Ropar and Yamunanagar whereas it measured 0.041% in fly ash from Choudwar. Sulphur in fly ash from all the three sources measured less than generally reported values of ESP ash in literature. Calcium, magnesium and sodium in ESP ash from Choudwar were within range as reported for fly ash in the literature (Table 1b). Calcium in ESP fly ash of Ropar and Yamunanagar was less than the reported values. Magnesium and sodium in ESP fly ash of Yamunanagar and Ropar were less than values generally reported in literature; however magnesium content in ESP fly ash of Ropar and sodium in fly ash of Yamunanagar was within range. Sulphur and sodium were highest in ESP fly ash of Yamunanagar. However magnesium and sodium were the dominant secondary elements in fly ash from Ropar and Choudwar, respectively. Sulphur measured higher than calcium in ESP fly ash from Ropar and Yamunanagar (Table 1b).

Micronutrients (Cu, Zn, Mn, Fe and Mo)

Iron measured 0.676 % in the ESP ash from Choudwar and was within range as reported in the literature. However, it ranged from 0.219 to 0.236 % in the ESP fly ash from Ropar and Yamunanagar and was below the generally reported values (Table 1b). Copper ranged from 19.9 to 20.0 mg/kg in fly ash from Choudwar and Ropar and was 38.6 mg/kg in fly ash from Yamunanagar. Zinc measured 210 mg/kg in ESP ash from Ropar and ranged from 79 to 92.0 mg/kg in ESP ash from Choudwar and Yamunanagar. Manganese was 739 mg/kg in the ESP ash from Choudwar and was the highest compared to 530 and 105 mg/kg in ESP fly ash from Ropar and Yamunanagar respectively. Copper, zinc and manganese in fly ash from all three sources were within range as reported in the literature. Molybdenum ranged from 4.0 to 4.9 mg/kg in all fly ash samples studied and was within permissible limits (Table 1b) and much lower than values reported in the literature. Iron and manganese were highest in fly ash from Choudwar among all three fly ashes studied. However copper and molybdenum were highest in ESP fly ash from Yamunanagar. Zinc measured highest in ESP fly ash from Ropar. Micronutrients in the ESP fly ash from the three sources varied as Fe>Mn>Zn>Cu>Mo (Table 1b).

Heavy metals (As, Se, Pb, Ni, Cd, Cr and Co)

Arsenic and selenium ranged from 6.2 to 7.0 and 3.6 to 4.6 mg/kg occurring within permissible limits in fly ash from three different sources (Table 1b). Lead measured 67.1 mg/kg in ESP fly ash from Yamunanagar and ranged from 28.5 to 35.0 mg/kg in fly ash from Choudwar and Ropar. Nickel ranged from 13.0 to 15.9 mg/kg in fly ash from Choudwar and Yamunanagar and 25.3 mg/kg in ESP ash from Ropar. Both lead and nickel in fly ash were within range as reported in the literature (Table 1b). Chromium was more than nickel and lead among the fly ashes studied corresponding to 330 mg/kg in ESP ash from Choudwar and ranged from 33.3 to 49.4 mg/kg in fly ash from Ropar and Yamunanagar occurring within range as reported for fly ash in the literature (Table 1b). Cobalt measured 58.0 mg/kg in ESP fly ash from Choudwar and ranged from 10.9 to 15.1 mg/kg in ESP ash from Ropar and Yamunanagar, falling within range as reported in the literature. Cadmium was below detection limits in ESP ash from Ropar and ranged from 1.9 and 6.73 mg/kg in the ESP ash from Choudwar and Yamunanagar (Table 1b). Chromium and cobalt were highest in fly ash from Choudwar corresponding to 330 and

58 mg/kg respectively. ESP ash of Ropar contained 210 mg/kg of zinc which was highest among the three fly ashes studied. Seven mg/kg arsenic, 4.6 mg/kg selenium, 67.1 mg/kg lead and 6.73 mg/kg cadmium were highest in ESP fly ash from Yamunanagar. Nickel and chromium in ESP fly ash from Ropar measured higher compared to ESP ash of Yamunanagar. Arsenic measured more than selenium and 67.1 mg/kg lead in ESP fly ash of Yamunanagar was highest among the three fly ashes studied. Radioactivity in ESP fly ash from Choudwar measured 100, 141 and 376 Bq kg⁻¹ for Ra ²²⁶, Ac ²²⁸ and K⁴⁰ respectively (Table 1b).

Physical properties

Bulk density

Bulk density of ESP ash from Choudwar measured 0.9 g cm⁻³ and ranged from 0.36 to 0.41 g cm⁻³ in fly ash from Ropar and Yamunanagar respectively (Table 1c).

Hydraulic conductivity

Hydraulic conductivity measured 23.1 cm min⁻¹ in ESP ash from Choudwar and ranged from 14 to 16 cm min⁻¹ in fly ash from Ropar and Yamunanagar (Table 1c).

Water holding capacity

Water holding capacity of fly ash from Choudwar and Yamunanagar was almost equivalent, corresponding to 67 and 67.6 % while it was 73 % for ESP ash from Ropar (Table 1c).

Particle size distribution and Texture

Sand ranged from 84.6 to 87.5 %, silt from 5.5 to 12.0 % and clay from 3.0 to 7.4 % in ESP ash from Choudwar, Ropar and Yamunanagar. The texture of ESP fly ash from all the three sources was loamy sand (Table 1c).

Microbiological properties

Soil dehydrogenase activity

No dehydrogenase activity was detected in the fly ash from the three different sources (Table 1c).

Bacterial count

No microorganisms were observed in the fly ash from various sources (Table 1c).

Organic carbon

Organic carbon measured in fly ash from Choudwar measured 1% and in ESP ash from Ropar and Yamunanagar it was not detected (Table 1c).

Table 2a. Chemical characterization of soil used in nursery, field and laboratory trials.

Element	Soil (Literature values)*	Our study	
		Acidic soil (Orissa) ¹	Alkaline soil (Patiala)
PH		5.82	7.63
EC ($\mu\text{S cm}^{-1}$)		80	59
Major elements in %			
N	0.01-1.0	0.11-0.14	0.39-0.44
P	0.005-0.2	0.009-0.013	0.02-0.04
K	0.04-3.0	0.033-0.035	0.001-0.003
S	0.01-2.0	0.0001-0.0002	1.10-1.12
Ca	0.7-50	0.10-0.14	0.02-0.06
Mg	0.06-0.6	0.043-0.044	0.14-0.16
Na	0.04-3.0	0.0112-0.0114	0.002-0.004
Fe	0.7-55	2.69-3.22	0.15-0.21
Trace elements in mg/kg			
Cu	2-100	4.5-7.5	<0.025
Zn (300)	10-300	67-80	25.0-45.3
Mn	100-4000	1033-1090	202-475
Mo (2-40)	0.2-5.0	2.6-3.0	3.2
As (20-50)	0.1-40	3.5	3.9
Se (5-10)	0.1-2.0	2.2-2.5	2.4
Pb (100)	2-100	153-163	18.2-25.2
Ni (50)	10-1000	10.7-56.0	19.0-31.4
Cd	0.01-7.0	1.4-2.1	<0.009
Cr (10)	5-3000	48-54	33.3-38.6
Co	1-40	81-135.5	5.9-17.1
Radioactivity in Bq kg ⁻¹			
Ra ²²⁶ (370)	N.A.	31	-
Ac ²²⁸ (2-40)	N.A.	75	-
K ⁴⁰ (810-925)	N.A.	260	-

(*Page et al., 1979; Goyal et al., 2002¹). (Values in parenthesis are permissible limits: Pendias and Pendias, 1984). (N.A.: Not available; - : Not done)

Table 2b. Physical characteristics and microbiological properties of acidic soil

<i>Physical properties</i>	<i>Acidic soil (Orissa)</i>	<i>Alkaline soil (Patiala)</i>
Water holding capacity (%)	45.0	33.6
Bulk density (g cm ⁻³)	1.3	0.87
Hydraulic conductivity (cm min ⁻¹)	14.8	21.0
<i>Particle size distribution (Hydrometer Method)</i>		
Sand %	5	67.1
Silt %	18	13.6
Clay %	77	19.1
Texture	Clay	Sandy loam
<i>Microbiological properties</i>		
Bacterial count (cfu g ⁻¹ soil)	< 10 ⁶	3.1x10 ⁶
Soil dehydrogenase activity (µgTPFg ⁻¹ soil day ⁻¹)	3.7	0.62
Organic carbon (%)	0.37	0.29

used in field trial and alkaline soil used in nursery and laboratory trials.

Chemical properties of soil

pH and electrical conductivity

pH of the Orissa and Patiala soils was 5.82 and 7.63 and the electrical conductivity was 80 and 59 $\mu\text{S cm}^{-1}$, respectively (Table 2a).

Essential elements (N, P, K)

Nitrogen ranged from 0.39 to 0.44 % in the alkaline soil, which was within the range generally found in common cropland soil (Table 2a). Phosphorus and potassium ranged from 0.009 to 0.013 and 0.033 to 0.035 % respectively in the acidic soil occurring within range as reported for cropland soil in the literature. Nitrogen and phosphorus were higher in the alkaline soil. Nitrogen was highest followed by potassium in the acidic soil and phosphorus in the alkaline soil.

Secondary elements (S, Mg, Ca, Na)

Sulphur and magnesium ranged from 1.10 to 1.12 and 0.14 to 0.16%, respectively, with a higher concentration in the alkaline soil falling within range as given in the literature (Table 2a). Calcium and sodium ranged from 0.10 to 0.14 and 0.011 to 0.0114% with higher concentrations in the acidic soil and both were below range as reported for normal cropland soil. In the acidic soil the concentrations ranged as $\text{Ca} > \text{Mg} > \text{Na} > \text{S}$ while in the alkaline soil the trend was $\text{S} > \text{Mg} > \text{Ca} > \text{Na}$ (Table 2a).

Micronutrients (Cu, Mn, Fe, Zn, Mo)

Iron in the acidic soil was greater compared to the alkaline soil and ranged from 2.69 to 3.22 %, which was within range as reported for normal cropland soil (Table 2a). Copper, zinc and manganese were also present at a higher concentration in the acidic soil, ranging from 4.5 to 7.5, 67 to 80 and 1033 to 1090 mg/kg respectively and were within range as observed in normal cropland soil. Molybdenum in the acidic and alkaline soils ranged from 2.6 to 3.2 mg/kg and was within permissible limits. All micronutrients except Mo were present at a higher concentration in the acidic soil and varied as $\text{Fe} > \text{Mn} > \text{Zn}$ in both the acidic and alkaline soils (Table 2a).

Heavy metals (As, Se, Pb, Ni, Cd, Cr and Co)

Arsenic and selenium ranged from 3.5 to 3.9 mg/kg and 2.3 to 2.4 mg/kg respectively in the acidic and alkaline soils at concentrations below permissible limits (Table 2a). Lead, nickel and chromium ranged from 153 to 163, 10.7 to 56.0 and 48 to 54 mg/kg respectively, in the acidic soil. Cobalt ranged from 81 to 135.5 and 5.9 to 17.1 mg/kg in the acidic and alkaline soil respectively. Both lead and cobalt exceeded the range as reported in the literature for normal cropland soil. Cadmium was below detection limits in the alkaline soil. All heavy metals except As and Se were greater in acidic soil. In both the acidic and alkaline soils $Pb > Co$ and $Cr > Ni$ with 158 mg/kg lead and 51 mg/kg chromium. In the acidic and alkaline soil the range was $As > Se > Cd$ respectively (Table 2a).

Physical properties

Bulk density

Bulk density of the acidic soil was 1.3 g cm^{-3} , which was much higher than that of alkaline soil at 0.87 g cm^{-3} (Table 2b).

Water holding capacity

Water holding capacity was 45 % in the acidic soil and 33.6 % in the alkaline soil (Table 2b).

Particle size distribution and texture

Sand, silt and clay percentage was 5, 18 and 77 % in the acidic soil while in the alkaline soil it was 67.1, 13.6 and 19.1% respectively. The texture of the acidic soil was clay and that of the alkaline soil was sandy loam (Table 2b).

Hydraulic conductivity

Hydraulic conductivity of the acidic and alkaline soil was 14.8 cm min^{-1} and 21.0 cm min^{-1} , respectively (Table 2b).

Microbiological properties

Soil dehydrogenase activity

Soil dehydrogenase activity was $3.7\mu\text{gTPFg}^{-1}\text{ soil day}^{-1}$ in the acidic soil and was lower in the alkaline soil corresponding to $0.62\mu\text{g TPFg}^{-1}\text{ soil day}^{-1}$ (Table 2b).

Bacterial counts

Bacterial counts in the acidic soil was less than 10^6 cfu g^{-1} soil and nitrogen-fixing bacteria were not detected. In the alkaline soil the bacterial count was $3.1\times 10^6\text{ cfu g}^{-1}$ soil (Table 2b).

Organic carbon

Organic carbon measured 0.37% in acidic soil and 0.29 % in the alkaline soil (Table 2b).

2. To study the effect of fly ash amendment in soil on soil microbial activity.

Rate of carbon-dioxide evolution, microbial biomass, soil dehydrogenase activity and total organic carbon are sensitive indicators of soil quality and sustainability in understanding the complexities of the nutrient profile in soils and therefore were examined to study the effect of fly ash on microbial activity. Laboratory trials were conducted in which ESP fly ash from Yamunanagar was mixed with alkaline soil of Patiala at different concentrations and further analysed.

Effect of fly ash on rate of carbon-dioxide evolution

Rate of carbon dioxide evolution ranged from 70.8 to 87.5 $\text{mg CO}_2\ 100\ \text{g}^{-1}\ \text{soil day}^{-1}$ in the soil amended with 0 to 30% fly ash (Table 3). There was a marginal decrease in the rate of CO_2 evolution with addition of fly ash up to 30 % In soil without fly ash CO_2 evolution on day 4 of incubation was $80.9\ \text{mg CO}_2\ 100\ \text{g}^{-1}\ \text{soil day}^{-1}$ and it decreased to $72.9\ \text{mg CO}_2\ 100\ \text{g}^{-1}\ \text{soil day}^{-1}$ in soil amended with 30% fly ash.

Table 3. Rate of CO_2 evolution ($\text{mg CO}_2\ 100\ \text{g}^{-1}\ \text{soil day}^{-1}$) in alkaline soil amended with 0-30% fly ash at room temperature.

	Fly ash (%)				
Day	0	5	10	20	30
0	81.9	82.2	76.5	79.6	77.9

1	83.6	82.5	75.9	82.0	79.7
2	81.9	87.5	78.4	83.6	82.1
3	81.4	80.3	81.1	75.9	77.2
4	80.9	78.7	70.8	77.0	72.9

Source of ESP fly ash: Yamunanagar

In another set of experiments carried out with soil mixed with 0, 25, 50 and 75% fly ash on v/v basis there was a drastic reduction of up to 20.9 mg CO₂ 100 g⁻¹ soil day⁻¹ in soil mixed with 75% fly ash on the second day of incubation (Table 4). The optimum concentration of fly ash was 25% which did not cause any major decrease for rate of CO₂ evolution which ranged from 72.1 to 80.8 mg CO₂ 100 g⁻¹ soil day⁻¹. The range of carbon-dioxide evolution was observed to vary from 21.4 mg CO₂ 100 g⁻¹ soil day⁻¹ in soil amended with 75% fly ash on day 4 of incubation to 82.5 mg CO₂ 100 g⁻¹ soil day⁻¹ in soil without fly ash.

Table 4. Rate of CO₂ evolution (mg CO₂ 100 g⁻¹ soil day⁻¹) in alkaline soil amended with 0-75% fly ash at room temperature.

Day	Fly ash (%)			
	0	25	50	75
0	82.0	77.6	39.8	23.7
1	78.9	80.0	49.2	25.6
2	84.9	80.8	39.7	20.9
3	81.9	77.5	31.7	26.9
4	82.5	72.1	38.5	21.4

Source of ESP fly ash: Yamunanagar

Effect of 5% fly ash amendment in soil with varying concentration of glucose on rate of carbon-dioxide evolution

The effect of 5% fly ash in soil mixed with varying concentrations of glucose was studied on rate of CO₂ evolution. Carbon-dioxide evolution was 80.6 mg CO₂ 100 g⁻¹ soil day⁻¹ on day zero in soil without fly ash and glucose and was nearly same as observed on day 4 of incubation (Table 5). With addition of 5% fly ash, rate of CO₂ evolution decreased and ranged from 72.6 to 74.5 mg CO₂ 100 g⁻¹ soil day⁻¹. As glucose increased in soil from 1 to 4% a consistent rise in the rate of CO₂ evolution was observed from 72.6 to 79.7 mg CO₂ 100 g⁻¹ soil day⁻¹ on day 1 of incubation and from 74.5 to 80.9 mg CO₂ 100 g⁻¹ soil day⁻¹ on day 4 of incubation in the presence of 5% fly ash

Table 5. Rate of CO₂ evolution (mg CO₂ 100 g⁻¹ soil day⁻¹) in the alkaline soil mixed with 5% fly ash (v/v) and varying glucose percentage (w/w).

	Day				
	0	1	2	3	4
Soil	80.6	80.8	81.9	78.6	81.1
Soil+5% fa	73.4	72.6	73.1	73.7	74.5
Soil+5% fa+1%g	76.4	75.9	77.5	77.5	75.0
Soil+5% fa+2%g	78.4	79.7	78.1	77.0	78.9
Soil+5% fa+3%g	79.3	79.7	78.7	79.2	79.8
Soil+5% fa+4%g	80.1	78.1	80.0	81.7	80.9

fa: fly ash; g: glucose ; Source of ESP fly ash: Yamunanagar

Effect of 4% glucose addition in soil with varying concentrations of fly ash on rate of carbon-dioxide evolution

The effect of 4% glucose in soil mixed with varying concentration of fly ash from 1 to 10% was studied on rate of carbon-dioxide evolution (Table 6). On day zero the rate of CO₂ evolution ranged from 77.8 to 91.3 mg CO₂ 100 g⁻¹ soil day⁻¹ and eventually it increased to 91.3 mg CO₂ 100 g⁻¹ soil day⁻¹ after the addition of 4% glucose. On day 4 of incubation the rate of CO₂ evolution was 90.3 mg CO₂ 100 g⁻¹ soil day⁻¹ with no distinct variation as a result of increase in fly ash to 10% until day 4 of incubation. Overall the rate of CO₂ evolution ranged from 77 to 83.5 mg CO₂ 100 g⁻¹ soil day⁻¹ with no drastic variation in soil co-mixed with fly ash and glucose, indicating the beneficial effect of glucose as a carbon source for unhindered microbial activity.

Table 6. Rate of CO₂ evolution (mg CO₂ 100 g⁻¹ soil day⁻¹) in the alkaline soil mixed with 4% glucose and varying percentages of fly ash.

Treatment	Days				
	0	1	2	3	4
Soil	80.5	78.7	82.5	82.2	78.6
Soil+ 4% g	91.3	93.2	90.2	91.8	90.3
Soil+4%g+1%fa	81.3	79.2	80.2	83.5	82.5
Soil+4%g+3%fa	80.1	80.3	80.9	79.2	80.3
Soil+4%g+5%fa	79.7	80.9	78.7	81.4	78.1
Soil+4%g+10%fa	77.8	78.9	77.5	77.8	77.0

fa: fly ash; g: glucose ; Source of ESP fly ash: Yamunanagar

Effect of glucose on soil dehydrogenase activity

The effect of glucose on soil dehydrogenase activity (SDA) was studied without addition of fly ash (Table 7). Control soil without glucose was observed to have lowest dehydrogenase activity, corresponding to $2.5 \mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$. With an increase in glucose concentration up to 4%, the enzyme activity increased to $27.75 \mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$ (Fig.1a). The benefit of glucose is evident from the experiment since it acts as a carbon source for metabolic activities of the microorganisms.

Table 7. Effect of glucose on dehydrogenase activity in alkaline soil at varying percentages.

Treatment	Soil dehydrogenase activity ($\mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$)
Soil	2.50
Soil+1% g	7.75
Soil+2% g	10.25
Soil+3% g	23.50
Soil+4% g	27.75

Effect of 5% fly ash amendment on soil dehydrogenase activity with varying concentrations of glucose

The effect of 5% fly ash and varying glucose percentage on soil microbial activity in terms of dehydrogenase activity was analysed (Table 8). Addition of glucose as an organic carbon source resulted in a concentration-dependent increase in dehydrogenase activity in soil. 5% fly ash was added to soil containing glucose at 1, 2, 3 and 4% rates respectively. Dehydrogenase activity in soil without fly ash was $8.75 \mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$ (Table 8) and increased to $9.00 \mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$ with addition of 1% glucose. With further addition of glucose at 2, 3 and 4%, dehydrogenase activity increase rapidly up to 20.75, 23.25 and $29.50 \mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$ respectively (Fig. 1b). Highest enzyme activity was observed in soil amended with 4 % glucose and 5% fly ash, which could be the result of synergistic interaction between the carbon source and fly ash, which provides all necessary nutrients.

Table 8. Effect of 5% fly ash on dehydrogenase activity in alkaline soil with varying percentage of glucose.

Treatment	Soil dehydrogenase activity ($\mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$)
Soil	8.75
Soil + 5% fa	2.25
Soil + 5% fa + 1% g	9.00
Soil + 5% fa + 2% g	20.75
Soil + 5% fa+ 3% g	23.25
Soil + 5% fa+ 4% g	29.50

fa: fly ash; g: glucose ; Source of ESP fly ash: Yamunanagar

Effect of 4% glucose addition in soil on soil dehydrogenase activity with varying concentrations of fly ash

An experiment was conducted to study the effect of 4% glucose on soil dehydrogenase activity with varying concentrations of fly ash. Enzyme activity in soil without fly ash and glucose was 2.5 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ (Table 9). Soil mixed with 4% glucose showed the highest enzyme activity, corresponding to 23.3 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ (Fig. 1c). As the fly ash concentration increased to 10%, enzyme activity declined.

Table 9. Effect of 4% glucose on dehydrogenase activity in alkaline soil with varying percentages of fly ash.

Treatment	Soil dehydrogenase activity ($\mu\text{g TPFg}^{-1} \text{ soil day}^{-1}$)
Soil	2.50
Soil + 4% g	23.3
Soil+4%g+1%fa	19.0
Soil+4%g+3%fa	16.0
Soil+4%g+5%fa	8.50
Soil+4%g+10%fa	1.25

fa: fly ash; g: glucose ; Source of ESP fly ash: Yamunanagar

Effect of fly ash on inoculated bacteria *E.coli S-17*

Table 10. Populations of *E.coli S-17 lac Z⁺* in sterile and non-sterile alkaline soil.

Days	Fly ash (%)	Sterile (cfu x10 ⁶ g ⁻¹ soil)	Non-sterile (cfu x10 ⁶ g ⁻¹ soil)
0	0	60	200
	10	30	58.5
	30	10	23
6	0	65.5	180
	10	50	43
	30	24	20
12	0	60.3	210
	10	55.3	42.7
	30	20	23.25

E.coli S-17 lac Z⁺ inoculated in soil mixed with 0 to 30% fly ash under sterile and non-sterile conditions was monitored for a period of twelve days (Table 10). Bacterial count in non-sterile soil was 200 x10⁶cfu g⁻¹ soil, which was higher compared to 60x10⁶cfu g⁻¹soil in soil without fly ash. On day zero the count decreased to 30x 10⁶ and 10 x10⁶cfu g⁻¹ soil in sterile soil and 58.5 x10⁶ and 23 x10⁶cfu g⁻¹ soil in non-sterile soil respectively (Fig.1d). With an increase in fly ash percentage up to 30%, a consistent decrease in population of *E.coli* was observed in sterile and non-sterile soil. Over a period of twelve days bacterial populations did not show any drastic reduction but higher counts were observed in non-sterile soil. Bacterial counts ranged from 42.7 x10⁶ to 55.3 x10⁶ cfu g⁻¹ soil in sterile and non-sterile soil mixed with 10% fly ash. The lowest bacterial count was 20x10⁶ and 23x10⁶cfu g⁻¹ soil in soil amended with 30% fly ash under sterile and non-sterile conditions.

Effect of fly ash on bacterial counts, soil microbial biomass, available phosphorus, and organic carbon in soil inoculated with phosphate-solubilizing bacterial isolate S2 and transformant S2: pMMB277

Soil inoculated with S2

Bacterial counts

Enumeration of bacteria in soil inoculated with phosphate-solubilizing isolate S2 was carried out on nutrient agar plates. Bacterial counts ranged from 110×10^6 to 400×10^6 cfu g^{-1} soil just after inoculation (Table 11). After 8 days of incubation it ranged from 58×10^6 to 547×10^6 cfu g^{-1} soil in inoculated and uninoculated soil under sterile and non-sterile conditions and thereafter remained consistent. After 60 days of incubation the count ranged from 120×10^6 to 360×10^6 cfu g^{-1} soil under sterile conditions. In sterile uninoculated soil it ranged from 35×10^6 to 190×10^6 cfu g^{-1} soil on day zero of incubation and increased from 85×10^6 to 460×10^6 cfu g^{-1} soil on day 60. Inoculated soil showed higher bacterial count compared to uninoculated soil under sterile conditions; however, a reverse trend was observed in non-sterile soil (Fig. 2a-4a). Bacterial populations in soil amended with 10% fly ash ranged from 68×10^6 to 480×10^6 cfu g^{-1} soil and 105×10^6 to 550×10^6 cfu g^{-1} soil under sterile and non-sterile conditions.

Microbial biomass

Microbial biomass immediately after inoculation ranged from 153 to 637 $\mu g g^{-1}$ soil under sterile conditions and was higher compared to non-sterile soil, where it ranged from 172 to 295 $\mu g g^{-1}$ soil, respectively (Table 11). No drastic reduction in biomass was observed from day 8 onwards and it ranged from 115 to 456 $\mu g g^{-1}$ soil until day 50. Microbial biomass in inoculated soil ranged from 286 to 418 and 280 to 437 $\mu g g^{-1}$ soil in sterile and non-sterile conditions after 60 days of incubation. A similar pattern of increase was observed in uninoculated soil where microbial biomass varied from 249 to 351 and 312 to 460 $\mu g g^{-1}$ soil after 60 days of incubation (Fig. 2b-4b). Soil amended with 10% fly ash contained 152 to 456 $\mu g g^{-1}$ soil and 213 to 472 $\mu g g^{-1}$ soil microbial biomass in sterile and non-sterile soil respectively.

Organic carbon

Organic carbon ranged from 0.28 to 0.52 and 0.33 to 0.77% in soil inoculated with S2 under sterile and non-sterile conditions, respectively (Table 12) and was higher in inoculated compared to uninoculated soil. Organic carbon measured 0.77% in non-sterile inoculated soil on day zero of incubation and ranged from 0.17 to 0.71% from day 8 onwards remaining fairly consistent. The overall range on day 60 was 0.30 to 0.49% in inoculated and uninoculated soil under both sterile and non-sterile conditions. Organic carbon was higher in inoculated soil compared to uninoculated soil under sterile conditions where the highest organic carbon concentration was recorded after 32 days of incubation (Fig. 2c-4c). No drastic reduction in organic carbon was observed in inoculated and uninoculated soil, where it ranged from 0.32 to 0.70% and 0.22 to 0.71% in soil amended with 10% fly ash under sterile and non-sterile conditions.

Available phosphorus

Available phosphorus in soil immediately after inoculation ranged from 13.0 to 57.0 mg/kg and was nearly the same in uninoculated soil (Table 12). In sterile soil available phosphorus ranged from 7.2 to 32.8 mg/kg and was comparatively less than non-sterile soil where it ranged from 28.7 to 98 mg/kg. After 32 days of incubation a decrease in available phosphorus was observed until day 60 after which it ranged from 6.8 to 43.7 mg/kg in sterile and non-sterile soil. Available phosphorus ranged from 25.0 to 61.5 mg/kg and 42.7 to 98.0 mg/kg in soil amended with 10% fly ash under sterile and non-sterile conditions (Fig. 2d-4d).

Soil inoculated with S2:pMMB277

Bacterial count

S2: pMMB277 enumeration was carried out on nutrient agar plates containing chloramphenicol ($10\mu\text{g ml}^{-1}$), IPTG and X-gal and the bacteria were identified as blue colonies. On day zero of incubation, bacterial populations ranged from 11×10^6 to 50×10^6 cfu g^{-1} soil and 18×10^6 to 46×10^6 cfu g^{-1} soil in sterile inoculated and uninoculated soil (Table 11). The bacterial counts in non-sterile soil ranged from 16×10^6 to 45×10^6 cfu g^{-1} soil. After 8 days of incubation 10×10^6 to 60×10^6 cfu g^{-1} soil c was recorded. After two months of incubation inoculated and uninoculated soil were found to have

populations varying from 14×10^6 to 48×10^6 cfu g^{-1} soil in sterile and non-sterile conditions respectively (Fig. 5a-7a). In soil mixed with 10% fly ash the bacterial count ranged from 12×10^6 to 46×10^6 and 10×10^6 to 48×10^6 cfu g^{-1} soil in sterile and non-sterile conditions.

Microbial biomass

Microbial biomass ranged from 135 to 264 and 105 to 275 $\mu g g^{-1}$ soil in the sterile inoculated and uninoculated soil on day zero of incubation (Table 11). An increase was observed by day 60 and the corresponding biomass ranged from 160 to 418 and 159 to 354 $\mu g g^{-1}$ soil. In the inoculated and uninoculated soil, microbial biomass ranged from 195 to 282 and 115 to 239 $\mu g g^{-1}$ soil under non-sterile conditions. After 60 days of incubation microbial biomass increased in inoculated and uninoculated soil under non-sterile conditions and ranged from 300 to 440 and 396 to 555 $\mu g g^{-1}$ soil respectively. Biomass in soil mixed with 10% fly ash ranged from 111 to 541 and 221 to 555 $\mu g g^{-1}$ soil (Fig. 5b-7b).

Organic carbon

In inoculated and uninoculated soil total organic carbon content ranged from 0.22 to 0.65 % and 0.17 to 0.77 % under sterile and non-sterile conditions, respectively (Table 12). On day zero of incubation organic carbon ranged from 0.28 to 0.60% and after 8 days remained fairly consistent without any drastic variation. By day 60 of incubation organic carbon varied from 0.33 to 0.52 % in inoculated and uninoculated soil under both sterile and non-sterile conditions. In inoculated and uninoculated soil amended with 10% fly ash organic carbon ranged from 0.31 to 0.60 and 0.32 to 0.65 % under sterile and non-sterile conditions (Fig. 5c-7c).

Available phosphorus

Available phosphorus in sterile inoculated and uninoculated soil ranged from 9.00 to 69.0 and 12.2 to 85.6 mg/kg under sterile and non-sterile conditions (Table 12). After 8 days of incubation available phosphorus ranged from 18.6 to 65.8 mg/kg in sterile and non-sterile conditions respectively. The available phosphorous eventually decreased with increasing incubation time and by day 60 it ranged from 9.1 to 23.1 mg/kg in sterile and

9.5 to 31.5 mg/kg in non-sterile soil, respectively. Available phosphorus in soil amended with 10% fly ash was 23.1 to 87 and 31.4 to 91.0 mg/kg in sterile and non-sterile conditions, respectively (Fig. 5d-7d).

Fly ash application at 10% was favourable for bacterial populations and microbial biomass along with total organic carbon and available phosphorous in sterile and non-sterile conditions inoculated with S2 and S2 : pMMB277.

Table 11. Enumeration of total bacterial population ($\times 10^6$ cfu g^{-1} soil) in alkaline soil inoculated with phosphate solubilizing bacterial isolate S2 and its transformant (S2: pMMB277) and estimation of microbial biomass (μg g^{-1} soil) amended with (0-30%) fly ash. (fa: fly ash; St: Sterile soil; Ns: Non-sterile soil; S2: Phosphate solubilizer; T: S2: pMMB277; I: Inoculated; U: Uninoculated; mb: microbial biomass; cfu : colony forming unit)

fa (%)	Treatment			Days															
				0		8		16		24		32		40		50		60	
				S2	T	S2	T	S2	T	S2	T	S2	T	S2	T	S2	T	S2	T
0	St	I	cfu	400	50	320	30	190	21	450	16	310	20	230	14	360	25	360	26
			mb	637	264	547	329	448	443	244	357	349	152	188	201	397	426	418	379
		U	cfu	35	46	427	10	405	55	400	15	440	19	465	10	460	13	460	16
			mb	257	275	530	207	374	179	195	314	210	139	115	247	333	258	344	354
	Ns	I	cfu	250	45	132	20	173	50	63	11	123	23	70	15	320	10	150	35
			mb	215	195	227	428	278	323	264	381	205	294	269	147	423	269	404	384
		U	cfu	200	35	312	60	63	10	115	14	165	32	60	27	180	26	100	40
			mb	284	197	283	219	370	286	361	257	214	303	320	458	251	350	460	404
10	St	I	cfu	275	30	544	28	265	28	510	13	315	22	190	46	480	22	160	29
			mb	202	154	456	275	427	423	310	413	245	279	367	459	438	541	370	418
		U	cfu	190	25	157	24	68	40	220	12	155	24	170	25	470	20	200	14
			mb	195	111	431	268	437	333	152	240	217	247	240	269	311	394	351	267
	Ns	I	cfu	310	19	210	20	198	14	105	17	550	33	200	48	240	35	150	48
			mb	295	282	321	463	404	295	380	221	213	348	219	449	352	350	437	440
		U	cfu	305	18	153	10	297	10	170	11	280	31	240	33	300	28	350	46
			mb	289	239	379	419	385	290	472	254	281	348	252	515	416	384	460	555
30	St	I	cfu	110	11	260	20	61	30	180	13	200	12	125	34	205	17	120	20
			mb	153	135	263	256	413	283	261	235	205	252	202	241	249	426	286	160
		U	cfu	115	18	150	50	83	20	150	12	95	10	110	27	245	16	85	17
			mb	139	105	177	247	328	258	176	152	208	177	125	146	263	274	249	159
	Ns	I	cfu	120	16	88	21	240	10	113	12	170	14	215	10	145	19	90.0	33
			mb	172	201	320	330	335	289	312	219	175	262	195	262	283	230	280	300
		U	cfu	100	10	58	11	70	40	100	13	120	17	147	12	140	21	110	31
			mb	262	115	117	217	314	128	229	237	278	233	121	443	301	298	312	396

Table 12. **Organic carbon (%) and available P (mg/kg) in alkaline soil inoculated with phosphate solubilizing bacterial isolate S2 and its transformant (S2: pMMB277) amended with (0-30%) fly ash. (fa: fly ash; St: Sterile soil; Ns: Non-sterile soil; S2: Phosphate solubilizer; T: S2: pMMB277; I: Inoculated; U: Uninoculated)**

fa (%)	Strain	Treatment	Organic Carbon (%)									Available P (mg/kg)							
			0	8	16	24	32	40	50	60	0	8	16	24	32	40	50	60	
Days>			0	8	16	24	32	40	50	60	0	8	16	24	32	40	50	60	
0	S2	St.	I	0.41	0.38	0.35	0.69	0.29	0.31	0.37	0.36	13.0	22.9	15.1	31.0	20.1	19.7	14.8	7.2
			U	0.28	0.32	0.43	0.40	0.31	0.23	0.28	0.30	13.7	14.4	11.4	15.0	13.9	11.4	11.1	6.8
		Ns	I	0.77	0.52	0.52	0.24	0.35	0.35	0.29	0.33	30.0	12.9	15.0	13.6	16.1	12.1	13.1	10.8
			U	0.33	0.53	0.53	0.40	0.17	0.29	0.38	0.39	28.7	22.9	13.0	15.0	13.5	11.5	11.7	8.4
	T	St.	I	0.42	0.44	0.35	0.33	0.25	0.22	0.44	0.34	13.8	37.2	21.0	17.9	27.0	15.0	18.7	9.12
			U	0.39	0.41	0.25	0.26	0.13	0.17	0.23	0.42	9.00	18.6	15.4	17.2	13.1	15.7	17.1	9.4
		Ns	I	0.28	0.50	0.56	0.40	0.50	0.25	0.29	0.33	12.2	17.2	10.0	12.9	41.3	10.0	19.4	9.54
			U	0.46	0.55	0.47	0.37	0.26	0.29	0.35	0.40	14.4	14.7	19.0	13.6	14.5	14.0	15.9	9.83
10	S2	St.	I	0.60	0.52	0.59	0.51	0.70	0.49	0.46	0.44	57.0	59.0	90.3	61.5	40.0	39.3	36.5	35.6
			U	0.50	0.45	0.55	0.40	0.32	0.41	0.34	0.47	48.4	38.6	50.6	55.8	25.0	37.9	31.5	32.8
		Ns	I	0.52	0.55	0.61	0.38	0.71	0.47	0.34	0.42	82.6	89.4	94.3	86.5	58.0	48.6	41.5	43.7
			U	0.63	0.54	0.47	0.22	0.43	0.40	0.29	0.49	98.0	67.0	87.2	68.0	58.0	67.9	50.0	42.7
	T	St.	I	0.60	0.59	0.59	0.56	0.54	0.46	0.49	0.50	69.0	65.8	55.8	75.0	87.0	60.8	55.1	23.2
			U	0.44	0.50	0.61	0.48	0.31	0.40	0.44	0.44	44.9	43.0	53.0	87.3	65.1	33.6	50.8	23.1
		Ns	I	0.50	0.64	0.65	0.45	0.37	0.46	0.52	0.52	85.6	59.4	85.7	91.0	83.0	42.9	64.0	31.4
			U	0.50	0.50	0.62	0.65	0.32	0.43	0.41	0.47	83.5	88.0	83.1	91.0	72.0	42.2	65.1	31.5
30	S2	St.	I	0.61	0.55	0.49	0.39	0.59	0.35	0.35	0.34	39.8	38.6	30.9	40.0	34.0	34.3	21.0	20.7
			U	0.52	0.45	0.43	0.36	0.74	0.29	0.20	0.44	36.4	30.0	16.4	45.6	25.5	31.5	21.2	19.5
		Ns	I	0.44	0.68	0.46	0.37	0.73	0.32	0.31	0.44	58.3	60.8	58.0	55.1	40.1	35.8	16.9	30.8
			U	0.38	0.27	0.47	0.36	0.46	0.37	0.20	0.33	56.6	56.5	27.0	40.8	52.2	65.1	22.2	31.3
	T	St.	I	0.47	0.56	0.44	0.30	0.28	0.29	0.37	0.50	32.9	42.9	31.0	65.8	31.0	39.0	20.0	25.2
			U	0.32	0.50	0.40	0.22	0.34	0.31	0.32	0.43	31.4	35.0	30.0	32.9	10.9	35.8	16.5	33.1
		Ns	I	0.41	0.65	0.61	0.40	0.61	0.46	0.25	0.40	65.1	51.5	45.1	53.0	51.5	34.0	26.5	21.9
			U	0.44	0.56	0.44	0.42	0.44	0.38	0.43	0.40	52.3	54.0	37.0	49.3	36.5	34.4	30.0	27.5

3. To screen fly ash resistant soil microorganisms such as free- living nitrogen-fixers and phosphate solubilizers.

Rhizosphere soil of *Acacia auriculiformis* and *Eucalyptus tereticornis* was collected from a three year old plantation and screened for phosphate solubilizing and free-living nitrogen-fixing bacteria on Pikovskya and Jensen's media respectively (Table 13). Overall population of phosphate-solubilizing and nitrogen-fixing bacteria ranged from 9×10^6 to 27.5×10^6 and 0.02×10^6 to 2.60×10^6 cfu g⁻¹ soil in rhizosphere soil of *Acacia* and *Eucalyptus* both with and without chemical fertilizers.

Table 13. Enumeration of phosphate solubilizing and free-living nitrogen-fixing bacteria in fly ash (0-24%) amended rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) without chemical fertilizers (T1) and with chemical fertilizers (T2).

Fly ash	Species	Treatment	Phosphate-solubilizers (x 10 ⁶ cfu g ⁻¹ soil)	Nitrogen-fixers (x 10 ⁶ cfu g ⁻¹ soil)
F0 (0%)	<i>A.a</i>	T1	14.5	-
		T2	14.5	0.40
	<i>E.t</i>	T1	22.0	-
		T2	21.5	-
F1 (6%)	<i>A.a</i>	T1	17.0	0.55
		T2	22.5	2.60
	<i>E.t</i>	T1	20.0	0.30
		T2	24.0	-
F2 (12%)	<i>A.a</i>	T1	19.5	-
		T2	21.0	-
	<i>E.t</i>	T1	19.5	0.03
		T2	27.5	-
F3 (18%)	<i>A.a</i>	T1	17.5	-
		T2	16.5	-
	<i>E.t</i>	T1	19.5	0.02
		T2	17.0	-
F4 (24%)	<i>A.a</i>	T1	9.0	-
		T2	13.5	-
	<i>E.t</i>	T1	18.0	-
		T2	19.0	-

Nitrogen-fixing bacteria

Populations of free-living nitrogen-fixing bacteria isolated from rhizosphere soil of both tree species was much lower than phosphate-solubilizing bacteria in the presence and absence of chemical fertilizers and ranged from 0.02×10^6 to 2.60×10^6 cfu g^{-1} soil (Table 13). The count was negligible beyond 6% fly ash amendment and populations were essentially absent in the rhizosphere soil of both the tree species amended with 18 to 24% fly ash both with and without chemical fertilizers. The population of free-living nitrogen fixing bacteria in soil was less, therefore, further studies were mainly concentrated on phosphate-solubilizing bacteria.

Phosphate solubilizing bacteria

Acacia auriculiformis

Phosphate-solubilizing bacterial populations measured 14.5×10^6 cfu g^{-1} soil in (F0T1) rhizosphere control soil and (F0T2) fertilized control (Table 13). As the fly ash percentage increased up to 6 % the population of bacteria increased to 17×10^6 cfu g^{-1} soil and 22.5×10^6 cfu g^{-1} soil in F0T1 and F0T2, respectively. A similar rise in bacterial count was observed in the soil amended with 12% fly ash, which ranged from 19.5×10^6 to 21.0×10^6 cfu g^{-1} soil, in soil containing only fly ash and fly ash mixed with chemical fertilizers, respectively. A slight decrease ranging from 16.5×10^6 to 17.5×10^6 cfu g^{-1} soil was observed in soil amended with 18% fly ash. As the fly ash concentration increased to 24% the phosphate-solubilizing bacteria declined corresponding to 9×10^6 to 13.5×10^6 cfu g^{-1} soil in F0T1 and F0T2 rhizosphere soil of Acacia, respectively.

Eucalyptus tereticornis

Phosphate-solubilizing bacterial populations ranged from 21.5×10^6 to 22×10^6 cfu g^{-1} soil in rhizosphere control soil (F0T1) and fertilized control (F0T2), respectively (Table 13). As fly ash percentage increased up to 6 %, the population of bacteria increased to 20×10^6 and 24×10^6 cfu g^{-1} soil in F0T1 and F0T2, respectively. A marked increase in bacterial populations up to 27.5×10^6 cfu g^{-1} soil was observed in the rhizosphere soil mixed with 12% fly ash and chemical fertilizers. A slight decrease ranging from 17.0×10^6 to 19.5×10^6 cfu g^{-1} soil was observed in soil amended with 18% fly ash. As the fly ash concentration increased to 24% the phosphate solubilizing bacteria did not show much variation and ranged from 18×10^6 to 19×10^6 cfu g^{-1} soil in soil containing fly ash alone and fly ash mixed with fertilizers.

Table 14 Percentage solubilization of tricalcium phosphate by phosphate-solubilizing bacteria isolated from fly ash (0-24%) amended rhizosphere soil of *Acacia auriculiformis* and *Eucalyptus tereticornis*. (control: without bacterial isolate; Ava. P: 0.030 mg/kg; pH:7.0)

Strain	24 hrs.			48 hrs.			72 hrs.			96 hrs.		
	Ava. P. (mg/kg)	pH	% Sol. of TCP	Ava. P. (mg/kg)	pH	% Sol. of TCP	Ava. P. (mg/kg)	pH	% Sol. of TCP	Ava.P. (mg/kg)	pH	% Sol. of TCP
1	0.110	5.0	74.5	0.120	4.9	75.0	0.128	4.8	75.0	0.130	4.7	74.6
2	0.100	5.4	72.0	0.110	5.2	72.7	0.113	5.1	71.6	0.115	5.0	71.3
3	0.121	5.3	76.8	0.130	5.1	76.9	0.135	5.1	76.2	0.137	5.0	75.9
4	0.050	5.1	44.0	0.058	5.0	48.2	0.068	4.9	52.9	0.072	4.7	54.1
5	0.120	5.1	76.6	0.129	4.8	76.7	0.132	4.6	75.7	0.135	4.5	75.5
6	0.042	5.8	33.3	0.045	5.8	33.3	0.049	5.7	34.6	0.052	5.2	36.5
7	0.050	5.2	44.0	0.055	5.1	45.4	0.060	4.7	46.6	0.062	4.6	46.7
8	0.057	5.9	50.0	0.060	5.5	50.0	0.065	5.4	50.7	0.067	5.2	50.7
9	0.068	5.7	58.8	0.070	5.4	57.1	0.074	5.3	56.7	0.078	5.2	50.7
10	0.132	5.0	78.0	0.139	4.8	78.4	0.145	4.7	77.9	0.149	4.6	77.8
11	0.126	4.9	77.7	0.129	4.7	76.7	0.135	4.6	76.2	0.139	4.5	76.2
12	0.098	5.5	71.4	0.100	5.3	70.0	0.110	5.0	70.9	0.115	4.5	71.3
13	0.090	5.6	68.8	0.095	5.2	68.4	0.098	5.1	67.3	0.109	4.8	69.7
14	0.085	5.4	67.0	0.090	5.2	66.6	0.097	5.2	67.0	0.106	5.0	68.8
15	0.130	4.8	78.4	0.135	4.3	77.7	0.141	4.2	77.3	0.145	4.1	77.2
16	0.105	5.4	73.3	0.125	5.2	76.0	0.129	5.0	75.0	0.131	5.0	74.8
17	0.135	5.2	79.2	0.153	5.0	80.3	0.15	4.8	78.6	0.152	4.9	78.2
18	0.066	5.0	57.5	0.070	4.9	57.1	0.080	4.7	60.0	0.085	4.6	61.1
19	0.129	5.3	78.2	0.144	5.1	79.1	0.151	5.1	78.8	0.154	4.1	78.5
20	0.039	5.3	39.2	0.042	5.0	28.5	0.056	4.9	42.8	0.058	4.9	43.1
21	0.048	5.5	41.6	0.051	4.9	41.1	0.060	4.9	46.0	0.063	4.8	47.6
22	0.070	5.0	60.0	0.070	5.0	57.1	0.077	5.0	58.4	0.079	5.0	58.2
23	0.110	5.4	74.5	0.116	5.2	74.1	0.125	4.9	74.4	0.128	4.8	74.2
24	0.146	4.5	80.8	0.155	4.1	80.6	0.163	3.9	80.3	0.165	3.7	80.0
25	0.116	5.6	75.8	0.122	5.0	75.0	0.142	4.8	77.0	0.145	4.6	77.2
26	0.202	5.0	85.1	0.225	5.2	86.0	0.235	5.0	86.0	0.238	4.8	86.1
27	0.130	4.7	78.4	0.140	4.6	78.5	0.150	4.4	78.0	0.155	4.3	78.7
28	0.100	4.8	82.5	0.176	4.4	84.0	0.182	4.3	82.4	0.192	4.1	82.8

Phosphate solubilization

Phosphate-solubilizing bacteria were studied for their capacity to solubilize tricalcium phosphate (TCP) in Pikovskya broth over different time intervals and the subsequent pH changes were studied (Table 14). With increase in time of incubation the concentration of solubilized TCP was found to increase with a corresponding decrease in pH. In the beginning the pH ranged from 4.5 to 5.9 in the broth after which a drop was recorded which ranged from 3.7 to 5.2 respectively (Table 14). The range of percent solubilization of TCP was 36.5 to 86.1 % with the highest in isolate No. 19, 24, 26, 27, 28 designated as S1, S2, S3, S4 and S5. S1 and S4 were isolated from rhizosphere soil of Acacia and the remaining from that of Eucalyptus. The percentage solubilization of TCP was 78.5% by strain S1, 80.0% by strain S2, 86.1 by strain S3, 78.7 % by strain S4 and 82.8 % by strain S5.

Gram character and growth curve of S2, S3 and S5

Three bacterial strains S2, S3 and S5, showing higher percentage of solubilization of TCP, were examined for Gram character. S2 and S3 was Gram-negative whereas S5 was Gram-positive. The lag phase of bacterial isolate S2 lasted for 7 hours followed by the beginning of exponential phase (Fig.8). In this phase the cell division occurred steadily until 15 hours, after which an abrupt increase in rate of cell division was observed which continued linearly until 24 hours. Beyond this the stationary phase started which extended upto 31 hours. The lag phase of isolate S3 lasted up to 6 hours followed by the start of the exponential phase where a marked increase in rate of cell division was seen until 14 hours followed by a linear increase until 21 hours. Thereafter the stationary phase began which was observed until the 31st hour. Lag phase of strain S5 lasted for 10 hours followed by beginning of exponential phase where cell division occurred at a uniform rate and maximum growth occurred in the 23rd hour after which the stationary phase began (Fig.8).

4. Molecular tagging of beneficial soil bacteria for ecological monitoring in soil / fly ash amended soils.

Phosphate-solubilizing bacteria S1, S2, S3, S4 and S5 were studied for their antibiotic profile (Table 15). Strains S4 and S5 were resistant to streptomycin (50 µg ml⁻¹). All bacterial strains except S1 were also resistant to nalidixic acid (50µg ml⁻¹). Sensitivity to kanamycin (50µg ml⁻¹) and chloramphenicol (10µg ml⁻¹) was a common characteristic in addition to the lac Z^{vc} nature of all five strains. Strains S2, S3 and S5 were selected for transformation with plasmid pMMB277 (Fig.9) containing the *lacZ* and chloramphenicol molecular marker. Plasmid pMMB277 (Gene, 1997) is a low copy number with wide host range and controlled expression vector which was isolated from *E.coli* 2842 by the alkali lysis method. Fig.10 shows electrophoretogram on 0.7% agarose gel of the plasmid having size 9.237 Kb. The plasmid was purified from low melting agarose and used for transformation studies. The gram-negative strains were transformed with pMMB277 plasmid and as result the transformant was obtained only in the case of S2 which was named S2: pMMB277. It was checked for β-galactosidase expression on chloramphenicol +IPTG+X-gal plates and for plasmid pMMB277 by agarose gel electrophoresis (Fig. 11).

Table 15. Antibiotic profiling of bacterial isolates (S1-S5).

Antibiotic	S1	S2	S3	S4	S5
Str ₁ 50	-	-	-	+	+
Str ₂ 50	-	-	-	-	+
Kan ₁ 50	-	-	-	-	-
Kan ₂ 50	-	-	-	-	-
Nal ₁ 50	-	+	+	+	+
Nal ₂ 50	-	+	+	+	+
Chl ₁ 10	-	-	-	-	-
Chl ₂ 10	-	-	-	-	-

Str: Streptomycin, Kan: Kanamycin, Nal: Nalidixic acid, Chl: Chloramphenicol

5. To study the effect of fly ash on the growth of nursery seedlings of *Populus*.

ESP fly ash procured from Ropar was mixed with alkaline soil of Patiala at rates of 0 to 30% (v/v) and used as a soil ameliorating agent for nursery trial of *Populus deltoides* which has the ability to grow on marginal soils making harvesting of above ground biomass with subsequent resprouting possible. Fly ash amendment up to 20% was favourable for biomass production and growth without any adverse effect.

Effect of fly ash on biomass production of *Populus deltoides*

Effect of fly ash on growth of *Populus deltoides* was studied in terms of biomass production (Table 16). Approximately 70% rooting was observed in plants grown in soil without fly ash while 62.5 % rooting was observed in soil amended with 10% fly ash. Shoot biomass in soil without fly ash measured 5.73g, which increased to 6.88g in soil plus fly ash mixtures containing 20% fly ash. Leaf biomass was 1.26 g in soil receiving 10 % fly ash compared to 0.78 g in soil without fly ash. Root biomass increased to 0.28% in soil mixed with 20 % fly ash compared to 0.07 g in soil without fly ash (Table 16).

Table 16. Dry biomass of *Populus deltoides* (average of 20 replicates).

Fly ash (%)	Shoot biomass (g)	Leaf biomass (g)	Root biomass (g)
0	5.73±0.92	0.78 ±0.16	0.07 ±0.04
5	5.75 ±1.08	1.03 ±0.26	0.11 ±0.20
10	6.18 ±0.78	1.26 ±0.22	0.21 ±0.03
20	6.88 ±0.65	0.35 ±0.07	0.28 ±0.15
30	5.95 ±1.10	0.32 ±0.07	0.13 ±0.29

Effect of fly ash on physical properties of alkaline soil amended with ESP fly ash during nursery trial.

Bulk density in soil without fly ash was 1.24 g cm^{-3} which after the trial showed a slight increase to 1.34 g cm^{-3} . Bulk density in soil before and after the trial in various soil plus fly ash mixtures did not vary distinctly and ranged from 1.09 to 1.34 g cm^{-3} (Table 17).

Water holding capacity in soil without fly ash was 33.6% and increased to 61.3% in soil mixed with 30% fly ash after the trial (Table 17). The pattern of variation in water holding capacity was similar before and after the trial and overall water holding capacity ranged from 33.4 to 61.3% .

Hydraulic conductivity (HC), a parameter for determining the permeability of soil, ranged from 18 to 47 cm min^{-1} in soil without fly ash and soil plus fly ash mixtures during the trial. Highest hydraulic conductivity was 47 cm min^{-1} in soil amended with 20% fly ash (Table 17).

Texture of the soil was analyzed in terms of sand, silt and clay percentage, which was 74.6 , 15.0 and 10.4% in the soil without fly ash. Sand percentage in the soil without fly ash was 75.6% , which increased to 84.6% in soil plus fly ash mixture having 30% fly ash after the trial. Texture of soil remained loamy sand (Table 17).

Effect of fly ash on chemical properties of alkaline soil amended with ESP fly ash during nursery trial.

ESP fly ash used for amending alkaline soil was acidic in nature with pH 5.28 , as a result of which the soil pH became acidic, ranging from 6.68 to 6.92 with increase in fly ash percentage up to 30% . Overall no distinct variation was observed in pH with increasing fly ash percentage in the soil (Table 18).

Electrical conductivity in soil amended with 30% fly ash was $220.3 \mu\text{S cm}^{-1}$ compared to $152.5 \mu\text{S cm}^{-1}$ in soil without fly ash, thus showing a marked increase with rise in fly ash percentage after the trial, which can be attributed to the high soluble salt concentration in the fly ash (Table 18).

Organic carbon content was 0.37 and 0.73% in soil receiving a fly ash amendment of 10% (Fig. 12c) both before and after the trial. Increase in organic carbon content was

observed for the control soil corresponding to 0.44% after the trial, with a similar increase in soil plus fly ash mixtures (Table 18).

Total and available nitrogen ranged from 0.16 to 0.44 % and 0.001 to 0.007 % in the soil without fly ash and soil plus fly ash mixtures, respectively (Table 18). Though an increase in nitrogen was observed after the trial the highest nitrogen content, 0.44 % was observed in the control. Total and available phosphorus ranged from 254 to 1270 and 8.9 to 32.9 mg/kg in soil without fly ash and soil plus fly ash mixtures, showing an increase after the trial. Potassium ranged from 1090 to 1530 and 40.3 to 81.0 mg/kg in the soil without fly ash and soil plus fly ash mixtures with a marked increase in available potassium after the trial. Total and available sulphur ranged from 140 to 271 and 2.3 to 24 mg/kg in the soil without fly ash and soil plus fly ash mixtures and increased with increase in fly ash percentage (Table 18).

Highest organic carbon content was observed in soil amended with 10% fly ash along with an increase in nitrogen, phosphorus, potassium and sulphur contents with increase in fly ash up to 20%. For biomass production also 20% fly ash rate was most favourable, as a source of nutrients. Available potassium was the dominant essential element followed by nitrogen and phosphorus in the order K>N>P>S.

Nutrient status in terms of total secondary elements, micronutrients and heavy metals in alkaline soil amended with ESP fly ash during nursery trial

Secondary elements (Ca, Mg, Na)

Calcium, magnesium and sodium concentrations ranged from 225 to 6948, 1488 to 10858 and 25.6 to 47.4 mg/kg in soil without fly ash and in soil plus fly ash mixtures showing a marked increase after the trial (Table 19). Magnesium increased to 10858 mg/kg in soil mixed with 30 % fly ash. However, highest sodium was recorded after the trial in soil amended with 10 % fly ash at 47.4 mg/kg. Calcium in the control soil was 288 mg/kg and increased to 6948 mg/kg with increase in fly ash percentage to 30%. Secondary elements in soil and soil plus fly ash mixtures were in the order of Mg> Ca> Na. Calcium was within the range reported for cropland soil, magnesium was higher than generally reported values while sodium was lower (Table 2a).

Micronutrients (Fe, Zn, Mn, Mo)

Iron and manganese were present at a higher concentration in soil and soil plus fly ash mixtures compared to zinc and molybdenum and ranged from 2180 to 7175 and 310 to 555 mg/kg respectively after the trial (Table 19). Fe and Mn in soil without fly ash measured 2180 and 475 mg/kg and a steady increase was observed in concentration with increasing fly ash level, the highest observed at 30 % fly ash amendment corresponding to 7175 and 555 mg/kg respectively. On the other hand, zinc and molybdenum varied from 25 to 55.3 and 2.6 to 3.9 mg/kg respectively, in soil with and without fly ash after the trial (Table 19). Concentrations of these two micronutrients did not vary much with increasing fly ash percentage in the soil and overall order was Fe>Mn>Zn>Mo with all elements falling within range as found in normal cropland soil (Table 2a).

Heavy metals (Pb, Ni, Cr, Co, As, Se)

Lead, nickel and chromium ranged from 15.2 to 67.3, 4.8 to 19.0 and 32.3 to 55.5 mg/kg in soil without fly ash and soil plus fly ash mixtures, respectively, after the trial (Table 19). Among the heavy metals chromium was highest in soil followed by lead and nickel. Lead measured 21.3 mg/kg in soil without fly ash and increased to 67.3 mg/kg in soil amended with 6% fly ash after the trial, thereafter showing an irregular decrease. Nickel was highest in control soil corresponding to 19 mg/kg followed by a decrease in soil plus fly ash mixtures. However nickel in soil amended with 30% fly ash measured 18.4 mg/kg, nearly the same as control. Chromium measured 43.2 and 38.3 mg/kg in the soil without fly ash before and after trial with no distinct variation observed with increasing fly ash percentage. Cobalt, arsenic and selenium ranged from 5.6 to 8.4, 3.8 to 4.8 and 2.4 to 3.5 mg/kg in soil without fly ash and fly ash plus soil mixtures after the trial (Table 19). Overall heavy metals varied as Cr>Pb>Ni>Co>As>Se with all elements except arsenic falling within range and permissible limits as reported in the literature (Table 2a).

Macroelements (mg/kg) in leaves and stem of *Populus deltoides*

The plants were studied for metal uptake in the stem and leaves, which were observed to fall within the nutrient bracket generally reported with Fe as an exception. Calcium, sodium and magnesium in the stem ranged from 4020 to 8140, 337 to 505 and 3714 to

4620 mg/kg tissue while in leaves the observed range was 4120 to 8970, 310 to 618 and 1617 to 2739 mg/kg respectively (Table 20). In stem and leaves calcium was highest in soil without fly ash and sodium was lowest in soil amended with 5% fly ash.

Iron, zinc and manganese in the stem tissue ranged from 612 to 2832, 55.3 to 80.7 and 21.1 to 42.9 mg/kg, while in leaves they ranged from 459 to 2671, 30.7 to 69.7 and 24.2 to 32.2 mg/kg respectively. In soil amended with 10 % fly ash, 2832 and 2671 mg/kg iron was observed which was highest while lowest uptake was recorded for manganese corresponding to 21.1 and 24.2 mg/kg in soil amended with 5 to 10 % fly ash (Table 20). Elemental concentration in leaves was higher compared to stems and calcium was the dominant secondary element followed by magnesium in both stem and leaves. Among the micronutrients iron uptake was highest compared to zinc and manganese. Overall order of metal uptake in stem and leaves was $\text{Ca} > \text{Mg} > \text{Fe} > \text{Na} > \text{Zn} > \text{Mn}$ (Table 20).

Effect of fly ash on microbiological properties of alkaline soil amended with ESP fly ash during nursery trial

Soil dehydrogenase activity (SDA) is a measure of the microbial activity for which no distinct variation was observed in the fly ash-amended soil. SDA was nearly the same in control soil ranging from 0.40 to 0.50 $\mu\text{g TPFg}^{-1}\text{soil day}^{-1}$ and in soil containing 10% fly ash it varied from 1.50 to 2.75 $\mu\text{g TPFg}^{-1}\text{soil day}^{-1}$ (Table 21). With increase in fly ash up to 30% the enzyme activity ranged from 0.20 to 0.37 $\mu\text{gTPFg}^{-1}\text{soil day}^{-1}$ (Fig.12b).

Total bacterial counts in soil without fly ash was 3.82×10^6 cfu g^{-1} soil before the beginning of the trial and 2.75×10^6 cfu g^{-1} soil after the trial, respectively (Table 21). The population of bacteria in soil plus fly ash mixtures ranged from 4.63×10^6 to 11.6×10^6 cfu g^{-1} soil in soil amended with 10% fly ash and a corresponding increase was observed after the trial (Fig. 12a).

Microbial biomass of soil ranged from 103.8 to 134.0 $\mu\text{g g}^{-1}$ soil before and 110.7 to 359.3 $\mu\text{g g}^{-1}$ soil after the nursery trial, in soil without fly ash and soil plus fly ash mixtures (Table 21). Biomass in control soil increased from 104.8 $\mu\text{g g}^{-1}$ soil to 148 $\mu\text{g g}^{-1}$ soil after the trial. A marked increase corresponding to 359.3 $\mu\text{g g}^{-1}$ soil in soil amended with 10% fly ash was observed (Fig.12d). A correlation was observed between organic carbon, bacterial counts and microbial biomass where 10% fly ash was optimum for microbial activities.

Rate of carbon dioxide evolution is was measured over a period of four days in soil with and without fly ash (Table 22). Overall the range was from 75.1 to 83.6 mg CO₂100 g⁻¹soil day⁻¹ during the trial (Fig.13a, 13b). No distinct variation was observed in rate of carbon dioxide evolution in soil without fly ash and in soil plus increasing fly ash percentage. After three days of incubation, the rate of CO₂ evolution was 83.6 mg CO₂ 100 g⁻¹ soil day⁻¹ in the presence of 10% fly ash, which was optimum (Table 22) and did not bring about any drastic changes in the microenvironment of the soil. With increasing fly ash percentage up to 30%, no drastic reduction in the rate of carbon dioxide evolution occurred and this pattern remained consistent over the period.

Table 17. Bulk density, water holding capacity, hydraulic conductivity, particle size distribution and texture of alkaline soil with and without fly ash used in nursery trial of *Populus deltoides*.

Fly ash (%)	Bulk density (g cm ⁻³)	Water holding capacity (%)	Hydraulic conductivity (cm min ⁻¹)	Sand %	Silt %	Clay %	Texture
0	1.24 (1.34)	39.8 (33.6)	26 (18)	75.6 (74.6)	12.0 (15.0)	12.4 (10.4)	Loamy sand
5	1.21 (1.26)	44.5 (33.4)	32 (46)	78.6 (79.6)	7.0 (8.0)	14.4 (12.4)	Loamy sand
10	1.19 (1.25)	46.6 (41.2)	21 (27)	77.6 (80.6)	7.0 (8.0)	15.4 (11.4)	Loamy sand
20	1.13 (1.15)	51.0 (39.8)	47 (43)	82.6 (81.6)	5.0 (8.0)	12.4 (10.4)	Loamy sand
30	1.10 (1.095)	61.3 (44.05)	40 (39)	84.6 (83.6)	6.0 (3.0)	9.4 (13.4)	Loamy sand
M±SE	1.2±0.02 (1.2±0.04)	48.7±3.6 (38.4±2.1)	33.2±4.4 (34.6±5.3)	79±1.6 (80±1.5)	7.4±1.2 (8.4±1.9)	12.8±1.0 (11.6±0.6)	
C.V	5.03 (7.96)	16.7 (12.2)	31.5 (33.9)	4.6 (4.2)	36.5 (50.9)	17.9 (11.2)	

(Values in parenthesis: Initial values) M±SE: mean±standard error of mean; C.V: coefficient of variation

Table 18. Chemical characterisation of alkaline soil with and without fly ash used in nursery trial of *Populus deltoides*.

Fly ash (%)	pH	EC	Organic carbon (%)	N (%)		P (mg/kg)		K (mg/kg)		S (mg/kg)	
				Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
0	7.28 (7.20)	152.5 (146.3)	0.44 (0.20)	0.44 (0.33)	0.004 (0.003)	254 (261)	14.8 (1.42)	1090 (980)	40.3 (25.5)	140 (206)	8.8 (0.43)
5	6.85 (6.74)	169.2 (174.8)	0.41 (0.23)	0.41 (0.16)	0.002 (0.001)	568 (356)	21.2 (2.40)	1150 (1100)	45.3 (36.6)	183 (163)	2.54 (4.90)
10	6.74 (6.80)	180.4 (166.1)	0.73 (0.37)	0.35 (0.27)	0.007 (0.003)	684 (404)	32.9 (5.83)	1180 (1070)	60.4 (32.9)	226 (173)	3.11 (2.23)
20	6.78 (6.76)	199.2 (171.6)	0.66 (0.33)	0.39 (0.24)	0.007 (0.004)	1270 (421)	11.9 (5.30)	1530 (1400)	81.0 (44.0)	271 (190)	24.05 (9.01)
30	6.92 (6.68)	220.3 (187.8)	0.40 (0.30)	0.23 (0.18)	0.002 (0.001)	1050 (312)	8.9 (1.93)	1360 (1290)	70.9 (43.0)	235 (172)	2.32 (3.01)
M±SE	6.9±0.09 (6.8±0.09)	184±11.7 (169±6.7)	0.5±0.06 (0.3±0.03)	0.36±0.03 (0.23±0.03)	0.004±0.001 (0.002±0.0006)	765±179 (350±29)	17.9±4.3 (3.4±0.9)	1262±80.7 (1168±76)	59.6±7.6 (36.4±3.4)	211±22.6 (180±7.6)	7.1±2.6 (4.8±1.1)
C.V	3.1 (3.0)	14.3 (8.9)	29.4 (24.5)	21.9 (87.0)	57 (14.9)	52.4 (18.8)	53.0 (60.3)	14.3 (14.7)	28.6 (20.9)	23.9 (9.5)	103 (61.0)

(Values in parenthesis: Initial values) M±SE: mean±standard error of mean; C.V: coefficient of variation; Avail.: Available

Table19. Nutrient status in terms of secondary elements, micronutrients and heavy metals in alkaline soil with and without fly ash used in nursery trial of *Populus deltoides*.

Fly ash (%)	Secondary element (mg/kg)			Micronutrients (mg/kg)				Heavy metals (mg/kg)					
	Ca	Na	Mg	Fe	Zn	Mn	Mo	Pb	Ni	Cr	Co	As	Se
0	288 (198)	40.9 (11.1)	1488 (1533)	2180 (2171)	25.0 (30.8)	475 (345)	3.2 (3.0)	21.3 (18.2)	19.0 (17.0)	43.2 (38.3)	5.9 (4.1)	3.9 (4.0)	2.4 (2.6)
5	225 (162)	25.6 (17.2)	1712 (1403)	3500 (4385)	34.6 (27.4)	520 (380)	3.0 (3.2)	67.3 (23.5)	4.8 (8.0)	32.3 (31.7)	5.6 (2.8)	3.8 (4.1)	2.5 (2.8)
10	318 (240)	47.4 (15.0)	3511 (1248)	5795 (5460)	43.1 (31.1)	310 (460)	3.4 (3.3)	15.2 (23.4)	8.9 (8.0)	39.4 (42.7)	8.4 (6.1)	4.6 (4.0)	2.6 (3.1)
20	1461 (397)	35.5 (25.6)	7184 (1240)	6040 (5365)	55.3 (37.2)	540 (350)	2.6 (3.6)	22.2 (23.8)	10.8 (4.8)	55.5 (31.5)	7.8 (6.7)	4.8 (4.3)	3.5 (3.5)
30	6948 (432)	39.4 (54.8)	10858 (1653)	7175 (6850)	48.3 (34.4)	555 (315)	3.9 (3.7)	28.5 (10.3)	18.4 (15.1)	49.7 (37.1)	7.7 (7.2)	4.6 (4.7)	3.4 (3.2)
M±SE	1848±1296 (285±54)	38±3.6 (25±8)	4951±1795 (1415±80)	4938±911 (4617±979)	41±5.3 (32±2)	480±44 (370±25)	3.2±0.2 (3.4±0.1)	27±10.2 (23±1.6)	12.4±2.8 (11±2.3)	44±4.0 (36±2.1)	7.0±0.5 (4.9±0.9)	4.3±0.2 (4±0.07)	2.8±0.2 (3.0±0.1)
C.V	157 (42.5)	21.3 (71.2)	81.0 (12.7)	41.3 (47.5)	28.6 (11.6)	20.7 (14.9)	14.9 (8.6)	84.0 (15.5)	49.8 (49.2)	20.4 (13.0)	17.7 (36.5)	10.6 (3.5)	18.5 (13.0)

(Values in parenthesis: Initial values) M±SE: mean±standard error of mean; C.V: coefficient of variation

Table 20. Macroelements (mg/kg) in leaves and stem of *Populus deltoides*.

Fly ash (%)	Ca		Na		Mg		Fe		Zn		Mn	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
0	8140	8970	478	466	4434	1805	1154	1372	67.4	32.8	28.9	27.1
5	5290	8440	337	310	3714	1617	612	1454	55.3	30.7	24.6	24.2
10	4020	5780	505	618	3983	1761	2832	2671	68.4	39.6	21.1	32.2
20	5670	4120	493	460	4620	2739	665	660	80.7	69.7	42.9	30.5
30	4940	4180	427	498	4618	2112	864	459	59.3	53.7	34.2	28.0
M±SE	5612±688	6298±1030	448±30	470±49	4274±181	2007±200	1225±412	1323±388	66.2±4.4	105±35	30.3±3.8	28.4±1.4
C.V	27.4	36.6	15.4	23.4	9.52	22.3	75.3	65.7	14.8	74.7	28.2	10.9

(Values in parenthesis: Initial values) M±SE: mean±standard error of mean; C.V: coefficient of variation

Table 21. Microbiological properties of alkaline soil with and without fly ash used in nursery trial of *Populus deltoides*.

Fly ash %	Soil dehydrogenase activity ($\mu\text{g TPFg}^{-1} \text{soil day}^{-1}$)	Microbial enumeration ($\times 10^6 \text{cfu g}^{-1} \text{soil}$)	Microbial biomass ($\mu\text{g g}^{-1} \text{soil}$)
0	0.50 (0.40)	2.75 (3.82)	148 (104.8)
5	1.30 (1.00)	2.51 (1.70.)	174.1 (126.0)
10	2.75 (1.50)	11.6 (4.63)	359.3 (134.0)
20	0.75 (0.50)	5.34 (2.75)	154.7 (118.0)
30	0.37 (0.20)	0.43 (0.38)	110.7 (103.8)
M \pm SEM	1.13 \pm 0.43 (0.72 \pm 0.23)	4.52 \pm 1.93 (2.65 \pm 0.75)	189.4 \pm 43.7 (117.3 \pm 5.8)
C.V	85.6 (73.1)	95.5 (63.4)	51.6 (11.2)

(Values in parenthesis: Initial values) M \pm SE: mean \pm standard error of mean; C.V: coefficient of variation

Table 22. Rate of CO₂ evolution (mg CO₂100g⁻¹ soil day⁻¹) in alkaline soil with and without fly ash used in nursery trial of *Populus deltoides*.

Day	Fly ash (%)					
	0	5	10	20	30	M±SE
0	81.1 (79.8)	80.4 (79.6)	81.7 (79.2)	80.8 (79.1)	81.2 (79.8)	81.0±0.21 (79.5±0.14)
1	79.7 (80.6)	81.9 (81.4)	81.4 (80.4)	81.8 (80.9)	80.8 (82.5)	81.1±0.40 (81.1±0.37)
2	82.2 (81.4)	82.9 (78.4)	83.0 (79.2)	78.1 (78.6)	80.6 (80.8)	80.8±0.74 (79.6±0.60)
3	81.4 (81.1)	80.9 (82.5)	83.6 (81.1)	81.1 (81.9)	81.95 (80.60)	81.7±0.48 (81.4±0.33)
4	81.1 (76.2)	82.1 (75.1)	80.6 (76.2)	82.2 (75.2)	81.7 (75.6)	81.3±0.27 (75.6±0.23)
M±SE	81.1±0.52 (79.8±1.2)	81.4±0.27 (79.3±1.7)	81.7±0.6 (79.2±1.0)	80.8±0.9 (79.1±1.4)	81.2±0.3 (79.8±1.5)	
C.V	1.2 (3.0)	0.6 (4.2)	1.5 (2.7)	2.3 (3.7)	0.8 (3.7)	

(Values in parenthesis: Initial values) M±SE: mean±standard error of mean; C.V: coefficient of variation

6. To study the effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in established field plantation.

Effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* was monitored in a field plantation established in 1998, on a twenty-acre area in village Durgaprasad, Distt. Dhenkanal (Orissa) on partially degraded soil belonging to order Ultisols having reddish brown lateritic characteristics with a clay texture. Soil samples from the rhizospheric zone were collected for physicochemical and microbiological analysis. The plant growth (biometric) parameters collar diameter, girth at breast height (GBH) and height of the inner block of nine plants in three replications were recorded at yearly intervals and analysed statistically using Graph pad software.

pH

pH of soil remained acidic over the years along with increasing fly ash percentage (Table 23). pH ranged from 5.36 to 6.35 in the rhizosphere control soil (F0T1) of *Acacia* and *Eucalyptus* without any distinct variation with increased fly ash addition. In the rhizosphere fertilized control soil (F0T2) of *Acacia* and *Eucalyptus*, pH ranged from 5.62 to 6.20 respectively, and with increase in fly ash from 6 to 24% pH ranged from 5.20 to 6.63 respectively.

Electrical conductivity

Electrical conductivity (EC) in rhizosphere control soil (F0T1) of *Acacia* and *Eucalyptus* ranged from 71.9 to 166.7 and 24.6 to 70.0 $\mu\text{S cm}^{-1}$, respectively, and in the rhizosphere fertilized control soil (F0T2) it varied from 40 to 136.0 and 43.2 to 85.9 $\mu\text{S cm}^{-1}$ in *Acacia* and *Eucalyptus*, respectively (Table 24). A distinct rise in EC was observed especially in the rhizosphere soil of *Acacia auriculiformis*, with increasing fly ash percentage due to presence of soluble salts. Overall EC ranged from 20.0 to 260 $\mu\text{S cm}^{-1}$ in both tree species over the four-year study period.

Organic carbon

Organic carbon in rhizosphere control soil (F0T1) of *Acacia* (Fig.14a) and *Eucalyptus* (Fig.14c) varied from 0.31 to 0.58 and 0.38 to 0.71%, respectively (Table 25). As the fly ash percentage increased from 6 to 24% in soil without fertilizer no distinct variation was observed over the four-year study period and it ranged from 0.32 to 0.88% in soil of both

tree species. In the rhizosphere fertilized control soil (F0T2) of *Acacia* and *Eucalyptus* organic carbon ranged from 0.55 to 0.76 (Fig.14b) and 0.32 to 0.61 % (Fig.14d) respectively. The increased addition of fly ash in soil mixed with fertilizer led to an increase in organic carbon in soil, which ranged from 0.52 to 1.20 % in both the tree species. Organic carbon in rhizosphere soil of *Acacia* was slightly greater compared to *Eucalyptus* in soil mixed with fly ash and chemical fertilizers and highest organic carbon was observed in soil amended with 12% fly ash for both tree species.

Bacterial count

Bacterial counts in rhizosphere control soil (F0T1) of *Acacia* (Fig. 15a) and *Eucalyptus* (Fig. 15c) varied from 0.76×10^6 to 1.60×10^6 and 0.22×10^6 to 3.50×10^6 cfu g⁻¹ soil respectively (Table 26). With increase in fly ash percentage in soil, populations varied from 0.09×10^6 to 13.6×10^6 cfu g⁻¹ soil in the rhizosphere soil of both tree species. In rhizosphere fertilized control (F0T2) of *Acacia* (Fig. 15b) and *Eucalyptus* (Fig.15d) bacterial counts ranged from 0.11×10^6 to 1.57×10^6 and 0.05×10^6 to 2.30×10^6 cfu g⁻¹ soil. The addition of fly ash with fertilizers resulted in a slight increase in bacterial counts in *Acacia* and *Eucalyptus* soil both which ranged from 0.08×10^6 to 6.0×10^6 cfu g⁻¹ soil. Bacterial populations were greater in rhizosphere soil of *Acacia* compared to *Eucalyptus* and increased with increase in fly ash percentage in soil and over the four-year study period. Bacterial populations decreased slightly with 12% fly ash amendment in soil which can be nullified by mixing of fly ash and fertilizer at an optimum dose to maintain the microbial population, which is necessary for maintaining soil fertility. The population of free-living nitrogen-fixing bacteria was much lower comparatively as judged on Jensen's media and ranged from 0.01×10^6 to 1.30×10^6 and 0.01×10^6 to 4.5×10^6 cfu g⁻¹ soil in rhizosphere soil of *Acacia* and *Eucalyptus* (Table 27).

Soil dehydrogenase activity

Dehydrogenase activity (SDA) in rhizosphere control soil (F0T1) varied from 0.11 to 0.83 and 0.06 to 0.79 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ for *Acacia* (Fig. 16a) and *Eucalyptus*, (Fig. 16c) respectively (Table 28). With increase in fly ash up to 24% the enzyme activity ranged from 0.12 to 0.70 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ in *Acacia* and 0.01 to 0.72 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ in *Eucalyptus*, respectively. In the rhizosphere fertilized control soil (F0T2) SDA ranged from 0.25 to 0.82 and 0.23 to 0.93 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ in *Acacia* (Fig. 16b) and *Eucalyptus* (Fig. 16d), respectively. With increase in fly ash percentage along with

mixing of fertilizers the enzyme activity ranged from 0.06 to 0.85 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ in rhizosphere soil of *Acacia* and 0.03 to 0.78 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$ in rhizosphere soil of *Eucalyptus*. Soils amended with 6 to 12% fly ash had the highest dehydrogenase activity in *Acacia* and *Eucalyptus* which ranged from 0.19 to 0.63 and 0.37 to 0.47 $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$. No marked variation was observed in the enzyme activity in soil of *Eucalyptus* with increasing fly ash percentage and over the four-year study period.

Dehydrogenase activity in rhizosphere soil of *Acacia* was higher compared to *Eucalyptus* in soil with and without chemical fertilizers. Fly ash amendment in soil was found to have a favourable impact on microbiological parameters such as soil dehydrogenase activity at 6% while 12% fly ash was recorded as optimum for bacterial counts and organic carbon. All three parameters exhibited a common behaviour where organic carbon, bacterial count and SDA activity were higher in soil containing fly ash mixed with chemical fertilizers in *Acacia auriculiformis*. Soil dehydrogenase activity was observed to be correlated with organic carbon level in the rhizosphere soil of *Eucalyptus* where both were higher in soil without chemical fertilizers.

Table 23. pH of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)					M±SE	T2 (Chemical fertilizers)					M±SE
		2001	2002	2003	2004			2001	2002	2003	2004		
F0 (0)	<i>A.a</i>	5.99	6.35	5.36	5.36	5.7±0.2	6.20	6.36	5.62	6.12	6.0±0.1		
	<i>E.t</i>	5.73	5.56	5.63	5.50	5.6±0.03	3.74	6.20	5.85	5.36	5.2±0.5		
F1 (6)	<i>A.a</i>	6.64	6.49	5.76	5.20	6.0±0.2	5.91	6.21	5.44	5.34	5.7±0.2		
	<i>E.t</i>	5.58	5.48	5.73	5.41	5.6±0.06	2.30	6.63	5.26	5.46	4.8±0.9		
F2 (12)	<i>A.a</i>	6.41	5.75	5.80	5.62	5.9±0.1	6.07	6.39	5.84	5.45	6.0±0.1		
	<i>E.t</i>	5.56	5.57	5.65	5.65	5.6±0.02	5.00	5.70	5.42	5.49	5.4±0.1		
F3 (18)	<i>A.a</i>	6.50	6.38	5.74	5.34	6.0±0.2	5.82	6.26	5.62	5.56	5.8±0.1		
	<i>E.t</i>	5.71	5.86	5.75	5.45	5.8±0.03	3.06	5.50	5.55	5.10	4.9±0.6		
F4 (24)	<i>A.a</i>	6.44	6.23	5.76	5.76	6.0±0.2	5.76	6.47	5.59	5.50	5.8±0.2		
	<i>E.t</i>	5.66	5.49	5.86	5.86	5.7±0.08	4.96	6.13	5.31	5.24	5.4±0.2		
M±SE	<i>A.a</i>	6.4±0.1	6.2±0.1	5.7±0.08	5.9±0.08		5.8±0.08	6.3±0.04	5.6±0.06	5.6±0.12			
	<i>E.t</i>	5.6±0.03	5.6±0.06	5.7±0.04	5.7±0.04		3.8±0.5	6.0±0.2	5.5±0.1	5.3±0.1			
CV%	<i>A.a</i>	3.81	4.63	3.21	2.74		3.05	1.64	2.54	5.06			
	<i>E.t</i>	1.34	2.77	1.60	2.80		31	7.36	4.31	1.90			

Table 24.Electrical conductivity ($\mu\text{S cm}^{-1}$) of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)				M \pm SE	T2 (Chemical fertilizers)				M \pm SE
		2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0)	<i>A.a</i>	130	71.9	166.7	115.1	120.9 \pm 19.6	40.0	136	95.8	128.9	100.1 \pm 21.8
	<i>E.t</i>	70.0	24.6	56.3	42.7	48.4 \pm 9.7	80.0	45.2	85.9	43.2	63.6 \pm 11.3
F1 (6)	<i>A.a</i>	60.0	26.8	46.6	93.6	56.7 \pm 14.0	90.0	90.3	77.3	142.2	99.9 \pm 14.4
	<i>E.t</i>	30.0	21.5	48.0	55.7	38.8 \pm 7.9	63.0	24.5	58.2	49.1	48.7 \pm 8.56
F2 (12)	<i>A.a</i>	90.0	50.9	50.2	113.5	76.1 \pm 15.5	50.0	69.7	79.1	96.6	73.8 \pm 9.70
	<i>E.t</i>	20.0	27.6	22.4	37.5	26.8 \pm 3.8	90.0	22.0	56.8	23.7	48.1 \pm 16.0
F3 (18)	<i>A.a</i>	50.0	84.0	124.1	116.2	93.6 \pm 16.9	60.0	141.6	69.2	67.5	84.6 \pm 19.1
	<i>E.t</i>	50.0	29.6	62.9	26.9	42.3 \pm 8.6	160	32.2	58.9	28.8	69.9 \pm 31.0
F4 (24)	<i>A.a</i>	260	65.8	128.5	110.2	141.1 \pm 41.7	80.0	159.8	168.3	107.7	128.9 \pm 21.1
	<i>E.t</i>	80.0	31.2	22.8	28.9	40.7 \pm 13.2	100	40.8	132.8	30.2	75.9 \pm 24.3
M \pm SE	<i>A.a</i>	118 \pm 38	59.8 \pm 9.8	103 \pm 23.9	109 \pm 4.1		64 \pm 9.3	119.5 \pm 16.9	97.9 \pm 18.1	108.6 \pm 12.9	
	<i>E.t</i>	50 \pm 11.4	26.9 \pm 1.74	42.4 \pm 8.45	38.3 \pm 5.2		98.6 \pm 16.5	32.9 \pm 4.5	78.5 \pm 14.6	35 \pm 4.7	
CV%	<i>A.a</i>	72.3	36.7	51.0	8.5		32.45	31.6	41.4	26.8	
	<i>E.t</i>	50.9	14.5	44.5	30.3		37.5	30.4	41.6	30.5	

Table 25. Organic carbon (%) of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1(Control)					M±SE	T2 (Chemical fertilizers)					M±SE
		2001	2002	2003	2004			2001	2002	2003	2004		
F0 (0)	<i>A.a</i>	0.33	0.58	0.31	0.52	0.44±0.06	0.62	0.63	0.76	0.55	0.6±0.04		
	<i>E.t</i>	0.64	0.46	0.71	0.38	0.54±0.07	0.42	0.32	0.61	0.40	0.4±0.06		
F1 (6)	<i>A.a</i>	0.36	0.78	0.82	0.75	0.70±0.10	0.60	0.52	0.78	0.75	0.6±0.06		
	<i>E.t</i>	0.57	0.72	0.32	0.50	0.52±0.08	0.39	0.16	0.91	0.70	0.5±0.16		
F2 (12)	<i>A.a</i>	0.41	0.62	0.68	0.73	0.61±0.07	0.64	0.59	0.97	0.56	0.7±0.09		
	<i>E.t</i>	0.68	0.67	0.88	0.59	0.70±0.06	0.53	0.47	0.76	0.83	0.6±0.08		
F3 (18)	<i>A.a</i>	0.35	0.69	0.79	0.75	0.64±0.10	0.63	1.00	0.98	0.79	0.8±0.08		
	<i>E.t</i>	0.70	0.72	0.65	0.92	0.74±0.05	0.49	0.51	1.20	0.99	0.8±0.17		
F4 (24)	<i>A.a</i>	0.49	0.79	0.64	0.59	0.62±0.06	0.65	0.83	0.57	0.65	0.7±0.05		
	<i>E.t</i>	0.75	0.62	0.85	0.73	0.73±0.04	0.45	0.55	1.12	0.80	0.7±0.14		
M±SE	<i>A.a</i>	0.4±0.02	0.7±0.04	0.64±0.09	0.7±0.04		0.62±0.008	0.71±0.08	0.81±0.07	0.66±0.04			
	<i>E.t</i>	0.7±0.03	0.6±0.04	0.7±0.10	0.6±0.09		0.5±0.02	0.40±0.07	0.9±0.1	0.74±0.09			
CV%	<i>A.a</i>	16.5	13.53	31.35	15.9		3.06	27.60	20.93	16.46			
	<i>E.t</i>	10.12	16.9	32.81	33.51		12.17	40.01	26.64	29.40			

Table 26. Bacterial counts ($\times 10^6$ cfu g^{-1} soil) in rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

		T1 (Control)						T2 (Chemical fertilizers)					
Fly ash (%)	Species	2001	2002	2003	2004	M \pm SE	2001	2002	2003	2004	M \pm SE		
F0 (0)	<i>A.a</i>	1.00	0.76	1.60	1.40	1.2 \pm 0.19	0.83	0.11	1.57	0.70	0.8 \pm 0.3		
	<i>E.t</i>	0.55	0.22	3.50	2.50	1.7 \pm 0.8	1.21	0.05	0.30	2.3	0.9 \pm 0.5		
F1 (6)	<i>A.a</i>	1.10	0.92	1.85	4.72	2.14 \pm 0.8	0.10	0.10	1.42	3.80	1.3 \pm 0.8		
	<i>E.t</i>	0.23	0.09	0.12	5.0	1.4 \pm 1.2	0.13	0.23	0.12	1.30	0.4 \pm 0.3		
F2 (12)	<i>A.a</i>	1.90	13.6	0.10	3.10	4.7 \pm 3.0	6.0	0.08	1.15	6.00	3.30 \pm 1.6		
	<i>E.t</i>	0.80	0.15	0.45	0.65	0.5 \pm 0.1	0.39	0.12	0.42	4.80	1.4 \pm 1.1		
F3 (18)	<i>A.a</i>	0.70	0.75	0.10	3.90	1.4 \pm 0.8	0.10	0.25	0.40	2.10	0.7 \pm 0.4		
	<i>E.t</i>	0.63	0.16	2.00	2.80	1.4 \pm 0.6	1.10	0.52	0.10	3.00	1.2 \pm 0.6		
F4 (24)	<i>A.a</i>	0.30	0.46	5.30	3.60	2.41 \pm 1.2	1.20	0.18	0.15	1.65	0.8 \pm 0.4		
	<i>E.t</i>	1.21	0.09	4.0	0.88	1.5 \pm 0.8	3.25	0.10	3.75	1.50	2.1 \pm 0.8		
M \pm SE	<i>A.a</i>	1.0 \pm 0.3	3.3 \pm 2.5	1.8 \pm 0.9	3.3 \pm 0.5		1.6 \pm 1.1	0.14 \pm 0.03	0.9 \pm 0.3	2.8 \pm 0.9			
	<i>E.t</i>	0.7 \pm 0.2	0.14 \pm 0.02	1.8 \pm 0.65	2.4 \pm 0.8		1.2 \pm 0.5	0.19 \pm 0.08	0.9 \pm 0.7	2.6 \pm 0.6			
CV%	<i>A.a</i>	59.16	174.69	118.75	36.95		150.67	48.76	67.15	73.29			

	<i>E.t</i>	52.57	38.38	86.6	74.11		100.82	92.4	168	54.77	
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Table 27. Nitrogen-fixing bacteria ($\times 10^6$ cfu g^{-1} soil) in rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)				T2 (Chemical fertilizers)			
		2001	2002	2003	2004	2001	2002	2003	2004
F0 (0)	<i>A.a</i>	-	-	0.01	0.03	0.40	-	0.20	0.07
	<i>E.t</i>	-	-	0.01	-	-	-	0.03	-
F1 (6)	<i>A.a</i>	0.55	-	-	0.15	2.60	-	0.20	0.02
	<i>E.t</i>	0.30	-	-	0.02	-	-	0.10	0.02
F2 (12)	<i>A.a</i>	-	-	-	-	-	-	-	0.03
	<i>E.t</i>	0.03	-	-	-	-	-	1.01	0.01
F3 (18)	<i>A.a</i>	-	-	0.20	0.04	-	-	0.20	-
	<i>E.t</i>	0.02	-	0.10	-	4.5	-	-	0.03
F4 (24)	A.a	-	-	0.30	0.02	-	-	1.00	0.01
	E.t	-	-	1.30	0.03	-	-	0.51	0.01

Table 28. Soil dehydrogenase activity ($\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$) of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)					M \pm SE	T2 (Chemical fertilizers)					M \pm SE
		2001	2002	2003	2004			2001	2002	2003	2004		
F0 (0)	<i>A.a</i>	0.13	0.31	0.11	0.83	0.34 \pm 0.2	0.25	0.66	0.47	0.82	0.55 \pm 0.12		
	<i>E.t</i>	0.40	0.36	0.06	0.79	0.40 \pm 0.1	0.23	0.26	0.93	0.72	0.53 \pm 0.17		
F1 (6)	<i>A.a</i>	0.55	0.55	0.12	1.30	0.63 \pm 0.2	0.20	0.36	0.12	0.85	0.38 \pm 0.2		
	<i>E.t</i>	0.20	0.01	0.08	0.47	0.19 \pm 0.1	0.50	0.10	0.09	0.17	0.21 \pm 0.09		
F2 (12)	<i>A.a</i>	0.50	0.48	0.13	0.70	0.45 \pm 0.1	0.17	0.75	0.20	0.79	0.47 \pm 0.16		
	<i>E.t</i>	0.55	0.36	0.11	0.57	0.39 \pm 0.1	0.15	0.54	0.03	0.78	0.37 \pm 0.17		
F3 (18)	<i>A.a</i>	0.41	0.54	0.12	0.60	0.41 \pm 0.1	0.15	0.20	0.62	0.49	0.36 \pm 0.11		
	<i>E.t</i>	0.45	0.19	0.09	0.46	0.3 \pm 0.09	0.10	0.75	0.31	0.40	0.39 \pm 0.13		
F4 (24)	<i>A.a</i>	0.50	0.08	0.44	0.31	0.3 \pm 0.09	0.11	0.36	0.06	1.01	0.38 \pm 0.21		
	<i>E.t</i>	0.72	0.02	0.09	0.27	0.27 \pm 0.1	0.28	0.75	0.08	0.10	0.30 \pm 0.15		
M \pm SE	<i>A.a</i>	0.4 \pm 0.07	0.4 \pm 0.08	0.2 \pm 0.06	0.7 \pm 0.16		0.2 \pm 0.05	0.5 \pm 0.1	0.3 \pm 0.10	0.8 \pm 0.08			
	<i>E.t</i>	0.5 \pm 0.08	0.18 \pm 0.07	0.08 \pm 0.008	0.5 \pm 0.08		0.1 \pm 0.04	0.5 \pm 0.1	0.3 \pm 0.1	0.4 \pm 0.1			
CV%	<i>A.a</i>	40.37	57.3	77.87	48.55		29.9	49.35	81.76	23.86			
	<i>E.t</i>	41.3	91.78	21.12	37.02		56.9	60.04	130	71.3			

Particle size distribution

Control soil (F0T1) of *Acacia* with no fly ash had 80.6 % sand, 9.0 % silt and 10.4 % clay (Table 29a) while soil in *Eucalyptus* (F0T1) had 71.1 sand, 13.0% silt and 15.9% clay. The sand, silt and clay percentage in the soil of *Acacia* (Fig. 17a) ranged from 75.6 to 89.6, 1.0 to 11.0 and 8.4 to 20.4 % whereas in the soil of *Eucalyptus* (Fig.17c) it was 71.1 to 87.6, 2.0 to 14.8 and 7.4 to 20.4 % (Table 29 b) respectively.

No distinct variation was observed in the sand, silt and clay percentage with increasing fly ash percentage over the four-year study period in the rhizosphere soil of both tree species. In other words no drastic change in soil particle size distribution was observed with the addition of fly ash up to 24%.

Hydraulic conductivity

Hydraulic conductivity, a parameter for studying the permeability in soil, was 26.1 and 8.36 cm min⁻¹ in control soil (F0T1) of *Acacia auriculiformis* (Fig.17b) and *Eucalyptus tereticornis* (Fig.17d) and overall ranged from 11.0 to 70.6 and 8.36 to 72.0 cm min⁻¹, respectively (Table 30). An increase by 21% was observed in the rhizosphere soil of *Acacia* and by 45% in the fly ash mixed rhizosphere soil of *Eucalyptus*. In soil amended with 12% fly ash highest hydraulic conductivity corresponding to 55.6 and 51.0 cm min⁻¹ was observed in *Acacia* and *Eucalyptus*, respectively.

Water holding capacity

Water holding capacity in the control soil (F0T1) of *Acacia* (Fig.18a) and *Eucalyptus* (Fig. 18b) ranged from 22.8 to 58.4 and 37.4 to 48.9% respectively (Table 31) without any marked variation with increasing fly ash percentage over the four-year study period. However, the rhizosphere soil of *Acacia* was observed to have a higher water holding capacity as compared to *Eucalyptus* especially by the year 2003. The highest water holding capacity was 49.9 and 44.5% in soil amended with 12% fly ash in *Acacia* and *Eucalyptus*, respectively.

Bulk density

Bulk density of rhizosphere control soil (F0T1) ranged from 1.16 to 1.22 and 1.14 to 1.27 g cm⁻³ in Acacia (Fig. 18c) and Eucalyptus (Fig.18d), respectively, with increase in fly ash up to 30% (Table 32). Overall the bulk density ranged from 0.58 to 1.24 and 0.97 to 1.27 g cm⁻³ in rhizosphere soil of Acacia and Eucalyptus with increasing fly ash percentage from 6 to 24% without any marked variation with increasing fly ash percentage and over the four-year study period in rhizosphere soil of both tree species. Highest bulk density corresponding to 1.23 and 1.19 g cm⁻³ was observed in rhizosphere soil of Acacia and Eucalyptus amended with 24% fly ash.

Table 29 a. Particle size distribution (%) of rhizosphere soil of *Acacia auriculiformis* (A.a) at yearly intervals (fa: fly ash; TR: Treatment; T1: Control; T2: Chemical fertilizers).

fa (%)	TR	2001			2002			2003			2004			M±SE		
		Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
F0 (0)	T1	80.6	9.0	10.4	84.6	3.0	12.4	82.6	5.0	12.4	81.6	8.0	10.4	82±0.8	6.3±1.3	11±0.6
	T2	75.6	10.0	14.4	89.6	1.0	9.4	81.6	9.0	9.4	84.6	3.0	12.4	83±2.9	5.8±2.2	11±1.2
F1 (6)	T1	81.6	5.4	13.4	79.6	7.0	13.4	76.6	3.0	20.4	78.6	6.0	15.4	79±1.0	5.4±0.8	16±1.6
	T2	77.6	10.5	11.9	84.6	3.0	12.4	79.6	8.0	12.4	79.6	3.0	10.4	80±1.5	6.1±1.9	12±0.5
F2 (12)	T1	84.6	6.0	9.4	76.6	8.0	15.4	78.6	4.0	17.4	77.6	4.0	18.4	79±1.8	5.5±0.9	15±2.0
	T2	78.6	9.0	12.4	82.6	5.0	12.4	86.6	3.0	10.4	82.6	5.0	12.4	83±1.6	5.5±1.3	12±0.5
F3 (18)	T1	81.6	10.0	8.4	87.6	4.0	8.4	86.6	5.0	8.4	84.6	5.0	10.4	85±1.3	6.0±1.3	8.9±0.5
	T2	76.6	9.5	13.9	79.6	11.0	9.4	84.6	2.0	13.4	78.6	7.0	14.4	80±1.7	7.4±1.9	12.5±1.3
F4 (24)	T1	79.1	7.0	13.9	87.6	2.0	10.4	84.6	5.0	10.4	79.6	5.0	15.4	83±2.0	4.8±1.0	13.1±1.4
	T2	77.6	10.0	12.4	86.6	3.0	10.4	79.6	3.0	17.4	78.6	9.0	12.4	81±2.0	6.3±1.9	
M±SE	T1	82±0.9	7.5±0.8	11±1.1	83±2.2	4.8±1.0	12±1.2	82±2	4.4±0.4	14±2.2	80±1.2	5.6±0.7	14±2			
	T2	77±0.5	9.8±0.2	13±0.5	85±2	4.6±1.7	11±0.6	82.4±1.4	5.0±1.4	13±1.4	81±1.2	5.4±1.1	12±0.6			
C.V	T1	2.5	26.2	21.9	5.92	53.9	22.5	5.0	20.3	36.0	3.5	27.0	25.0			
	T2	1.48	5.32	8.34	4.50	83.6	14.0	3.78	64.8	24.7	3.32	48.2	11.7			

Table 29 b. Particle size distribution (%) of rhizosphere soil of *Eucalyptus tereticornis* (E.t) at yearly intervals (fa: fly ash; TR:Treatment; T1: Control; T2: Chemical fertilizers).

fa (%)	TR	2001			2002			2003			2004			M±SE		
		Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
F0 (0)	T1	71.1	13.0	15.9	87.6	4.0	8.4	79.6	5.0	15.4	84.6	3.0	12.4	81±3.6	6.3±2.3	13.0±1.7
	T2	72.8	14.8	12.4	85.6	5.0	9.4	79.6	5.0	15.4	78.6	6.0	15.4	79±2.6	7.7±2.4	13.1±1.4
F1 (6)	T1	74.6	9.0	16.4	85.6	5.5	8.9	82.6	2.0	15.4	81.6	4.0	14.4	81±2.3	5.1±1.5	13.7±1.7
	T2	77.6	12.0	10.4	79.6	10.0	10.4	77.6	2.0	20.4	76.6	3.0	20.4	78±0.6	6.8±2.5	15.4±2.9
F2 (12)	T1	75.1	11.5	13.4	86.6	6.0	7.4	71.2	2.0	13.4	81.6	5.0	13.4	79±3.4	6.1±1.9	11.9±1.5
	T2	75.1	9.7	15.2	76.6	12.0	11.4	79.6	7.0	13.4	80.6	6.0	13.4	78±1.3	8.6±1.3	13.4±0.7
F3 (18)	T1	77.6	7.5	14.9	85.6	4.0	10.4	85.6	4.0	10.4	79.6	8.0	12.4	82±2.0	5.8±1.0	12.0±1.0
	T2	86.8	12.0	11.4	84.6	4.0	11.4	76.6	.8.0	15.4	76.6	6.0	17.4	81±2.6	5.7±2.3	13.9±1.5
F4 (24)	T1	76.6	9.0	14.4	82.6	5.0	12.4	82.6	5.0	12.4	81.6	8.0	10.4	81±1.4	6.7±1.0	12.4±0.8
	T2	77.6	11.0	11.4	79.6	5.0	15.4	77.6	.5.0	17.4	77.6	7.0	15.4	78±0.5	5.8±2.1	15±1.3
M±S	T1	75±1.1	10±1.0	15±0.5	86±0.8	4.9±0.4	9.5±0.8	80±2.5	3.6±0.6	13±0.9	82±0.8	5.6±1.0	13±0.6			
	T2	78±2.4	11.9±0.8	13±0.8	81±1.7	7.2±1.6	11.6±1.0	78±0.6	3.0±1.3	16±1.18	78±0.7	5.6±0.6	16.4±1.2			
C.V	T1	3.31	22.0	7.96	2.19	18.3	20.5	6.87	42.1	15.8	2.19	41.1	11.7			
	T2	6.82	15.7	15.1	4.7	49.5	19.6	1.72	92.5	16.1	2.15	27.0	16.1			

Table 30. Hydraulic conductivity (cm min^{-1}) of rhizosphere soil of *Acacia auriculiformis* (A.a) and *Eucalyptus tereticornis* (E.t) at yearly intervals.

Fly ash (%)	Species	T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE
		2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0)	A.a	26.1	68.0	43.0	23.3	40.1±10.2	24.0	45.0	40.0	36.0±4.5	
	E.t	8.36	30.0	41.0	32.0	27.8±6.92	32.0	46.0	33.0	33.5±4.72	
F1 (6)	A.a	31.7	17.0	44.0	54.6	36.8±8.0	28.0	49.0	25.0	30.2±6.5	
	E.t	23.67	61.0	46.0	29.0	39.9±8.48	17.0	45.0	38.6	31.1±6.43	
F2 (12)	A.a	39.9	44.0	66.0	20.9	42.7±9.2	61.0	53.0	70.6	55.6±6.9	
	E.t	21.3	20.0	37.0	49.0	31.8±6.90	86.0	46.0	49.2	51.0±13.0	
F3 (18)	A.a	28.0	40.0	46.0	19.7	33.4±5.9	12.0	68.0	41.8	37.8±11.7	
	E.t	13.28	47.0	61.0	49.0	42.5±10.2	28.0	47.0	57.0	38.7±8.00	
F4 (24)	A.a	17.5	43.0	47.0	11.0	29.6±9.0	23.0	45.0	62.0	38.4±9.4	
	E.t	27.12	21.0	48.0	35.4	32.8±5.8	49.0	72.0	22.0	42.5±11.4	
M±SE	A.a	28.6±3.6	42.4±8.0	49.2±4.25	25.9±7.4		29.6±8.3	52.0±4.3	47.8±8.2		
	E.t	18.7±3.4	35.8±7.9	46.6±4.0	38.8±4.2		42.4±12	51.2±5.2	39.9±6.1		
CV%	A.a	28.6	42.7	19.4	64.5		62.6	18.3	38.2		
	E.t	41.2	49.6	19.6	24.5		63.6	22.6	34.2		

Table 31. Water holding capacity (%) of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE
		2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0)	<i>A.a</i>	37.9	34.0	58.4	22.80	38.3±7.4	41.0	45.32	51.7	42.64	45.1±2.3
	<i>E.t</i>	44.1	37.8	48.9	37.4	42±2.75	39.9	40.7	62.5	35.5	44.6±6.0
F1 (6)	<i>A.a</i>	35.5	47.4	48.2	33.24	41±3.9	41.3	41.0	43.7	38.41	41.1±1.0
	<i>E.t</i>	36.7	34.0	46.5	43.0	40.1±2.8	42.6	34.0	38.2	42.8	39.3±2.0
F2 (12)	<i>A.a</i>	36.4	42.1	44.6	33.03	39.0±2.6	62.2	41.5	52.1	43.81	49.9±4.6
	<i>E.t</i>	33.3	21.4	53.6	39.5	36.9±6.7	33.6	42.0	61.0	41.4	44.5±5.8
F3 (18)	<i>A.a</i>	38.8	62.8	48.2	35.35	46.3±6.1	41.5	42.5	56.7	38.62	44.8±4.0
	<i>E.t</i>	28.1	40.1	45.2	40.9	38.5±3.6	30.7	28.3	55.9	37.4	38.0±6.2
F4 (24)	<i>A.a</i>	38.3	42.9	44.5	34.63	40.0±2.2	40.9	52.0	68.4	21.66	45.7±9.8
	<i>E.t</i>	38.4	68.8	30.3	29.9	41.8±9.1	34.9	32.7	49.7	29.4	36.6±4.5
M±SE	<i>A.a</i>	37.4±0.6	45.8±4.7	48.7±2.5	31.8±2.3		45.4±4.2	44.4±2.0	54.5±4.0	37.0±3.9	
	<i>E.t</i>	36.1±2.6	40.4±7.7	44.9±3.9	38.1±2.2		36.4±2.1	35.5±2.5	53.4±4.4	37.3±2.3	
CV%	<i>A.a</i>	3.69	23.21	11.64	16.12		20.7	10.19	16.62	24.08	
	<i>E.t</i>	16.6	43.1	19.5	13.2		13.3	16.1	18.5	14.2	

Table 32. Bulk density (g cm^{-3}) of rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals.

Fly ash (%)	Species	T1 (Control)				M \pm SE	T2 (Chemical fertilizers)				M \pm SE
		2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0)	<i>A.a</i>	1.15	1.22	1.18	1.16	1.18 \pm 0.01	1.30	1.16	1.40	1.12	1.24 \pm 0.06
	<i>E.t</i>	1.26	1.27	1.14	1.27	1.24 \pm 0.03	1.24	1.17	1.11	1.16	1.17 \pm 0.02
F1 (6)	<i>A.a</i>	1.06	1.16	1.16	1.18	1.14 \pm 0.02	1.17	1.26	1.12	1.18	1.18 \pm 0.02
	<i>E.t</i>	1.14	1.13	1.14	1.22	1.15 \pm 0.02	1.18	1.21	0.97	1.14	1.12 \pm 0.05
F2 (12)	<i>A.a</i>	1.17	0.58	1.14	1.15	1.01 \pm 0.14	1.30	1.19	1.06	1.17	1.18 \pm 0.04
	<i>E.t</i>	1.20	1.18	1.15	1.22	1.18 \pm 0.01	1.18	1.26	1.08	1.21	1.18 \pm 0.03
F3 (18)	<i>A.a</i>	0.98	1.14	1.13	1.16	1.10 \pm 0.04	1.10	1.31	1.13	1.09	1.15 \pm 0.05
	<i>E.t</i>	1.17	1.16	1.03	1.21	1.14 \pm 0.03	1.02	1.13	1.10	1.13	1.18 \pm 0.03
F4 (24)	<i>A.a</i>	1.05	1.23	1.06	1.15	1.12 \pm 0.04	1.33	1.25	1.19	1.14	1.23 \pm 0.04
	<i>E.t</i>	1.20	1.17	1.15	1.24	1.19 \pm 0.02	1.09	1.13	0.98	1.25	1.11 \pm 0.05
M \pm SE	<i>A.a</i>	1.08 \pm 0.03	1.07 \pm 0.1	1.13 \pm 0.02	1.16 \pm 0.006		1.24 \pm 0.04	1.23 \pm 0.02	1.18 \pm 0.05	1.14 \pm 0.01	
	<i>E.t</i>	1.19 \pm 0.01	1.18 \pm 0.02	1.12 \pm 0.02	1.23 \pm 0.01		1.14 \pm 0.03	1.18 \pm 0.02	1.05 \pm 0.03	1.17 \pm 0.02	
CV%	<i>A.a</i>	7.2	25	4.0	1.1		8.0	4.8	11.1	3.2	
	<i>E.t</i>	3.7	4.4	4.6	1.9		8.0	4.8	6.5	4.3	

Essential elements (N, P, K)

Nitrogen

Total and available nitrogen in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 0.02 to 0.12 and 0.005 to 0.01 %, respectively (Table 33a). As the concentration of fly ash increased from 6 to 24%, total and available nitrogen, ranged from 0.02 to 0.12 and 0.006 to 0.01% (Fig.19a), respectively, with no significant variation. In fertilized control soil (F0T2) total and available nitrogen ranged from 0.05 to 0.08 and 0.005 to 0.01 % (Fig.19b), respectively. Overall no significant variation was observed in nitrogen in rhizosphere soil of *Acacia* and *Eucalyptus* (Table 33b).

In the rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* total and available nitrogen ranged from 0.02 to 0.12 and 0.004 to 0.009 %, respectively (Table 33a). Total and available N in the soil increased and ranged from 0.02 to 0.17 and 0.003 to 0.01 %, respectively, with increase in fly ash from 6 to 24 %. In the rhizosphere-fertilized control soil (F0T2) total and available N (Fig. 19c) ranged from 0.04 to 0.12 and 0.002 to 0.01 %, respectively (Table 33b). Soil amended with 18% fly ash and co-mixed with fertilizers had 0.17% nitrogen, which was highest and with increase in fly ash from 6 to 24 % total and available nitrogen ranged from 0.04 to 0.17 and 0.004 to 0.008 %, respectively (Fig. 19d).

A slight increase in nitrogen was observed over the four-year study period and with increased fly ash addition up to 12 % after which it became non-significant in soil without chemical fertilizers (T1) and soil co-mixed with fly ash and chemical fertilizers (T2). Nitrogen in rhizosphere soil of *Acacia* was slightly higher compared to *Eucalyptus* but the difference was statistically non-significant.

Phosphorus

Total and available phosphorus in rhizosphere control soil (F0T1) of *Acacia* ranged from 50 to 384 and 0.05 to 6.29 mg/kg respectively (Table 34a) which ranged from 60 to 456 and 0.74 to 63.9 mg/kg (Fig. 20a) with rise in fly ash from 6 to 24%. In the year 2002 a depressing effect of fly ash addition on phosphorus uptake by soil was observed. In rhizosphere fertilized control soil (F0T2) phosphorus ranged from 20 to 545 and 1.8 to 5.70 mg/kg respectively. Total and available phosphorus (Fig. 20b) ranged from 50 to 581 and 2.96 to 68.7 mg/kg, respectively, with addition of fly ash from 6 to 24% (Table 34b).

In rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* total phosphorus ranged from 60 to 435 mg/kg and available P ranged from 0.55 to 5.0 mg/kg (Table 34a). Phosphorous levels declined in 2002 in the fly ash amended soil on a pattern similar to that observed in *Acacia*. With increased levels of fly ash from 6 to 24 % total and available phosphorus (Fig. 20c) ranged from 37.4 to 545 and 1.90 to 54.5 mg/kg respectively. The rhizosphere fertilized control soil (F0T2) showed an increase in total P from 67.4 to 419 and available phosphorus from 1.05 to 6.99 mg/kg which continued with increasing fly ash percentage from 6 to 24% resulting in total and available phosphorus (Fig. 20d) ranging from 40.0 to 706 and 0.95 to 61.0 mg/kg respectively (Table 34b).

Overall, phosphorus content was greater in the rhizosphere soil of *Eucalyptus* compared to *Acacia* and 18% fly ash amendment increased its concentration in soil. In soil without fertilizers (T1) a significant increase was observed after 2002 while the variation with increasing fly ash percentage was statistically non-significant. In fly ash treated soil co-mixed with chemical fertilizers (T2) an increase was observed over the four-year study period.

Potassium

Total and available potassium in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 1434 to 12080 and 50 to 127 mg/kg respectively (Table 35a). Potassium was highest in 2001 followed by a decrease in subsequent years and ranged from 1300 to 29270 and 94.8 to 300 mg/kg with increase in fly ash from 6 to 24 % (Fig. 21a). In the rhizosphere fertilized control soil (F0T2) total and available K (Fig. 21b) ranged from 2062 to 6450 and 60 to 172 mg/kg respectively and with increase in fly ash percentage from 6 to 24 % total and available K ranged from 1410 to 22930 and 84.4 to 335 mg/kg, respectively (Table 35b).

In rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* total potassium ranged from 1410 to 12230 mg/kg and available K (Fig. 21c) ranged from 58 to 170 mg/kg (Table 35a). With increase in fly ash percentage from 6 to 24% total and available potassium ranged from 1055 to 18520 and 72.6 to 315 mg/kg, respectively. Total and available potassium in rhizosphere fertilized control soil (F0T2) varied from 1830 to 6630 and 85.4 to 150 mg/kg, respectively. In the soil receiving increased dose of fly ash and mixed with fertilizers, the total and available

potassium (Fig. 21d) ranged from 1280 to 82500 and 64.9 to 340 mg/kg, respectively (Table 35b).

Available potassium increased significantly over the period of four-years in the rhizosphere soil of *Acacia* and *Eucalyptus*. Highest potassium in the rhizosphere soil of both tree species was observed in the presence of 18% fly ash, which helped in maintaining nutrient status of soil. Potassium level in rhizosphere soil of *Acacia* was greater compared to *Eucalyptus* in soil containing fly ash alone (T1) and in soil co-mixed with fly ash and chemical fertilizers (T2).over the four-year study period with increasing fly ash percentage at rates 0 to 24%.

Secondary element (S)

Sulphur

Total and available sulphur in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 140 to 436 and 0.39 to 7.7 mg/kg respectively (Table 36a). Sulphur increased over the four-year study period with highest observed in 2004. Total and available sulphur (Fig. 22a) ranged from 120 to 739 and 0.35 to 12.1 mg/kg respectively in soil amended with increased fly ash dose from 6 to 24 %. Total and available sulphur in the fertilized control (F0T2) ranged from 150 to 336 and 0.22 to 15.5 mg/kg respectively. In soil receiving increased fly ash percentage from 6 to 24 % and co-mixed with fertilizers (Table 36b) the total and available sulphur (Fig. 22b) varied from 185 to 672 and 0.30 to 31.5 mg/kg and increased over the four-year study period.

In rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* total sulphur ranged from 106 to 369 mg/kg and available sulphur ranged from 0.29 to 6.0 mg/kg (Table 36a).With increase in fly ash concentration from 6 to 24 % total sulphur ranged from 109 to 569 mg/kg and available S (Fig. 22c) ranged from 0.21 to 12.1 mg/kg. Total and available sulphur in rhizosphere fertilized control (F0T2) ranged from 112.7 to 470 and 0.49 to 11.7 mg/kg, respectively. In soil receiving increased dose of fly ash and co-mixed with fertilizers, total and available sulphur (Fig. 22d) ranged from 105 to 638 and 0.44 to 11.8 mg/kg, respectively (Table 36b).

Overall sulphur increased in rhizosphere soil of both tree species with greater rise in *Eucalyptus* characterized by a distinct variation with fly ash amendment up to 18%. In the rhizosphere soil of *Acacia* as well as *Eucalyptus* potassium was highest in total as well as available form followed by nitrogen. Total sulphur was greater than phosphorous and the trend was reverse for

available sulphur. No significant variation was observed in nitrogen over the years and with increase in fly ash percentage whereas 12 to 18% fly ash was optimum for maintaining phosphorous, potassium and sulphur level in soil.

Table 33 a. Total and available (Avail.) nitrogen (%) in fly ash amended rhizosphere soil (T1) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total N in soil: 0.14 % and fly ash: 0.19 %; Initial Avail. N in soil: 0.005 % and fly ash: 0.002 %; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Ava	Total	Ava
F0 (0%)	<i>A.a</i>	0.06	0.01	0.12	0.0089	0.05	0.0056	0.02	0.007	0.06±0.02	0.007±0.001
	<i>E.t</i>	0.12	0.008	0.12	0.0095	0.04	0.0042	0.02	0.0067	0.07±0.02	0.007±0.001
F1 (6%)	<i>A.a</i>	0.08	0.009	0.10	0.0078	0.07	0.0062	0.09	0.01	0.08±0.006	0.008±0.0008
	<i>E.t</i>	0.16	0.007	0.17	0.0072	0.05	0.0031	0.04	0.0078	0.10±0.04	0.006±0.001
F2 (12%)	<i>A.a</i>	0.04	0.01	0.12	0.0084	0.06	0.0087	0.07	0.009	0.07±0.01	0.009±0.0003
	<i>E.t</i>	0.02	0.008	0.10	0.01	0.06	0.0042	0.03	0.0086	0.05±0.01	0.007±0.001
F3 (18%)	<i>A.a</i>	0.06	0.008	0.10	0.0095	0.06	0.0067	0.05	0.009	0.06±0.01	0.008±0.0003
	<i>E.t</i>	0.02	0.007	0.12	0.0095	0.08	0.0045	0.05	0.0086	0.06±0.02	0.007±0.001
F4 (24%)	<i>A.a</i>	0.02	0.008	0.05	0.0078	0.07	0.0070	0.06	0.009	0.05±0.01	0.007±0.0004
	<i>E.t</i>	0.05	0.008	0.08	0.0078	0.07	0.0059	0.07	0.0086	0.06±0.006	0.007±0.0006
M±SE	<i>A.a</i>	0.05±0.01	0.009±0.0004	0.09±0.01	0.008±0.0003	0.06±0.003	0.006±0.0005	0.05±0.01	0.008±0.0004		
	<i>E.t</i>	0.07±0.02	0.009±0.0004	0.09±0.01	0.0081±0.0005	0.06±0.003	0.004±0.0004	0.05±0.01	0.008±0.0003		
CV%	<i>A.a</i>	43.85	11.11	29.22	8.64	13.49	17.07	44.6	12.45		
	<i>E.t</i>	85.25	7.21	28.36	13.89	26.35	22.91	69.60	10.37		

Table 33b. Total and available (Avail.) nitrogen (%) in fly ash plus chemical fertilizer amended rhizosphere soil (T2) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total N in soil: 0.14 % and fly ash: 0.19 % ; Initial Avail. N in soil: 0.005 % and fly ash: 0.002 %; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	0.06	0.01	0.08	0.01	0.07	0.0059	0.05	0.0098	0.06±0.005	0.008±0.001
	<i>E.t</i>	0.10	0.006	0.12	0.01	0.05	0.0020	0.042	0.007	0.07±0.01	0.006±0.001
F1 (6%)	<i>A.a</i>	0.09	0.009	0.08	0.0089	0.06	0.0076	0.05	0.0098	0.07±0.009	0.008±0.0005
	<i>E.t</i>	0.08	0.007	0.17	0.0084	0.06	0.0045	0.040	0.007	0.08±0.02	0.006±0.0008
F2 (12%)	<i>A.a</i>	0.16	0.008	0.01	0.0089	0.07	0.0067	0.07	0.0118	0.07±0.03	0.008±0.001
	<i>E.t</i>	0.11	0.007	0.20	0.0079	0.07	0.0056	0.049	0.008	0.10±0.03	0.007±0.0005
F3 (18%)	<i>A.a</i>	0.03	0.008	0.09	0.0084	0.08	0.0067	0.06	0.0112	0.06±0.01	0.008±0.0009
	<i>E.t</i>	0.12	0.008	0.17	0.0084	0.07	0.0053	0.056	0.009	0.10±0.02	0.007±0.000
F4 (24%)	<i>A.a</i>	0.07	0.009	0.10	0.0095	0.07	0.0070	0.058	0.0095	0.07±0.009	0.008±0.0006
	<i>E.t</i>	0.08	0.008	0.12	0.0067	0.08	0.0059	0.058	0.006	0.08±0.01	0.006±0.0005
M±SE	<i>A.a</i>	0.08±0.02	0.008±0.0003	0.07±0.015	0.009±0.0002	0.07±0.003	0.006±0.0002	0.05±0.003	0.01±0.0004		
	<i>E.t</i>	0.06±0.02	0.007±0.0003	0.008±0.0005	0.008±0.0005	0.06±0.005	0.004±0.0007	0.04±0.002	0.007±0.0005		
CV%	<i>A.a</i>	59.37	9.51	49.50	6.77	10.10	9.06	14.4	9.75		
	<i>E.t</i>	81.38	11.62	14.33	14.33	17.28	33.81	13.98	15.41		

Table 34a. Total and available (Avail.) phosphorus (mg/kg) in fly ash amended rhizosphere soil (T1) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total P in soil: 117 mg/kg and fly ash: 1084 mg/kg; Initial Avail. P in soil: 0.02 mg/kg and fly ash: 5.12 mg/kg; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	205	0.16	50	6.29	328	0.05	384	3.3	242±74.	2.5±1.5
	<i>E.t</i>	153	2.5	60	0.63	435	0.55	402	5.0	263±92	2.17±1.0
F1 (6%)	<i>A.a</i>	225	0.74	80	6.09	327	1.20	366	3.3	249±64	2.8±1.2
	<i>E.t</i>	204	3.2	37.4	1.93	274	5.30	456	14.1	242±87	6.13±2.7
F2 (12%)	<i>A.a</i>	260	1.78	60	5.76	328	1.40	330	15.6	244±64	6.13±3.3
	<i>E.t</i>	236	3.3	130	4.87	166	1.90	491	9.8	256±81	4.96±1.7
F3 (18%)	<i>A.a</i>	295	2.97	67	7.06	274	2.60	456	38.5	273±79	12.7±8.6
	<i>E.t</i>	321	6.2	50.5	4.43	328	6.20	545	26.3	311±101	10.7±5.2
F4 (24%)	<i>A.a</i>	250	3.97	65	6.95	274	1.40	402	63.9	247±69	19.0±14.9
	<i>E.t</i>	280	3.8	52.5	15.2	166	3.80	456	54.5	239±86	19.3±12.0
M±SE	<i>A.a</i>	247±15	1.9±0.7	61.4±4.8	6.4±0.2	306±13	1.33±0.4	387±21	25±11.6		
	<i>E.t</i>	239±29	3.8±0.6	66.0±16	5.4±2.8	274±51	3.6±1.0	470±23	22±9.0		
CV%	<i>A.a</i>	13.9	81.4	16.9	8.70	9.60	68.0	12.0	104.		
	<i>E.t</i>	27.3	37.3	55.4	106.1	41.7	65.8	11.2	90.4		

Table 34b. Total and available (Avail.) phosphorus (mg/kg) in fly ash plus chemical fertilizer amended rhizosphere soil (T2) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total P in soil: 117 mg/kg and fly ash: 1084 mg/kg; Initial Avail. P in soil: 0.02 mg/kg and fly ash: 5.12 mg/kg; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	220	4.55	20.0	3.42	166	5.70	545	1.8	238±111	3.86±0.8
	<i>E.t</i>	419	3.5	67.4	6.99	274	1.05	384	3.5	286±79.1	3.7±1.2
F1 (6%)	<i>A.a</i>	210	4.64	60.0	4.55	220	15.70	509	32.2	250±94	14.2±6.5
	<i>E.t</i>	261	4.9	40.0	6.19	167	0.95	706	18.9	294±145	7.7±3.8
F2 (12%)	<i>A.a</i>	290	2.96	50.0	8.90	221	5.55	527	68.7	272±98	21.5±16
	<i>E.t</i>	217	7.3	60.0	6.55	300	1.05	527	15.3	276±97	7.5±2.9
F3 (18%)	<i>A.a</i>	280	7.98	70.0	4.66	281	6.20	581	43.2	303±105	15.5±9.2
	<i>E.t</i>	419	12.0	70.0	18.6	327	29.2	627	22.3	360±115	20.5±3.6
F4 (24%)	<i>A.a</i>	325	9.03	150	9.80	328	2.45	473	44.2	319±66	16.3±9.4
	<i>E.t</i>	498	9.4	160.0	13.0	380	16.55	670	61.0	427±107	25±12
M±SE	<i>A.a</i>	265±22	5.8±1.1	70±22	6.3±1.3	243±28	7.1±2.2	527±18	38±11		
	<i>E.t</i>	363±53	7.4±1.5	79.4±20	10.2±2.4	289±35.0	9.7±5.7	563±58	24.2±9.7		
CV%	<i>A.a</i>	18.39	43.8	69.2	45.87	25.69	70.49	7.64	63.78		
	<i>E.t</i>	32.7	46.0	58.5	52.9	27.2	130.95	22.2	89.9		

Table 35a. Total and available (Avail.) potassium (mg/kg) in fly ash amended rhizosphere soil (T1) of *Acacia Auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total K in soil: 342 mg/kg and fly ash: 1075 mg/kg; Initial Avail. K in soil: 69 mg/kg and fly ash: 70.7 mg/kg; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	12080	103.7	3155	127	1930	50	1434	65	4649±2503	86.4±17.6
	<i>E.t</i>	12230	104.2	1410	170	1628	100	1646	58	4228±2667	108±23.1
F1 (6%)	<i>A.a</i>	9820	128.3	2840	174	2394	117	1684	118	4184±1893	134.3±14
	<i>E.t</i>	10280	141.3	1055	148	1836	124	1382	102	3638±2219	129±10.3
F2 (12%)	<i>A.a</i>	7830	94.8	2420	193	2252	134	2056	206	3639±1398	156±25.9
	<i>E.t</i>	11330	96.5	3495	146	1886	143	1708	200	4604±2277	146±21.1
F3 (18%)	<i>A.a</i>	29270	138.3	3450	160	2062	193	1962	282	9186±6703	193±31
	<i>E.t</i>	9000	81.6	1910	170	1928	178	1454	284	3573±1812	178±41.4
F4 (24%)	<i>A.a</i>	2960	131.4	1300	154	2496	203	1728	300	2121±373	197±37.4
	<i>E.t</i>	18520	72.6	2370	194	1776	193	1536	315	6050±4160	194±49.5
M±SE	<i>A.a</i>	12390±4480	119±8.5	2633±375	161±10.9	2227±104	139±28	1773±109	194±46		
	<i>E.t</i>	12270±1652	99±12	2048±424	165±8.7	1811±52.3	148±170	1545±59.8	192±50		
CV%	<i>A.a</i>	80.8	15.9	31.8	15.1	10.4	44.6	13.8	53		
	<i>E.t</i>	30.1	26.7	46.4	11.8	6.4	25.9	8.6	58.1		

Table 35 b. Total and available (Avail.) potassium (mg/kg) in fly ash plus chemical fertilizer amended rhizosphere soil (T2) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total K in soil: 342 mg/kg and fly ash: 1075 mg/kg; Initial Avail. K in soil: 69 mg/kg and fly ash: 70.7 mg/kg; Sp : Species)

Fly ash (%)	Sp.	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	6450	124.6	3210	172	2062	60	2098	69	3455±1083	106±26
	<i>E.t</i>	5810	85.4	6630	150	1830	93	2016	100	4071±1252	107±15
F1 (6%)	<i>A.a</i>	15060	84.4	2975	169	2170	118	1604	125	5452±3214	124±17
	<i>E.t</i>	8250	64.9	1435	125	1574	132	2250	126	3377±1633	111±16
F2 (12%)	<i>A.a</i>	8050	100.1	1910	143	2302	196	1960	214	3555±1500	163±26
	<i>E.t</i>	1440	87.1	1965	160	2258	175	3522	199	2296±442	155±24
F3 (18%)	<i>A.a</i>	22930	124.7	2295	128	2394	200	2720	298	7584±5715	188±41
	<i>E.t</i>	7030	62.6	1280	155	2134	207	1470	226	2978±1362	163±37
F4 (24%)	<i>A.a</i>	11920	135.7	1410	171	1922	225	2666	335	4479±2493	217±44
	<i>E.t</i>	9550	70.9	2015	127	1854	245	1614	340	3758±1932	196±60
M±SE	<i>A.a</i>	12880±2927	114±9.4	2360±332	156±8.9	2170±84	160±31	2210±213	208±50		
	<i>E.t</i>	6416±1391	74±54	2665±1002	143.4±7.3	1930±121	170.4±27	2174±364	198±42		
CV%	<i>A.a</i>	50.81	18.4	31.5	12.78	6.64	43.0	21.59	53.9		
	<i>E.t</i>	48.47	15.4	84.04	11.36	13.99	35.2	37.48	47.7		

Table 36a. Total and available (Avail.) sulphur (mg/kg) in fly ash amended rhizosphere soil (T1) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total S in soil: 2.9 mg/kg and fly ash: 10.7 mg/kg ; Initial Avail. S in soil: 0.018 mg/kg and fly ash: 2.12 mg/kg; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	140	0.39	336	7.7	201	3.26	436	6.7	278±66	4.51±1.7
	<i>E.t</i>	106	0.29	369	5.37	302	2.98	201	6.0	244±57	3.6±1.3
F1 (6%)	<i>A.a</i>	125	0.35	369	4.7	235	3.86	369	8.0	274±58	4.22±1.6
	<i>E.t</i>	153	0.28	268	6.38	168	9.61	302	10.1	222±37	6.6±2.3
F2 (12%)	<i>A.a</i>	145	0.38	302	9.7	285	7.56	504	6.4	309±74	6.0±1.9
	<i>E.t</i>	116	0.26	235	8.06	403	8.78	235	12.1	247±59	7.3±2.5
F3 (18%)	<i>A.a</i>	135	0.43	201	12.0	336	3.20	705	5.7	344±127	5.33±2.5
	<i>E.t</i>	109	0.26	336	9.74	369	4.75	369	7.4	295±63	5.5±2.0
F4 (24%)	<i>A.a</i>	120	0.47	396	11.4	300	5.30	739	11.4	388±130	7.14±2.6
	<i>E.t</i>	105	0.21	537	11.4	569	3.31	470	10.4	420±10.7	6.3±2.7
M±SE	<i>A.a</i>	133±4.63	0.40±0.02	320±33	9.1±1.3	271±19.6	4.6±0.8	550±73.3	7.6±1.0		
	<i>E.t</i>	118±9.0	0.26±0.01	349±53	8.2±1.1	362±65	5.8±1.4	315±48	9.2±1.09		
CV%	<i>A.a</i>	7.80	11.56	23.5	32.6	19.6	39.7	29.7	29.6		
	<i>E.t</i>	17.1	11.85	33.7	29.88	40.4	52.79	34.1	26.7		

Table 36 b. Total and available (Avail.) sulphur (mg/kg) in fly ash plus chemical fertilizer amended rhizosphere soil (T2) of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total S in soil: 2.9 mg/kg and fly ash: 10.7 mg/kg Initial Avail. S in soil: 0.018 mg/kg and fly ash: 2.12 mg/kg; *Sp*: Species)

Fly ash (%)	<i>Sp.</i>	2001		2002		2003		2004		Mean	
		Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.	Total	Avail.
F0 (0%)	<i>A.a</i>	150	0.22	336	6.4	168	15.5	336	3.0	247±51	6.3±3.3
	<i>E.t</i>	112.7	0.49	302	11.7	470	3.43	235	7.1	280±74	5.6±2.4
F1 (6%)	<i>A.a</i>	185	0.30	235	13.8	269	31.5	604	3.7	323±95	12.3±7.0
	<i>E.t</i>	105	0.56	369	10.0	403	6.41	403	11.8	320±72	7.1±2.5
F2 (12%)	<i>A.a</i>	220	0.20	201	10.0	269	10.7	571	4.9	315±86	6.4±2.5
	<i>E.t</i>	106.5	0.44	268	6.4	403	3.81	537	8.4	328±92	4.7±1.7
F3 (18%)	<i>A.a</i>	195	0.13	268	10.0	235	8.3	638	4.4	334±12	5.7±2.1
	<i>E.t</i>	114.1	0.44	470	6.0	168	5.52	504	15.1	314±100	6.7±3.0
F4 (24%)	<i>A.a</i>	190	0.29	403	7.9	336	8.7	672	7.4	400±100	6.0±1.9
	<i>E.t</i>	121.8	0.46	638	5.7	336	4.61	604	9.7	424±121	5.1±1.9
M±SE	<i>A.a</i>	188±11	0.22±0.03	288±36	9.6±1.2	255.4±27	14.9±4.3	564±59	4.6±0.7		
	<i>E.t</i>	112.0±3	0.47±0.02	409±66	7.9±1.2	356±51.	4.75±0.5	456±64	10.4±1.4		
CV%	<i>A.a</i>	13.38	30.61	28.1	28.9	23.9	64.86	23.5	35.9		
	<i>E.t</i>	5.99	10.5	36.4	34.1	32.41	25.7	31.4	30.13		

Micronutrients (Fe, Zn, Mn)

Iron

Total and DTPA-extractable iron in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 5315 to 37060 and 18.6 to 65.6 mg/kg, respectively (Table 37). With increase in fly ash from 6 to 24%, increase in total iron was from 5810 to 86500 mg/kg (Fig. 23a) and DTPA-extractable Fe ranged from 10.8 to 72.6 mg/kg. Iron showed a decrease until 2003, with a reverse trend for increasing fly ash percentage. Iron in fertilized control soil (F0T2) in total and DTPA-extractable forms ranged from 16785 to 50130 and 32.5 to 79.8 mg/kg respectively. With increase in fly ash percentage from 6 to 24% total iron ranged from 7180 to 79100 mg/kg (Fig. 23b) and DTPA-extractable iron varied from 11.4 to 86.3 mg/kg. Over the four-year study period a decrease in total Fe was observed while DTPA-extractable iron increased with no significant variation with rise in fly ash.

Total and DTPA-extractable iron in rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* ranged from 11265 to 48300 and 11.8 to 56.0 mg/kg respectively (Table 37). Total Fe (Fig. 23c) ranged from 5500 to 71400 mg/kg and DTPA-extractable iron varied from 9.7 to 52.8 mg/kg with increase in fly ash percentage up to 24% in soil. Total iron did not show any distinct variation with increasing fly ash percentage. In *Eucalyptus tereticornis* total and DTPA-extractable Fe in rhizosphere fertilized control soil (F0T2) ranged from 9330 to 43410 and 20.4 to 80.0 mg/kg respectively. With increase in fly ash from 6 to 24 %, iron ranged from 8050 to 87900 mg/kg with no distinct variation (Fig. 23d). The DTPA-extractable iron ranged from 12.1 to 75.0 mg/kg showing an irregular variation with no discernible trend.

Zinc

Total and DTPA-extractable zinc in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 21.1 to 87.0 and 1.6 to 10.1 mg/kg respectively (Table 38). With increase in fly ash from 6 to 24 % total Zn (Fig. 24a) was observed to vary from 23.0 to 95.0 mg/kg while DTPA-extractable Zn ranged from 0.4 to 21.4 mg/kg, respectively, over the four-year study period. Total and DTPA-extractable zinc decreased

over the four-year study period and no distinct variation was observed with increasing fly ash percentage.

Total and DTPA-extractable zinc in rhizosphere fertilized control soil (F0T2) ranged from 23.2 to 38.0 and 1.2 to 7.5 mg/kg. With increased addition of fly ash from 6 to 24% total and DTPA-extractable zinc varied from 17.9 to 56.0 and 0.7 to 13.3 mg/kg, respectively. Total (Fig. 24b) and DTPA-extractable zinc did not exhibit any distinct variation with increasing fly ash percentage.

In rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* total and DTPA-extractable zinc varied from 13.1 to 41.9 and 1.0 to 12.6 mg/kg, respectively (Table 38). With elevated level of fly ash in soil from 6 to 24% total and DTPA-extractable zinc ranged from 16.6 to 59 and 0.6 to 8.6 mg/kg, respectively. Total Zn (Fig. 24c) decreased in 2002 followed by an increase while no distinct variation was observed with increasing fly ash percentage with a similar pattern observed for DTPA-extractable zinc.

Total and DTPA-extractable zinc in fertilized control soil (F0T2) varied from 22.8 to 42.9 and 1.2 to 14.0 mg/kg respectively. With increase in fly ash percentage from 6 to 24%, total zinc (Fig. 24d) ranged from 20.9 to 50.1 mg/kg and DTPA-extractable zinc varied from 0.5 to 24.8 mg/kg. Total zinc in soil containing fertilizers decreased in 2002 followed by an increase with a similar trend observed for DTPA-extractable zinc.

Manganese

Total and DTPA-extractable manganese in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 320 to 1500 and 23.6 to 118 mg/kg respectively (Table 39). With rise in concentration of fly ash from 6 to 24% total and DTPA-extractable manganese ranged from 195 to 5306 and 7.6 to 174 mg/kg, respectively. Total Mn (Fig. 25a) showed an irregular variation over the four-year study period with an increase with rise in fly ash percentage in soil. DTPA-extractable manganese showed a significant variation over the years but with rise in fly ash percentage it was non-significant.

Total and DTPA-extractable manganese ranged from 305 to 3527 and 15.1 to 284 mg/kg respectively in fertilized control soil (F0T2) of *Acacia auriculiformis*. With increase in fly ash percentage from 6 to 24%, total manganese (Fig. 25b) varied from 175 to 5162

mg/kg and DTPA-extractable manganese ranged from 7.8 to 288 mg/kg. Total Mn increased with increasing fly ash percentage while the trend was the opposite for DTPA-extractable manganese.

Total and DTPA-extractable Mn in rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* ranged from 360 to 5021 and 66 to 129 mg/kg, respectively (Table 39). With increasing fly ash percentage from 6 to 24 %, total manganese (Fig. 25c) ranged from 325 to 5260 mg/kg and DTPA-extractable manganese from 38 to 169 mg/kg. No discernible trend was observed for total Mn while DTPA-extractable manganese decreased over the years.

Total and DTPA-extractable manganese in fertilized control soil (F0T2) ranged from 400 to 844 and 52 to 133 mg/kg respectively. As the fly ash percentage increased from 6 to 24% manganese ranged from 355 to 5578 and 56 to 271 mg/kg. Total manganese (Fig. 25d) increased until 12% fly ash amendment followed by a decrease with increase in fly ash up to 24%. DTPA-extractable manganese decreased over a period of four-years and with increasing fly ash percentage in the soil.

In rhizosphere soil without chemical fertilizers (T1) and soil mixed with fly ash and chemical fertilizers (T2) for both *Acacia auriculiformis* and *Eucalyptus tereticornis* the DTPA-extractable micronutrients varied in as Mn>Fe>Zn and total elemental concentration varied as Fe>Mn>Zn, respectively.

Heavy metals (Ni, Cr, Pb)

Nickel

Total nickel in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 26.8 to 32.9 mg/kg (Table 40). With increase in fly ash percentage from 6 to 24%, nickel was observed to range from 18.6 to 116 mg/kg (Fig. 26a) with an increase in soil amended with 12% fly ash. A decline in nickel concentration was observed over the years while it increased with increasing fly ash percentage. DTPA-extractable nickel was below detection limits i.e, less than 0.04 mg/kg for the years 2003 and 2004 and overall was less than 1.2 mg/kg.

Nickel in the rhizosphere fertilized control soil (F0T2) ranged from 19.7 to 65 mg/kg and with increased fly ash concentration from 6 to 24% it varied from 17.1 to 72.9 mg/kg with no significant variation with increasing fly ash percentage (Fig. 26b) and a decrease over the years. DTPA-extractable nickel was very low or below the detection limit.

In rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* (Fig.26c) nickel ranged from 15.4 to 54.0 and 12.3 to 72.4 mg/kg in soil receiving an increased fly ash dose from 6 to 24% (Table 40). Over the years the nickel concentration declined in soil receiving increased applications of fly ash. In fertilized control soil (F0T2) Ni ranged from 15.9 to 26.7 mg/kg. With increase in fly ash percentage from 6 to 24% nickel in soil mixed with fertilizers ranged from 19.0 to 56.1 mg/kg with a decrease over the years (Fig. 26d). The DTPA-extractable nickel was low or below detection limit over the four-year study period.

Chromium

Chromium in rhizosphere control soil (F0T1) of *Acacia auriculiformis* ranged from 12.8 to 76.6 mg/kg (Table 41) and from 16 to 99 mg/kg with increase in fly ash from 6 to 24%. Total chromium showed an irregular trend in soil over the four-year study period (Fig. 27a) and with increasing fly ash percentage. In fertilized control (F0T2) chromium ranged from 22.8 to 34.2 mg/kg and from 16.5 to 78.9 mg/kg in soil amended with fly ash up to 24% and mixed with fertilizers (Fig. 27b). No significant variation was observed in chromium concentration with increased fly ash addition and DTPA-extractable chromium was below the detection limit.

Cr in rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* ranged from 20.5 to 54.3 mg/kg and with increase in fly ash percentage from 6 to 24 %, it ranged from 14.8 to 62.3 mg/kg (Table 41). Total chromium (Fig. 27c) did not vary significantly with increasing fly ash percentage. Cr in fertilized control soil (F0T2) ranged from 22.8 to 34.2 mg/kg without fly ash. With increase in fly ash percentage in soil from 6 to 24% the observed range was 13.5 to 68.2 mg/kg. An irregular trend in total chromium was observed over the four-year study period (Fig. 27d) and DTPA-extractable chromium was below the detection limit.

Lead

Total and DTPA-extractable lead in rhizosphere control soil (F0T1) of *Acacia auriculiformis* was 11.0 to 27.0 and 1.8 to 4.2 mg/kg respectively (Table 42). Increased application of fly ash in the soil from 6 to 24% resulted in an increase which ranged from 14.4 to 82.2 mg/kg (Fig. 28a). A slight increase in lead was observed with increasing fly ash percentage. DTPA-extractable lead varied from 1.1 to 7.5 mg/kg with rise in fly ash up to 24% showing a non-significant variation with increased fly ash level in soil beyond year 2002.

Lead in rhizosphere fertilized control (F0T2) ranged from 15.3 to 36.0 mg/kg in the soil mixed with fly ash and chemical fertilizers and with increase in fly ash percentage from 6 to 24%, it ranged from 17.0 to 97.2 mg/kg (Fig. 28b). A slight increase in lead was observed with increasing fly ash percentage in soil. The DTPA-extractable lead varied from 1.5 to 3.5 mg/kg in control (F0T2) and ranged from 1.0 to 4.2 mg/kg with increased fly ash percentage. The variation observed in DTPA-extractable lead was statistically non-significant with increase in fly ash percentage and over the four-year study period.

Lead in rhizosphere control soil (F0T1) of *Eucalyptus tereticornis* ranged from 12.8 to 39.3 mg/kg (Fig. 28c) and in soil plus fly ash mixtures ranged from 16.8 to 58.2 mg/kg (Table 42). No distinct variation was observed with increasing fly ash percentage and over the years. DTPA-extractable lead ranged from 1.3 to 2.1 mg/kg in control (F0T1) and with increased fly ash percentage from 6 to 24%, it ranged from 0.6 to 2.7 mg/kg. In fertilized control soil (F0T2) lead ranged from 16.0 to 52.6 mg/kg (Fig. 28d) and 16.8 to 45.4 mg/kg with rise in fly ash from 6 to 24%. DTPA-extractable lead ranged from 1.5 to 2.1 mg/kg in fertilized control (F0T2) and with increased fly ash percentage the observed range was 1.3 to 4.2 mg/kg. Overall no significant variation was observed in lead with increasing fly ash percentage and over the years.

Overall iron (5315 to 87900 mg/kg), zinc (13.1 to 95.0 mg/kg), nickel (12.3 to 116 mg/kg), chromium (12.8 to 99 mg/kg) and lead (11.0 to 97.2 pm) occurred within the limits prescribed for normal cropland soils. All elements except manganese (175 to 5578 mg/kg) were higher in rhizosphere soil of *Acacia auriculiformis* and varied as Fe>Mn>Pb>Zn for both tree species. Nickel concentration was higher than that for chromium in *Acacia* with a reverse trend observed in rhizosphere soil of *Eucalyptus*.

Table 37. Total and DTPA-extractable iron (mg/kg) in rhizosphere soil of *Acacia auriculiformis* (A.a) and *Eucalyptus tereticornis* (E.t) at yearly intervals. (Initial total Fe in soil: 32420 mg/kg and fly ash: 6762 mg/kg)

Fly ash (%)	Species		T1 (Control)					T2 (Chemical fertilizers)				
			2001	2002	2003	2004	M±SE	2001	2002	2003	2004	M±SE
F0 (0%)	A.a	Total	28300	5315	29830	37060	25126±6874	50130	16785	19750	42980	32411±8317
		Ava.	24.1	43.2	18.6	65.6	37.9±10.6	32.5	62.8	35.4	79.8	52.6±11.3
	E.t	Total	38520	11265	34910	48300	33248±7854	21390	9330	32820	43410	26737±7340
		Ava.	33.1	56	11.8	40.9	36±9.2	24.2	80	20.4	61.8	46.6±14.5
F1 (6%)	A.a	Total	86500	18765	23290	56100	46163±15810	46900	15100	30990	41930	33730±7043
		Ava.	29.6	51.2	16.8	64.1	40.4±10.6	30.0	69.2	44.5	80.3	56±11.4
	E.t	Total	39680	7785	38120	44450	32508±8350	69500	8050	31480	42480	37877±12753
		Ava.	31.2	38	28.0	46.8	36±4.2	26.7	48	12.1	47.0	33.5±8.6
F2 (12%)	A.a	Total	66100	5810	29660	51160	38182±13128	79100	7180	29490	45920	40422±15140
		Ava.	28.9	36.4	18.4	72.6	39.0±11.8	23.6	28.8	24.8	77.5	38.6±12.9
	E.t	Total	41320	14375	38790	44660	34786±6909	68650	9605	34410	37720	37596±12103
		Ava.	32.2	42	13.3	52.8	35±8.4	32	35	10.6	32.5	27.5±5.7
F3 (18%)	A.a	Total	79700	11550	28440	50980	42667±14751	9900	20290	21420	35340	21737±5222
		Ava.	10.8	47.2	34.8	65.8	39.6±11.5	11.4	64	17.2	81.9	43.6±17.3
	E.t	Total	71400	18215	37910	38670	41548±11019	41910	14845	32090	28030	29218±5607
		Ava.	27.1	48	12.1	31.7	29.7±7.4	34.4	60	19.4	37.7	37.9±8.4
F4 (24%)	A.a	Total	81500	18850	27300	66840	48622±15148	56900	12680	24540	43580	34425±9829
		Ava.	23.4	16.4	31.2	62.3	33.3±10.1	22.9	51.6	13.8	86.3	43.6±16.3
	E.t	Total	62610	5500	41130	32680	35480±11813	87900	9315	31890	28500	39401±16912
		Ava.	34.7	64	9.7	28.9	34.3±11.2	29.1	47	14.7	75	41.4±12.9
M±SE	A.a	Total	68420±10580	12060±2965	27700±1194	52430±4801		48590±11180	14410±2190	25240±2195	41950±1777	
		Ava.	23.4±3.4	38.8±6.1	23.9±3.7	66±1.7		24±3.7	55.2±7.2	27.1±5.7	81.1±1.5	
	E.t	Total	50710±6812	11430±2271	38170±996	41750±2742		57870±11700	10230±1185	32540±515	36030±3314	
		Ava.	31.6±1.3	49.6±4.7	14.9±3.3	40.2±4.5		29.2±1.8	54.0±7.6	15.4±1.9	50.8±7.8	
CV%	A.a	Total	34.6	55	9.7	20.5		51.4	34	19.5	9.5	
		Ava.	32.3	35.2	35.0	5.9		34.0	29.2	47.0	4.0	
	E.t	Total	30.0	44.4	5.8	14.7		45.2	26.0	3.5	20.6	
		Ava.	9.0	21.2	49.3	25		13.8	31.5	28.1	35.0	

Table 38. Total and DTPA-extractable zinc (mg/kg) in rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total Zn in soil: 71.8 mg/kg and fly ash: 79 mg/kg)

Fly ash (%)	Species		T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE
			2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0%)	A.a	Total	87.0	21.1	42.7	27.4	44.5±14.8	38.0	23.2	34.5	31.1	31.7±3.2
		Ava.	2.2	5.8	10.1	1.6	4.9±1.9	1.2	4.6	7.5	2.0	3.8±1.4
	<i>E.t</i>	Total	13.1	28.6	41.9	27.2	27.7±5.8	25.2	22.8	42.9	29.9	30.2±4.5
		Ava.	1.4	1.0	12.6	2.0	4.25±2.8	1.7	1.2	14.0	1.7	4.6±3.1
F1 (6%)	<i>A.a</i>	Total	95.0	23.0	43.4	28.5	47.5±16.4	31.0	17.9	40.1	26.7	28.9±4.6
		Ava.	1.4	1.6	9.5	1.6	3.5±1.9	0.7	0.9	11.0	1.5	3.5±2.5
	<i>E.t</i>	Total	52.3	16.6	48.4	29.7	36.7±8.3	20.9	25.2	43.1	24.8	28.5±4.9
		Ava.	1.9	0.6	8.6	1.2	3.0±1.8	1.2	1.0	24.8	1.5	7.1±5.9
F2 (12%)	<i>A.a</i>	Total	69.0	30.6	37.0	29.5	41.5±9.3	47.0	20.3	32.7	30.6	32.6±5.5
		Ava.	0.7	0.8	10.3	1.6	3.3±2.3	3.6	1.1	13.3	1.9	4.9±2.8
	<i>E.t</i>	Total	49.7	36.8	50.8	27.6	41.2±5.5	21.8	23.0	49.8	29.4	31.0±6.5
		Ava.	0.6	0.9	4.8	1.2	1.9±0.9	0.5	0.6	7.9	1.3	2.6±1.8
F3 (18%)	<i>A.a</i>	Total	94.0	40.6	35.3	32.1	50.4±14.6	56.0	27.0	35.5	33.0	37.8±6.3
		Ava.	1.4	0.9	21.4	1.8	6.4±5.0	2.0	0.8	3.4	2.0	2.0±0.5
	<i>E.t</i>	Total	49.4	27.0	59.0	29.4	41.2±7.7	41.1	23.8	50.1	28.1	35.7±6.0
		Ava.	0.8	1.7	4.9	1.5	2.2±0.9	1.8	1.6	12.5	1.4	4.4±2.7
F4 (24%)	<i>A.a</i>	Total	91.0	28.5	36.4	39.3	48.8±14.3	53.0	34.1	28.8	31.2	36.7±5.5
		Ava.	0.4	0.9	6.9	1.9	2.5±1.5	2.4	0.7	7.0	2.6	3.2±1.3
	<i>E.t</i>	Total	46.9	26.8	53.9	25.7	38.3±7.1	48.9	29.6	43.6	30.5	38.1±4.8
		Ava.	2.0	0.7	2.8	1.4	1.7±0.4	2.6	0.7	6.5	1.4	3.0±1.2
M±SE	<i>A.a</i>	Total	87.2±4.8	28.6±3.3	38.9±1.7	31.3±2.1		45.0±4.7	24.5±2.8	34.3±1.8	30.5±1.0	
		Ava.	1.2±0.3	2.0±0.9	11.6±2.5	1.7±0.06		1.9±0.5	1.6±0.7	8.4±1.7	2.0±0.1	
	<i>E.t</i>	Total	42.2±7.3	27.1±3.2	50.8±2.8	27.9±0.7		31.5±5.6	24.8±1.3	45.9±1.6	28.5±1.0	
		Ava.	1.3±0.3	0.9±0.2	6.7±1.7	1.5±0.1		1.56±0.3	1.0±0.1	13.1±3.2	1.5±0.06	
CV%	<i>A.a</i>	Total	12.2	25.9	9.7	15.2		23.2	25.9	12.0	7.6	
		Ava.	57.4	107	48.3	8.3		56.7	103	45.3	19.7	
	<i>E.t</i>	Total	39.0	26.5	12.5	5.9		41.0	11.2	8.0	7.9	
		Ava.	47.0	44.1	57.7	22.5		49.8	39.5	54.9	10.4	

Table 39. Total and DTPA-extractable manganese (mg/kg) in rhizosphere soil of *Acacia auriculiformis*(A.a) and *Eucalyptus tereticornis*(E.t) at yearly intervals. (Initial total Mn in soil: 879 mg/kg and fly ash: 739 mg/kg)

Fly ash (%)	Species	T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE	
		2001	2002	2003	2004		2001	2002	2003	2004		
F0 (0%)	A.a	Total	415	320	1500	792	756±267	3527	305	1240	836	1477±709
		Ava.	23.6	82.8	107	118	82.8±21.0	15.1	103	284	133	134±55.9
	E.t	Total	5021	360	954	598	1733±1102	499	400	844	704	611±100
		Ava.	125	66	129	121	110±14.8	123	52	128	133	109±19.1
F1 (6%)	A.a	Total	5306	290	1828	958	2095±1115	2916	465	1090	760	1307±551
		Ava.	7.6	81.6	117	111	79.3±21.3	9.6	92.4	288	121	127±58.4
	E.t	Total	4013	325	1016	626	1495±851	370	355	644	730	524±95.3
		Ava.	169	38	88	125	105±27.8	138	58	156	111	115±21.4
F2 (12%)	A.a	Total	823	1265	1370	952	1102±128	4463	175	1030	718	1596±971
		Ava.	11.3	86.8	151	122	92.7±30.1	7.8	82.8	80	87	64.4±18.9
	E.t	Total	1362	345	1012	698	854±217	2960	425	842	786	1253±577
		Ava.	102	72	151	101	107±16.4	86	56	107	82	82.7±10.5
F3 (18%)	A.a	Total	2453	195	1116	842	1151±474	5162	250	1088	774	1818±1127
		Ava.	20.9	70.4	174	128	98.3±33.4	9.6	72.8	80	87	62.4±17.8
	E.t	Total	4004	395	1018	754	1542±830	1693	545	796	616	912±265
		Ava.	120	69	77	82	87±11.3	136	57	83	75	87.7±16.9
F4 (24%)	A.a	Total	3833	450	1400	880	1640±756	4012	425	852	548	1459±855
		Ava.	6.0	76.4	126	81.6	72.5±24.8	15	77.2	102	81.3	68.8±18.7
	E.t	Total	5260	435	1282	646	1905±1132	5578	455	930	532	1873±1239
		Ava.	141	62	60	70	83.3±19.4	271	68	96	57	123±50
M±SE	A.a	Total	2566±916	504±194	1443±15.2	884±31.9		4016±384	324±53.9	1060±62.5	727±48.6	
		Ava.	13.8±3.5	79.6±2.8	135±12.2	112±8.1		11.4±1.5	85.6±5.4	166±48	101±10.5	
	E.t	Total	3932±691	372±19.4	1056±57	664±27.7		2220±961	436±31.8	811±47	674±45	
		Ava.	731±11.2	61.4±6.1	101±16.9	99.8±10.7		150±31.5	58.2±2.6	114±12.8	91.6±13.5	
CV%	A.a	Total	79.8	86.3	17.9	8.0		21.4	37.2	13.2	14.9	
		Ava.	57.2	7.9	20.1	16.2		29.7	14.2	65.4	23.0	
	E.t	Total	39	11.7	12.2	9.3		96.8	16.3	12.9	14.9	
		Ava.	19.2	22.1	37.4	23.9		46.6	10.1	25.2	33.0	

Table 40. Total and DTPA-extractable nickel (mg/kg) in rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total Ni in soil: 28.25 mg/kg and fly ash: 13 mg/kg).

Fly ash (%)	Species	T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE	
		2001	2002	2003	2004		2001	2002	2003	2004		
F0 (0%)	A.a	Total	32.9	36.3	26.8	25.7	30.4±2.5	65	28.2	19.7	26.5	34.8±10.2
		Ava.	0.4	0.6	<0.04	<0.04	0.3±0.1	1.2	0.8	<0.04	<0.04	0.5±0.2
	E.t	Total	54.0	25.4	15.4	24.6	29.8±8.4	23	26.7	15.9	18.6	21±2.4
		Ava.	0.3	0.6	<0.04	<0.04	0.2±0.1	0.2	0.7	<0.04	<0.04	0.2±0.1
F1 (6%)	A.a	Total	40.8	24.9	27.5	24.2	29.6±3.8	72.9	26.8	21.7	30.3	37.9±11.8
		Ava.	0.5	0.5	<0.04	<0.04	0.2±0.1	0.8	0.8	<0.04	<0.04	0.4±0.2
	E.t	Total	40.0	12.3	28.7	20.6	25.4±5.9	56.1	25.6	20.2	26.2	32±8.1
		Ava.	0.4	0.5	<0.04	<0.04	0.2±0.1	0.1	0.7	<0.04	<0.04	0.2±.1
F2 (12%)	A.a	Total	47.6	29.7	28.6	32.1	26.5±9.7	31.7	17.1	26.6	26.5	25.4±3.0
		Ava.	0.6	0.5	<0.04	<0.04	0.3±0.1	0.6	0.7	<0.04	<0.04	0.3±0.1
	E.t	Total	72.4	27.2	26.8	28.2	38.6±111.2	40.0	24.7	27.3	25.6	29.4±3.6
		Ava.	0.1	0.5	<0.04	<0.04	0.17±0.1	0.2	0.5	<0.04	<0.04	0.19±0.10
F3 (18%)	A.a	Total	116	21.3	28.7	27.7	48.4±22.6	52.5	24.5	25.4	29.8	33±6.6
		Ava.	0.9	0.4	<0.04	<0.04	0.3±0.2	0.7	0.8	<0.04	<0.04	0.3±0.2
	E.t	Total	61.2	22.1	29.8	20.9	33.5±9.4	38.1	19.4	25.5	24.4	26.8±3.9
		Ava.	0.8	0.7	<0.04	<0.04	0.4±0.2	0.5	0.3	<0.04	<0.04	0.2±0.1
F4 (24%)	A.a	Total	103	18.6	30.1	27.9	44.9±19.5	43.0	23.1	24.5	35.9	31.6±4.7
		Ava.	0.1	0.2	<0.04	<0.04	0.09±0.03	0.4	0.3	<0.04	<0.04	0.19±0.09
	E.t	Total	56.0	20.0	27.5	24.7	32.0±8.1	23.1	19.0	26.6	19.3	22±1.8
		Ava.	0.4	0.7	<0.04	<0.04	0.2±0.1	0.7	0.8	<0.04	<0.04	0.4±0.2
M±SE	A.a	Total	68±17.2	26.1±3.1	28.3±0.5	21.4±5.3		53±7.4	23.9±1.9	23.5±1.3	29.8±1.7	
		Ava.	0.5±0.1	0.4±0.06	<0.04	<0.04		0.74±0.1	0.68±0.09	<0.04	<0.04	
	E.t	Total	56.7±5.3	21.4±2.6	25.6±2.6	23.8±1.4		36±6.1	23±1.6	23.1±2.1	22.8±1.6	
		Ava.	0.40±0.1	0.60±0.04	<0.04	<0.04		0.3±0.1	0.6±0.08	<0.04	<0.04	
CV%	A.a	Total	56.5	27	4.5	55.5		31.2	17.9	11.9	12.9	
		Ava.	58.3	34.5	<0.04	<0.04		40.0	31.8	<0.04	<0.04	
	E.t	Total	21	27	23	13.2		38.2	15.6	21.2	15.8	
		Ava.	63.7	16.7	<0.04	<0.04		73.8	33.3	<0.04	<0.04	

Table 41. Total and DTPA-extractable chromium (mg/kg) in rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) at yearly intervals. (Initial total Cr in soil: 58.63 mg/kg and fly ash: 330 mg/kg).

Fly ash (%)	Species	T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE	
		2001	2002	2003	2004		2001	2002	2003	2004		
F0 (0%)	<i>A.a</i>	Total	76.6	12.8	39.3	61.0	47.4±13.8	34.1	22.8	34.2	30.8	30.4±2.7
		Ava.	<0.05	0.6	<0.05	<0.05	0.18±0.13	<0.05	0.6	<0.05	<0.05	0.18±0.13
	<i>E.t</i>	Total	26.9	20.5	43.7	54.3	36.3±7.7	20.1	30.9	42.8	38.0	32.9±4.9
		Ava.	<0.05	2.5	<0.05	<0.05	0.66±0.61	<0.05	2.3	<0.05	<0.05	0.6±0.5
F1 (6%)	<i>A.a</i>	Total	25.8	17.0	42.5	99.0	46±18.4	30.2	17.9	27.3	78.9	38.5±13.7
		Ava.	<0.05	0.9	<0.05	<0.05	0.26±0.21	<0.05	1.3	<0.05	<0.05	0.36±0.31
	<i>E.t</i>	Total	16.1	14.5	60.3	65.8	39.1±13.8	18.9	15.9	41.2	62.3	34.5±10.8
		Ava.	<0.05	2.5	<0.05	<0.05	0.66±0.61	<0.05	2.5	<0.05	<0.05	0.66±0.61
F2 (12%)	<i>A.a</i>	Total	24.0	16.0	28.7	35.9	26.1±4.2	26.6	28.8	22.8	42.0	30.0±4.2
		Ava.	<0.05	1.2	<0.05	<0.05	0.3±0.2	<0.05	1.7	<0.05	<0.05	0.46±0.41
	<i>E.t</i>	Total	43.2	19.3	50.7	47.9	40.2±7.2	17.7	20.1	36.2	50.0	31.0±7.5
		Ava.	<0.05	2.8	<0.05	<0.05	0.7±0.6	<0.05	3.0	<0.05	<0.05	0.78±0.73
F3 (18%)	<i>A.a</i>	Total	51.7	24.3	43.7	38.3	39.5±5.7	28.6	31.0	38.9	22.3	30.2±3.4
		Ava.	<0.05	1.9	<0.05	<0.05	0.5±0.4	<0.05	1.7	<0.05	<0.05	0.46±0.41
	<i>E.t</i>	Total	16.2	21.8	56.3	68.2	40.6±12.7	29.9	14.4	46.7	31.4	30.6±6.6
		Ava.	<0.05	3.0	<0.05	<0.05	0.78±0.73	<0.05	3.1	<0.05	<0.05	0.8±0.7
F4 (24%)	<i>A.a</i>	Total	25.6	34.8	41.9	62.4	41.1±7.8	30.4	16.5	55.0	25.5	31.8±8.2
		Ava.	<0.05	1.6	<0.05	<0.05	0.4±0.3	<0.05	2.1	<0.05	<0.05	0.56±0.51
	<i>E.t</i>	Total	26.4	13.5	53.7	28.2	30.4±8.4	33.0	16.0	50.8	18.4	29.5±8.0
		Ava.	<0.05	3.6	<0.05	<0.05	0.9±0.8	<0.05	3.4	<0.05	<0.05	0.88±0.83
M±SE	<i>A.a</i>	Total	40.7±10.3	20.9±3.9	39.2±2.7	59.8±11.3		29.9±1.2	23.4±2.8	35.6±5.6	39.9±10.3	
		Ava.	<0.05	1.24±0.2	<0.05	<0.05		<0.05	1.48±0.3	<0.05	<0.05	
	<i>E.t</i>	Total	25.7±4.9	17.9±1.6	52.9±2.8	52.8±7.2		23.9±3.1	19.4±3.0	43.5±2.5	40±7.5	
		Ava.	<0.05	2.8±0.2	<0.05	<0.05		<0.05	2.8±0.2	<0.05	<0.05	
CV%	<i>A.a</i>	Total	57	41.9	15.5	43		9.2	27.4	34.9	57.8	
		Ava.	<0.05	42.1	<0.05	<0.05		<0.05	38.3	<0.05	<0.05	
	<i>E.t</i>	Total	42.9	20.6	11.8	30.4		29.3	34.6	12.7	42.2	
		Ava.	<0.05	15.8	<0.05	<0.05		<0.05	15.8	<0.05	<0.05	

Table 42. Total and DTPA-extractable lead (mg/kg) in fly ash amended rhizosphere soil of *Acacia auriculiformis* (*A.a*) and *Eucalyptus tereticornis* (*E.t*) yearly intervals. (Initial total Pb in soil: 32420 mg/kg and fly ash: 6762 mg/kg)

Fly ash (%)	Species		T1 (Control)				M±SE	T2 (Chemical fertilizers)				M±SE
			2001	2002	2003	2004		2001	2002	2003	2004	
F0 (0%)	A.a	Total	15.2	27.0	21.9	11.0	18.7±3.5	22.3	36.0	31.7	15.3	26.3±4.7
		Ava.	2.4	4.2	1.8	1.8	2.5±0.6	2.8	3.5	1.5	1.9	2.4±0.4
	E.t	Total	39.3	21.3	12.8	17.0	22.6±5.8	52.6	19.6	20.4	16.0	27.1±8.5
		Ava.	1.4	2.1	1.3	1.8	1.6±0.1	1.5	2.1	1.6	1.7	1.7±0.1
F1 (6%)	A.a	Total	14.4	20.5	26.2	14.9	19±2.7	30.0	17	35.3	17.3	24.9±4.6
		Ava.	2.5	3.2	1.5	1.6	2.2±0.4	3.2	3.2	1.8	1.7	2.5±0.4
	E.t	Total	35.5	33.9	24.1	19.5	28.2±3.8	16.8	21.5	25.6	18.0	20.4±1.9
		Ava.	1.0	2.5	0.6	1.6	1.4±0.4	2.6	1.8	1.8	1.9	2.0±0.1
F2 (12%)	A.a	Total	29.4	24.5	23.3	20.1	24.3±1.9	73.8	19.2	40.4	18.3	37.9±13.0
		Ava.	2.1	3.1	1.4	1.5	2.0±0.3	1.0	4.2	1.4	1.2	1.9±0.7
	E.t	Total	58.2	19.2	29.1	24.6	32.7±8.7	34.8	32.2	36.6	23.7	31.8±2.8
		Ava.	2.2	2.4	0.7	1.9	1.8±0.3	2.4	2.1	1.3	1.6	1.8±0.2
F3 (18%)	A.a	Total	73.6	26.0	26.2	24.0	37.4±12.0	97.2	19.0	44.0	20.7	45.2±18.2
		Ava.	1.1	3.0	1.2	1.5	1.7±0.4	1.5	3.2	1.0	1.6	1.8±0.4
	E.t	Total	24.4	39.2	33.4	24.1	30.2±3.7	33.2	18.9	42.2	25.9	30±4.9
		Ava.	1.4	2.6	1.0	1.9	1.7±0.3	2.4	1.7	1.4	1.8	1.8±0.2
F4 (24%)	A.a	Total	82.2	14.3	18.7	24.2	34.8±15.9	80.0	18.4	36.8	22.2	39.3±14.4
		Ava.	7.5	3.4	1.5	1.7	3.5±1.4	1.6	3.5	1.2	1.5	1.9±0.5
	E.t	Total	16.8	41.0	39.3	28.3	31.3±5.6	30.6	42.4	45.4	32.4	37.7±3.6
		Ava.	2.7	2.0	0.9	1.5	1.7±0.3	4.2	2.9	1.8	1.7	2.6±0.6
M±SE	A.a	Total	42.9±14.5	22.4±2.3	23.2±1.4	18.8±2.6		60.6±14.7	21.9±3.5	37.6±2.1	18.7±1.2	
		Ava.	3.1±1.1	3.4±0.2	1.4±0.09	1.6±0.05		2±0.4	3.5±0.1	1.4±0.1	1.5±0.1	
	E.t	Total	34.8±7.0	30.9±4.5	27.7±4.5	22.7±3.8		33.6±5.7	26.9±4.5	34.0±4.8	23.2±2.9	
		Ava.	1.7±0.3	2.3±0.1	0.9±0.1	1.7±0.08		2.6±0.4	2.1±0.2	1.5±0.1	1.7±0.05	
CV%	A.a	Total	75.8	23.1	13.6	30.7		54	36.1	12.6	14.6	
		Ava.	80.4	14.6	14.7	8.0		46.2	11.6	21.9	16.4	
	E.t	Total	45.4	32.7	36.2	19.6		38	37.8	31.5	28.1	
		Ava.	39.7	11.1	30.4	10.4		37.4	22.2	14.4	6.55	

Growth

The growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in an established field plantation involving various treatments was monitored by measuring the height and collar diameter of the inner block of nine plants in three replications during the initial years and when they attained breast height, then onwards height and GBH was measured. All biometric measurements were taken during the month of January each year.

Acacia auriculiformis

With the addition of 18% fly ash and chemical fertilizers (F3T2) there was significant rise in the collar diameter up to 2.6 cm corresponding to almost a 19.2% increase (Table 43). Fly ash alone at 18% (F3T1) resulted in 15% increase (Fig. 29a) in the year 1999. The GBH was measured from year 2002 onwards where it was 14.5 cm in (F0T1) soil without fly ash and chemical fertilizers. With rise in fly ash percentage up to 24% (F4T1), the GBH measured 16.5 cm showing no significant variation with respect to the control. In soil mixed with fly ash and chemical fertilizers, the GBH in the control (F0T2) was 13.4 cm, which increased to 15.4 cm in soil mixed with 18 % fly ash (F3T2).

The highest GBH measured 23.9 cm in (F1T1) soil amended with 6% fly ash and no fertilizers (Fig. 29a). Over the four-year study period the GBH in the control (F0T1) without fly ash and chemical fertilizer increased by as much as 31.6% while in (F0T2) soil mixed with fertilizers it rose by 36.1%. With increase in fly ash percentage up to 24% the GBH in soil (F4T1) without chemical fertilizers increased by 25.3 % and in soil with chemical fertilizers (F4T2) increased by 38.6%. A similar pattern of variation in GBH was observed in all treatments over the four-year study period with a significant rise with increasing fly ash percentage but the variation between the two treatments T1 and T2 was not statistically significant.

The height of *Acacia auriculiformis* in 1999 showed a distinct increase up to 186 cm in soil (F4T1) mixed with 24 % fly ash compared to 169 cm in (F0T1) rhizosphere control soil (Fig. 29b). After 1999 no significant distinction was maintained between the various treatments and growth pattern, the increase becoming statistically insignificant with

increase in fly ash percentage. Over the four-year study period a distinct increase in height was observed from 169 cm to 686 cm in rhizosphere soil (F0T1) while an addition of 24% fly ash (F4T1) resulted in an increase in height from 186 cm to 675 cm. A similar pattern in increase of height was observed in soil mixed with fly ash and chemical fertilizers. *Acacia* increase in height corresponding to 705 cm from an initial height of 193 cm (Table 43).

Eucalyptus tereticornis

With the addition of 24% fly ash and chemical fertilizers (F4T2) there was a significant rise in collar diameter up to 2.3 cm corresponding to almost a 30.4 % increase after six months of growth (Table 44). The collar diameter was measured up to year 2001 and no significant variation was observed with increasing fly ash percentage from 6 to 24% (Fig. 30a). The collar diameter in soil without fly ash (F0T1 and F0T2) ranged from 6.94 to 10.9 cm and in soil mixed with 24 % fly ash (F4T1 and F4T2) it ranged from 10.6 to 12.9 cm.

GBH was measured from 2002 onwards, which showed a significant increase in the soil without fertilizer and with chemical fertilizers over the years. The soil with fly ash alone (F4T1) and mixed with chemical fertilizers (F4T2) at 24 % fly ash concentration increased by 43.3 and 27.8 % in the GBH which was not statistically significant with increasing fly ash percentage. Over the four-year study period a significant increase in GBH was observed with increased fly ash percentage in soil (Fig. 30a).

The height of *Eucalyptus tereticornis* in 1999 showed an increase up to 202 cm in (F4T2) soil mixed with fly ash and chemical fertilizers, which was significant compared to 121 cm in (F0T2) soil without fly ash. After 1999 a significant increase was observed for various treatments with increasing fly ash percentage and over the four-year study period (Fig. 30b). A distinct increase in height was observed from 127 cm to 698 cm in rhizosphere control soil (F0T1) over the years. Addition of 24% fly ash (F4T1) resulted in an increase in height from 132 cm to 734 cm. A similar pattern for increase in height was observed in soil mixed with fly ash and chemical fertilizers where a height of 760 cm was observed in soil with 24% fly ash (F4T2) from an initial height of 202 cm (Table 44).

Growth of *Acacia auriculiformis* from 1999 until on 2004 increased by as much as 6.8 % while GBH was enhanced by 6.44% in soil mixed with fly ash alone and in presence of chemical fertilizers mixed with fly ash varying from 0 to 24%. On the other hand, growth increased significantly by 14.3% and GBH by as much as 15.1% in *Eucalyptus tereticornis* in soil without chemical fertilizers and in soil admixed with fly ash and chemical fertilizers. Mean Annual Increment (MAI) in *Acacia auriculiformis* and *Eucalyptus tereticornis* ranged from 113 to 129 and 111.6 to 128.6 cm (Table 45). With increased addition of fly ash in soil with and without fertilizers no drastic effect on the MAI in the tree species was observed and was higher in soil mixed with fly ash and fertilizers compared to fly ash alone. The Mean Annual Increment in the GBH ranged from 2.8 to 4.7 and 3.0 to 4.7 cm in *Acacia* and *Eucalyptus*, respectively, which was more in *Eucalyptus* compared to *Acacia* and the overall GBH was higher for the fly ash amended soil compared to control (F0T1) for both the tree species. Overall the effect of addition of chemical fertilizers for the MAI in height was greater in *Eucalyptus* as compared to *Acacia*. There was a synergistic effect of fly ash plus chemical fertilizers on MAI of *Eucalyptus tereticornis* and *Acacia auriculiformis*. No negative effect on the MAI of both the tree species was observed as a result of 6 to 12% fly ash amendment and it was rather more in fly ash-amended treatments as compared to control, indicating a higher growth rate and biomass production.

Table 43. Mean height (cm) and GBH (cm) of *Acacia auriculiformis* (A.a) at yearly intervals. (C.D: Collar diameter; Ht: Height; GBH: Girth at breast height)

Fly ash (%)	Treatment		1999	2001	2002	2003	2004
F0 (0)	T1	C.D	1.7±0.06		-	-	-
		Ht.	169±5.5	345.2±10.1	525±19.6	597±19	686±9.0
		GBH	-	-	14.5±0.54	16.8±0.60	21.2±0.69
	T2	C.D	2.1±0.08		-	-	-
		Ht.	180±5.6	337.9±14.4	503±4.4	589±26	637±32
		GBH	-	-	13.4±0.79	16.6±0.94	21.0±1.29
F1 (6)	T1	C.D	1.8±0.06		-	-	-
		Ht.	189±5.9	332.5±25.0	442±41.2	586±18	641±27
		GBH	-	-	14.5±1.46	18.0±0.95	23.9±1.14
	T2	C.D	2.3±0.11		-	-	-
		Ht.	190±6.0	387±15.8	487±16.4	577±22	681±16
		GBH	-	-	13.9±0.62	18.1±0.96	21.3±0.76
F2 (12)	T1	C.D	1.8±0.06		-	-	-
		Ht.	190±4.9	381.5±22.2	493±20.4	593±24	659±19
		GBH	-	-	14.2±0.75	18.6±0.69	21.8±0.86
	T2	C.D	2.5±0.07		-	-	-
		Ht.	192±4.0	394.6±18.7	479±20.7	594±22	684±22
		GBH	-	-	14.3±0.70	17.7±0.88	20.5±1.06
F3 (18)	T1	C.D	2.0±0.07		-	-	-
		Ht.	202±5.8	349.8±8.6	525±19.2	626±18	668±17
		GBH	-	-	15.6±0.64	17.6±0.67	22.9±0.88
	T2	C.D	2.6±0.09		-	-	-
		Ht.	200±3.8	354.8±11.6	513±15.3	600±24	678±18
		GBH	-	-	15.4±0.23	17.1±0.73	21.5±0.81
F4 (24)	T1	C.D	1.8±0.06		-	-	-
		Ht.	186±7.2	366.5±21.6	508±19.2	558±17	675±14
		GBH	-	-	16.5±0.80	18.9±0.82	22.1±0.79
	T2	C.D	2.5±0.09		-	-	-
		Ht.	193±5.6	302.0±14.8	403±54.7	403±55	705±38
		GBH	-	-	13.8±2.24	13.8±2.24	22.5±2.02

Table 44. Mean height (cm) and GBH (cm) of *Eucalyptus tereticornis* (E.t) at yearly intervals. (C.D: Collar diameter; Ht: Height; GBH: Girth at breast height)

Fly ash (%)	Treatment		1999	2000	2001	2002	2003	2004
F0 (0)	T1	C.D	1.0±0.5	2.4±0.1	6.94±0.38	-	-	-
		Ht.	127±3.2	181±7.0	260±9.0	398±24.3	491±26	698±29
		GBH	-	-	-	10.7±0.72	15.1±0.94	18.6±1.2
	T2	C.D	1.6±0.06	3.8±0.2	10.9±0.40	-	-	-
		Ht.	121±4.0	251±12	340±11	456±12.6	596±22	694±24
		GBH	-	-	-	13.7±0.58	18.2±0.69	20.1±1.0
F1 (6)	T1	C.D	0.9±0.05	2.1±0.1	7.46±0.41	-	-	-
		Ht.	121±4.0	173±11	248±10	365±24.3	555±33	704±29
		GBH	-	-	-	10.6±0.66	15.8±0.96	20.0±1.1
	T2	C.D	1.6±0.06	4.5±0.3	13.8±0.43	-	-	-
		Ht.	160±5.1	281±9.5	414±13.0	414±26.7	713±29	803±30
		GBH	-	-	-	17.1±0.76	21.5±1.09	23.8±1.5
F2 (12)	T1	C.D	1.0±0.05	3.0±0.2	6.84±0.61	-	-	-
		Ht.	120±2.5	223±16	246±9.6	406±27.0	572±29	720±29
		GBH	-	-	-	11.7±0.66	16.0±0.87	20.3±1.1
	T2	C.D	2.2±0.09	4.0±0.3	12.9±0.71	-	-	-
		Ht.	200±6.2	312±18	427±24	634±38.1	779±44	799±32
		GBH	-	-	-	16.9±0.95	20.2±1.18	23.8±1.3
F3 (18)	T1	C.D	1.02±0.04	2.5±0.1	8.04±0.56	-	-	-
		Ht.	134±10.0	178±8.0	267±24	438±24.4	557±30	733±34
		GBH	-	-	-	12.6±0.91	17.2±1.27	21.4±1.5
	T2	C.D	1.8±0.09	4.5±0.3	11.7±0.69	-	-	-
		Ht.	174±5.0	291±9.8	382±15	602±36.9	644±35	789±22
		GBH	-	-	-	16.9±0.96	20.0±1.08	22.9±1.3
F4 (24)	T1	C.D	1.03±0.05	2.5±0.1	10.6±0.78	-	-	-
		Ht.	132±2.9	214±8.0	290±15	408±29.2	570±32	734±31
		GBH	-	-	-	13.2±1.02	16.7±0.79	23.3±1.5
	T2	C.D	2.3±0.09	4.9±0.2	12.9±0.69	-	-	-
		Ht.	202±5.9	331±18.0	436±32	521±26.8	623±34	760±31
		GBH	-	-	-	15.8±0.94	19.9±0.53	21.9±1.3

Table 45. Mean Annual Increment (cm) in the height and GBH of *Acacia auriculiformis* (A.a.) and *Eucalyptus tereticornis* (E.t.) (1999-2004).

Fly ash (%)	Treatment	<i>Acacia auriculiformis</i>		<i>Eucalyptus tereticornis</i>	
		Height (cm)	GBH (cm)	Height (cm)	GBH (cm)
F0 (0)	T1	129.0	3.3	114.2	3.9
	T2	114.2	3.8	114.6	3.2
F1 (6)	T1	113.0	4.7	116.6	4.7
	T2	122.8	3.3	128.6	3.4
F2 (12)	T1	117.2	3.7	120.0	4.3
	T2	123.0	3.1	119.8	3.5
F3 (18)	T1	116.5	3.6	119.8	4.4
	T2	119.5	3.0	122.0	3.0
F4 (24)	T1	122.2	2.8	120.4	5.0
	T2	128.0	4.3	111.6	3.0

Discussion

1. Characterization of fly ash for its physicochemical properties.

Fly ash from electrostatic precipitator (ESP) fly ash collected from three different sources viz. BILT, Choudwar, Orissa; GGS Thermal Power Plant, Ropar, Punjab; BILT, Yamunanagar, Haryana was analyzed for physicochemical properties after air-drying. The acidic soil from village Durgaprasad, Distt. Dhenkanal, (Orissa) and alkaline soil from Thapar Technology Campus, Patiala (Punjab) collected from the topmost (0-30 cm) soil, was processed and subjected to physicochemical characterization.

Fly ash

Heterogenous chemistry of ESP fly ash obtained from the different sources is evident from the variation in concentration of the different elements. Essential elements were measured in the order N>P>K in fly ash from Choudwar and Yamunanagar while phosphorous was highest followed by nitrogen and potassium in ESP fly ash from Ropar. Sulphur was higher than calcium in fly ash from both Yamunanagar and Ropar. In ESP fly ash from Choudwar calcium was the dominant element (Sikka and Kansal, 1994) followed by sodium and magnesium (Maiti et al., 1990). On the other hand magnesium and sodium were highest in ESP fly ash from Ropar and Yamunanagar. The micronutrients varied as Fe>Mn>Zn>Cu>Mo and As were higher than Se in all three fly ash samples studied. Selenium (5-10 mg/kg) and arsenic (20-50 mg/kg) were below while molybdenum (2-40 mg/kg) was within critical levels reported in the literature (Pendias and Pendias, 1984). Cadmium was highest in ESP ash of Yamunanagar. Further, lead and chromium concentration were greater compared to nickel in ESP fly ash from all three sources.

The overall trend was Fe>Mn>Zn>Cr>Ni in all fly ashes characterized which implied an Fe and Mn-rich nature. Cobalt was highest in ESP fly ash from Choudwar among the three fly ashes studied. The presence of micronutrients in fly ash can thus be beneficial for maintaining nutrient balance in soil from the fertility angle, however, its dosage of application has to be properly determined (Hill and Lamp, 1980; Wallace et al., 1980,

Aitken and Bell, 1984; Sikka and Kansal, 1994). Overall, alkaline fly ash was richer in N, P, K and S while total Ca, Mg, Zn, Mn, Ni, Cr and Co were higher in acidic fly ash which might be due to pH (Sikka and Kansal, 1994). Among the gamma-emitting radionuclides Ra²²⁶ measured 100 Bq kg⁻¹ and K⁴⁰ was 376 Bq kg⁻¹ in ESP fly ash of Choudwar, below permissible limits of 370 and 810 to 925 Bq kg⁻¹ respectively (Pendias and Pendias, 1984). With regard to physical properties water holding capacity of ESP fly ash from Ropar and bulk density and hydraulic conductivity in fly ash from Choudwar were highest among the three fly ashes studied. Bulk density was within range from 1 to 1.8 g cm⁻³ as reported by Natusch and Wallace (1974). The texture of all three fly ashes studied was loamy sand as determined from texture triangle.

Microbiological activity in terms of bacterial counts and soil dehydrogenase activity was negligible in fly ash since fly ash has earlier been reported to be microbiologically inert and essentially sterile (Cope, 1962; Rippon and Wood, 1975; Klubek et al., 1992). The heterogeneity in the ESP ash from different sources can be attributed to the geothermal origin of the coal from which the particular fly ash residue has been generated, combustion and handling conditions, efficiency and type of emission and control devices.

Soil

Among the essential elements nitrogen and phosphorous were present in highest concentration in the acidic and alkaline soil respectively. Calcium was the dominant secondary element in acidic soil while sulphur was highest in the alkaline soil. Acidic soil of Orissa is degraded soil, hence was found to be low in nitrogen, phosphorous and sulphur compared to alkaline soil of Patiala. Micronutrients varied as Fe>Mn>Zn>Cu in the acidic as well as alkaline soil. Arsenic concentration was slightly greater than selenium and nickel concentration was less than lead and chromium in both acidic and alkaline soil (Howari, 2004). Molybdenum (2-40 mg/kg) was within the permissible level (Pendias and Pendias, 1984) while arsenic (20-50 mg/kg) and selenium (5-10 mg/kg) in comparison were present at lesser concentration (Pendias and Pendias, 1984). Water holding capacity and bulk density of acidic soil were higher while hydraulic conductivity was more in alkaline soil.

The release of micronutrients and heavy metals was higher in acidic fly ash and soil (Sikka and Kansal, 1994). Low pH conditions tend to increase the mobility and create a reducing atmosphere for ions while alkalinity results in conversion of ions to hydroxy ions followed by formation of insoluble hydroxides or oxides. Fly ash on analysis revealed the occurrence of all elements within reported range (Page et al., 1979, Goyal et al., 2002). except potassium and sulphur, which were below the generally reported values. Metal content of acidic and alkaline soil on the whole was within the range reported in literature except K, Ca and Na, which were below range (Page et al., 1979, Goyal et al., 2002). Nitrogen concentration in the alkaline soil of Patiala was much higher than the reported range, which can be attributed to the fertile nature of the native soil. Among the gamma-emitting radionuclides Ra^{226} measured 31 Bq kg^{-1} and K^{40} measured 260 Bq kg^{-1} in acidic soil both below permissible limits of 370 and 810 to 925 Bq kg^{-1} indicating no radioactivity hazard (Pendias and Pendias, 1984). The texture was clay in the acidic and loamy sand in alkaline soil, respectively.

2. To study the effect of fly ash amendment in soil on soil microbial activity.

Rate of carbon dioxide evolution, microbial biomass, soil dehydrogenase activity and total organic carbon are sensitive indicators for the assessment of soil and give a good insight into the complexities of the nutrient profile in soil and therefore were examined to study the effect of fly ash on microbial activity along with bacterial count. The best index of overall metabolic activity of soil microbial populations is carbon dioxide evolution or soil respiration, which can be rapidly and reproducibly determined. The inherent organic matter of soil plays an important role as a source of energy and nutrients for microorganisms thereby promoting microbial development (Smith and Paul, 1990).

The rate of carbon dioxide evolution was studied in soil amended with varying fly ash and glucose percentages. The presence of fly ash from 0 to 30% in soil did not have any drastic effect on rate of CO_2 evolution and it remained fairly consistent ranging from 70.8 to 87.5 $\text{mg CO}_2 \text{ 100 g}^{-1} \text{ soil day}^{-1}$. In another set of experiments fly ash was mixed in soil from 0 to 75 % and drastic reduction up to 20.9 $\text{mg CO}_2 \text{ 100 g}^{-1} \text{ soil day}^{-1}$ was observed on the second day of incubation. 25% fly ash was optimum for the rate of carbon dioxide

evolution which ranged from 72.1 to 80.8 mg CO₂ 100 g⁻¹ soil day⁻¹ implying the utility of fly ash as a soil ameliorant which would not have any adverse effect on soil respiration since it provides nutrients to the microorganisms for carrying out various metabolic activities (Wong and Wong, 1986).

Soil mixed with 5% fly ash and increasing glucose percentage from 1 to 4% showed a consistent increase in CO₂ production which ranged from 72.6 to 79.7 and 74.5 to 80.9 mg CO₂ 100 g⁻¹ soil day⁻¹ from day 1 to day 4, implying the synergistic interaction between glucose and fly ash. In another setup fly ash added at different concentrations from 1 to 10% in soil along with glucose at 4% did not show any distinct variation in rate of CO₂ evolution until day four of incubation and overall it ranged from 77.5 to 83.5 mg CO₂ 100 g⁻¹ soil day⁻¹. The respiration is generally high in the surface soil due to greater microbial activity (Saffigna et al., 1989).

Microbial enzyme activity assay is a simple and rapid method to indicate the cycling of nutrients in soil. Since enzymes may originate from biotic (viable cells) or abiotic (extracellular) components soil enzyme activities may not always show a strong positive correlation with other biological parameters (Dick, 1994). Addition of glucose as an organic carbon source resulted in concentration-dependent increase in soil dehydrogenase activity in soil amended with glucose from 1 to 4%. Maximum enzyme activity corresponding to 27.75 µg TPF g⁻¹ soil day⁻¹ was observed at 4% glucose, which could be correlated with increased microbial populations resulting from glucose acting as a carbon source.

Dehydrogenase activity in soil without fly ash and glucose measured 8.75 µg TPF g⁻¹ soil day⁻¹ compared to enzyme activity in soil amended with 5% fly ash and increasing glucose percentage from 1 to 4%, where it ranged from 9 to 29.50 µg TPF g⁻¹ soil day⁻¹. Dehydrogenase activity in control soil without fly ash and glucose was almost equivalent to soil containing 1% glucose and 5% fly ash. Thus presence of glucose in fly ash-amended soil helps survival of bacterial populations and higher enzyme activity. The increased microbial population can be correlated to 4 % glucose acting as a source of

carbon since it provided the nutrients necessary for sustaining microbial growth and activity (Molliner and Street, 1982; Fang et al., 1998).

Enzyme activity in soil amended with 1 to 10% fly ash and in the presence of 4% glucose ranged from 8.50 to 19.0 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ compared to 2.50 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ in soil alone. The available data indicates that microbial numbers and diversity generally increase as ash weathers and nutrients accumulate (Rippon and Wood, 1975; Klubek et al., 1992). Addition of an organic source like glucose would act as an energy source for microbial growth and development, resulting in large, diverse microbial populations (Rippon and Wood, 1975; Klubek et al., 1992), which in turn would be capable of performing all metabolic activities for survival.

Population increase of *E.coli* S-17 lac Z^+ was studied in soil amended with 0 to 30% fly ash under sterile and non-sterile conditions over a 12-day period. Bacterial counts in sterile soil was lower compared to non-sterile soil. In soil amended with 10 % fly ash the population ranged from 30×10^6 to 55.3×10^6 and 42.7×10^6 to 58.5×10^6 cfu g^{-1} soil beyond which a decrease was observed. No distinct variation in the population was observed over the 12-day study period under both sterile and non-sterile conditions.

Fly ash amendment at 10% was found to be optimum for all parameters including bacterial counts, microbial biomass, organic carbon and available phosphorous in both sterile and non-sterile soil. In soil inoculated with phosphate solubilizing bacterial isolate S2, bacterial count ranged from 68×10^6 to 480×10^6 and 105×10^6 to 550×10^6 cfu g^{-1} soil and microbial biomass ranged from 152 to 456 and 213 to 472 $\mu\text{g g}^{-1}$ soil in sterile and non-sterile soil amended with 10% fly ash. Soil fertility is strongly affected by microbial biomass, which comprises 1 to 3% of total organic carbon (Jenkinson and Ladd, 1981), which is both a source and sink of nutrients, and controls soil organic matter mineralization.

The chloroform extraction method offers advantages such as easy execution and analytical ability and its applicability to various soils (Brookes et al., 1985b; Vance et al., 1987; Jenkinson, 1988; Tate et al., 1988; Sparling and West, 1989; Ross, 1990). Bacterial count and microbial biomass were higher in soil inoculated with phosphate solubilizing

bacteria S2 compared to uninoculated soil under sterile conditions, which might be due to proliferation of the isolate in the soil in the absence of competition from native populations. However, the trend was reversed in non-sterile conditions where the uninoculated soil had a comparatively higher microbial biomass and bacterial count, which might be attributed to the activity of native microorganisms in soil.

Organic carbon ranged from 0.32 to 0.70 and 0.22 to 0.71% and available phosphorous from 25.0 to 61.5 and 42.7 to 98.0 mg/kg in sterile and non-sterile soil amended with 10% fly ash. No distinct variation was observed in the organic carbon content while available phosphorous decreased after day 32 in soil under sterile and non-sterile conditions. The microorganisms increased the availability of phosphorous as they might utilize the phosphorous present in soil and fly ash (Gaiind and Gaur, 1991). Overall bacterial counts and available phosphorous were higher in non-sterile soil which might be due to presence of native microorganisms while microbial biomass was greater in sterile soil with organic carbon content showing no distinct variation in both sterile and non-sterile soil.

A beneficial cumulative effect on microbial activities can be attributed to the predominantly inorganic fly ash at 10% and inherently present organic matter in soil resulting in a positive influence on the nutrient status of soil (Rippon and Wood, 1975; Page et al., 1979; Molliner and Street, 1982; Fang et al., 1998).

Similarly, in soil inoculated with transformant S2: pMMB277, 10% fly ash was observed to be optimum without having any adverse effect on the microbial activities. Bacterial counts ranged from 12×10^6 to 46×10^6 and 10×10^6 to 48×10^6 cfu g^{-1} soil and microbial biomass ranged from 111 to 541 and 221 to 555 $\mu g g^{-1}$ soil in sterile and non-sterile soil amended with 10% fly ash. The parameters were observed to be higher in inoculated soil in sterile and non-sterile conditions due to the cumulative effect of the inoculated bacteria and the native populations in the soil. In soil amended with 10% fly ash, organic carbon ranged from 0.31 to 0.60 and available phosphorous ranged from 23.1 to 87.0 mg/kg in sterile conditions while in non-sterile soil these varied from 0.32 to 0.65 % and 31.4 to 91.0 mg/kg respectively.

Overall microbial biomass, organic carbon content and available phosphorous was higher in non-sterile soil while bacterial counts were higher in sterile soil. As in soil inoculated with S2 no marked variation was observed in organic carbon but after day 32 of incubation available phosphorous decreased under sterile and non-sterile conditions which could be due to consumption of the phosphorous present in soil and fly ash by microbes for growth and metabolism (Gand and Gaur, 1991).

Fly ash when added at an optimum concentration of 10% (v/v) to soil did not impart any adverse effect on microbial activity. Fly ash can promote soil microbial activity and mixing with an organic substrate enhances its benefits, which assumes importance owing to eco-friendly disposal of fly ash. A consistency was observed in the rate of carbon dioxide evolution in the soil without fly ash and soil plus fly ash mixtures over a given time period and with increasing fly ash percentage. A positive correlation between soil microbial biomass and organic carbon with 10% fly ash amendment was observed. Beyond 10% some decrease in the microbial activity might be due to decrease in soil fungi-to-bacteria ratio (Baath et al., 1995; Wolters et al., 1995) or increased mobility of metals such as Zn, Cr, Ni, etc. (Klose et al., 2004).

3. To screen fly ash resistant soil microorganisms such as free-living N₂-fixers and phosphate solubilizers.

Phosphate solubilizing and free-living nitrogen-fixing bacteria in rhizosphere soil collected from an established plantation of *Acacia auriculiformis* and *Eucalyptus tereticornis* were screened on Pikovskya and Jensen's media where the population of the latter was negligible in rhizosphere soil of both tree species, which indicated the better adaptability of phosphate-solubilizing bacteria to fly ash amended soil. Overall populations of phosphate-solubilizing bacteria ranged from 9×10^6 to 27.5×10^6 cfu g⁻¹ soil and nitrogen-fixing bacteria were much less, varying from 0.02×10^6 to 2.6×10^6 cfu g⁻¹ soil in rhizosphere soil of *Acacia* and *Eucalyptus*. An increase in population of phosphate-solubilizing bacteria was observed with increasing fly ash percentage up to 12% which was most favourable and the presence of chemical fertilizers augmented the nutrient supply for growth and proliferation of the bacteria by acting synergistically with fly ash. Fly ash had inherent phosphorous which in due course of time might have been

utilized by the microorganisms and converted into available form. In addition to this nutrient mineralization from plant litter and soil organic matter might have contributed to increased adaptability of the phosphate solubilizers in soil micro-environment (Kourtev et al., 2002; Klose et al., 2004).

The synergistic effect of fly ash and chemical fertilizers comes to light, which in turn can help in maintenance of soil fertility. Besides, plants release a variety of photosynthesis-derived organic compounds in the rhizosphere that can serve as carbon sources for heterotrophic bacteria (Bowen and Rovira, 1991). It has been reported by several investigators that a high proportion of P-solubilizing microorganisms are concentrated in the rhizosphere of the plants (Gaur, 1990) and their activities are much higher in rhizosphere soil than in bulk soil (Seeling and Jungk, 1992; Vesquez et al., 2000).

Further studies were concentrated on phosphate-solubilizing bacterial isolates only and their percentage solubilization of tricalcium phosphate (TCP) in Pikovskya media at different time intervals. With increase in time of incubation the concentration of solubilized TCP increased with a corresponding decrease in pH of the media (Cunningham, 1992; Motsara, 1995; Illmer et al., 1995; Bar-Yosef et al., 1999) because of microbial solubilization of phosphates in liquid medium due to excretion of organic acids. The role of organic acids produced by PSB in solubilizing insoluble P may be due to lowering of pH, chelation of cations and by competing with P for adsorption sites in the soil (Kucey et al., 1989; Bar-Yosef 1991). The solubilization of phosphates can also be attributed to release of protons accompanying respiration or NH_4^+ assimilation (Taha et al., 1969; Illmer and Schinner, 1995).

Efficiency of percentage solubilization of TCP ranged from 36.5 to 86.1%, the highest being for five bacterial isolates designated S1, S2, S3, S4 and S5. Strain S1 and S4 were isolated from rhizosphere soil of Acacia while S2, S3 and S5 were isolated from Eucalyptus soil, respectively. The percentage solubilization of TCP was 78.5% by strain S1, 80.0% by strain S2, 86.1% by strain S3, 78.7 % by strain S4 and 82.8 % by strain S5. The phosphate solubilizing bacteria can convert large amounts of unavailable phosphorous to available forms using a phosphatic compound such as TCP (Barber,

1984; Subba Rao, 1984; Prerena-Akhuary et al., 1997; Raju and Reddy, 1999) for their own and the utilization of plants.

Microscopic analysis carried out using differential staining (Gram, 1889) revealed the Gram-negative character of S2 and S3 while S5 was Gram positive. During the study of growth curve, exponential phase of the bacterial isolates S2, S3 and S5 occurred in 24, 21 and 23 hours respectively. The lag phase of S2, S3 and S5 lasted for 7, 6 and 10 hours respectively during which the cells metabolized and each adjusted according to its physical environment. All cells at the end of this period divide but not simultaneously which is the reason for gradual increase in population until the end of this period (Pelczar, 1986).

During the exponential phase the cells divide at a constant rate and is nearly uniform in terms of chemical composition of cells, metabolic activities and physiological features. Finally the stationary phase began during which the growth begins to taper off due to exhaustion of nutrients or due to production of some toxins. The population remained constant afterwards, which can be attributed to the cessation of division of cells and balance of reproduction rate by an equivalent death rate (Pelczar, 1986).

4. Molecular tagging of beneficial soil bacteria for ecological monitoring in soil / fly ash amended soils.

Reporter gene systems have become indispensable tools for understanding gene regulation in prokaryotes and eukaryotes (Loper and Lindow, 1997). Reporter genes like lac Z are most popular on account of their easy detectability, sensitivity, specific activity and rapidity (Bronstein et al., 1994; Manafi et al., 1991; Zhang et al., 1991). Due to the ease and sensitivity of its detection and the large number of plasmid vectors and transposons available for making transcriptional and translational fusions, lac Z is the most commonly used reporter gene in microbial ecology (Slauch and Silhavy, 1991).

The present study examined the feasibility of using a wide-host range, control expression vector pMMB277 that retains all the properties of pMMB66 series chloramphenicol resistance (Cm^r) with the added capability of direct screening of plasmid by lacZ

expression. Further this reporter gene was inserted in beneficial soil bacteria such as phosphate solubilizers and monitored in terms of multiplication and colonization in fly ash amended soil after their introduction in order to assess the optimum dose of fly ash required for their survival and growth.

Antibiotic resistance has widely been used as a genetic marker in microbial ecology and for monitoring studies in soil (Williams and Davies, 1965; Lindow et al., 1988; Vandenhove et al., 1991; Nikado, 1994; Jacques et al., 1995). Antibiotic profiling of the isolates was carried out on media containing streptomycin ($50 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$), nalidixic acid ($50 \mu\text{g ml}^{-1}$) and chloramphenicol ($10\mu\text{g ml}^{-1}$) respectively. All strains were sensitive to kanamycin and chloramphenicol and were lacZ negative. Among these strains phosphate-solubilizing bacterial isolate S2 was successfully transformed with lac Z and chloramphenicol marker carrying plasmid pMMB277 from E.coli 2842 as per the method given by Mendel and Higa, (1970) and it was checked for β -galactosidase expression on chloramphenicol+IPTG+X-gal plates. Plasmid isolation from the transformant was carried out as per the method given by Kado and Liu (1981) to confirm the size of 9,237 bp followed by agarose gel electrophoresis.

Transformant S2: pMMB277 was inoculated in soil amended with 0 to 30% fly ash (v/v) under sterile and non-sterile conditions. The ecological monitoring in terms of population buildup was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient agar plates. Uninoculated control for sterile and non-sterile soil was run as a positive control. The soil maintained at 40-50% moisture level was incubated in the dark at 30°C for a period of two months and were periodically analysed for available phosphorous, organic carbon, soil microbial biomass and bacterial count.

The enumeration of inoculated bacteria on selective media indicated that with an increase in fly ash concentration there was a negative effect on soil microbial population and an optimum concentration of 10% is tolerable for microbes indicating that its application provides micronutrients for growth. A similar positive correlation was observed for microbial biomass, which increased with increasing fly ash percentage up to 5 to 10%.

5. To study the effect of fly ash on the growth of nursery seedlings of *Populus*.

Populus is a tolerant, fast-growing species capable of withdrawing water and nutrients for growth with the help of its matted root system (Dawson and Ehleringer, 1991).

Effect of fly ash on biomass production of *Populus deltoides*

Amendment of soil with 20% fly ash did not impart any adverse effect on the growth and biomass production of *Populus deltoides* popularly known as Eastern cottonwood and 62.5% rooting was observed in plants grown in 10% fly ash. Shoot biomass of 6.88g was recorded for plants grown in 20% fly ash which was higher than the control.

Effect of fly ash on physical properties of alkaline soil amended with fly ash

Bulk density in fly ash-amended soil ranged from 1.09 to 1.34 g cm⁻³ and decreased with fly ash addition as reported in other studies (Chang et al., 1977, 1989). Water holding capacity of the soil ranged from 33.4 to 61.3% and increased with increase in fly ash percentage, which could be due to dominance of silt-sized particles in fly ash and cenospheric nature of fly ash (Salter and Williams, 1967; Salter et al., 1971; Chang et al., 1977; Aitken et al., 1984; Aitken and Bell, 1985; Chang et al., 1989; Sharma et al., 1990). Hydraulic conductivity ranged from 18 to 47 cm min⁻¹ with some increase at 20% amendment which might be due to increase in capillary pores as compared to non-capillary pores (Campbell et al., 1983). Soil texture of the fly ash amended soil did not change and remained loamy sand because it is a basic property of the soil where proportion of each size groups can't be altered easily (Brady, 1995).

Effect of fly ash on chemical properties of alkaline soil amended with fly ash

Among the soil factors influencing the availability of heavy metals for root uptake, soil pH plays a key role (Dijkshoorn et al., 1983; Albasel & Cottenie, 1985; Kullie et al., 1999). The native soil was alkaline with pH 7.2 and its amelioration with acidic fly ash resulted in a shift towards acidity, which ranged from pH 6.68 to 6.92. Electrical conductivity ranged from 146.3 to 220.3 $\mu\text{S cm}^{-1}$ and increased due to enrichment with fly ash, which is composed of various essential and non-essential cations and anions (Wong

and Wong, 1989). Organic carbon in fly ash amended soil increased with addition of fly ash up to 10% (Fang et al., 1998).

Nitrogen content in the fly ash amended soil showed a significant increase ranging from 0.16 to 0.44 %. The native soil of Patiala is rich in nitrogen. The increased soil nitrogen can be attributed to decomposition of leaf litter, etc resulting in enzyme-aided nutrient mineralization carried out by the native microbial population (Klose et al., 2004). Phosphorous increased in soil as a result of fly ash amendment and ranged from 254 to 1270 mg/kg which can be attributed to fly ash itself and partly because of some native phosphate-solubilizers (Gand and Gaur, 1991).

Positive effect of fly ash application on crop productivity and increased phosphorous uptake may be attributed to reduced soil crust strength, improved texture of soil and water holding capacity besides presence of organic matter (Elsewi et al., 1978; Sims et al., 1995). The compounds present in organic matter react with interfering compounds thereby leaving the phosphates free and also help in the adsorption of phosphorous on humic compounds and silicate clays thus protecting the phosphates from microbial attack (Brady, 1995).

Potassium and sulphur ranged from 980 to 1530 mg/kg and 140 to 271 mg/kg respectively showing a marked increase with increasing fly ash percentage. Potassium showed the highest concentration compared to other elements as happens in soil generally (Brady, 1995). The increased availability of potassium could be due to breakdown of minerals by several organic and inorganic acids. Increase in sulphur can be attributed to acidic pH and presence of sulphur predominantly in SO_4 form as a result of which SO_4-S increases in proportion to fly ash added (Plank and Martens, 1974; Page et al., 1979; Natusch et al., 1975; Bern, 1976; Furr et al., 1977).

The geothermal origin of coal was anthracite from which ESP fly ash used in the study was generated. Anthracite is rich in sulphur and produces acidic flyash (Natusch et al., 1975; Bern, 1976; Furr et al., 1977; Page et al., 1979).

Nutrient status in terms of total secondary elements, micronutrients and heavy metals in alkaline soil amended with ESP fly ash during nursery trial

Secondary elements (Ca, Mg, Na)

Calcium, sodium and magnesium concentrations increased with increasing fly ash percentage the highest corresponding to 30% and ranged from 162 to 6948, 11.1 to 54.8 and 1240 to 10858 mg/kg in the soil respectively. Magnesium was present in highest concentration followed by calcium and sodium, which could be due to the high Mg concentration of fly ash itself. Fly ash increases the availability of Ca and Mg, balancing the ratio between monovalent and divalent cations $\text{Na}^+ + \text{K}^+ / \text{Ca}^{2+} + \text{Mg}^{2+}$ (Giardini, 1991). The ability of calcium to enhance flocculation/ aggregation of soil particles keeps the soil friable, enhances water penetration and allows roots to penetrate hard/compact soil layers. Calcium readily replaces Na at clay exchange sites to enhance soil flocculation and stability (Giardini, 1991).

Fly ash has been reported to increase yield of many plants apparently caused by increased availability of elements such as Ca^{2+} and Mg^{2+} (Adriano et al., 1978; Page et al., 1979) along with nullifying toxic effects of Al^{3+} and Mn^{2+} and other metallic ions by neutralizing soil acidity (Fail and Wochok, 1977; Capp and Engle, 1967). Inconsistencies in the uptake of K, Ca and Mg were reported which are probably caused by the interaction among these elements in the root-soil solution interface or within the plant system (Sikka and Kansal, 1995). Ca and Mg reduce uptake of K in plants grown on fly ash amended soil (Martens et al., 1970; Adriano et al., 1978). High concentration of these elements can be due to their presence in tap water used for irrigation.

Micronutrients (Fe, Zn, Mn, Mo)

Micronutrients such as Zn and Fe from fly ash may not be consistently available to plants. Depending on the chemical composition fly ash can alter soil pH levels, base saturation, carbon content, soluble salts and concentrations of major and heavy elements (Bellmann and Grote, 1998; Weisdorfer et al., 1998; Koch et al., 2002; Klose and Makeschin, 2003 a). Iron, manganese, zinc and molybdenum ranged from 2171 to 7175, 310 to 555, 25.0 to 55.3 and 3.0 to 3.9 mg/kg respectively. Variation in concentration was distinct for all elements except Mo and these were within permissible limits as reported in the literature (Page et al., 1979). Iron availability depends to a large extent on soil pH and

redox potential and is affected by several environmental conditions, including concentration of macronutrients and ratios between heavy metals (Chaney et al., 1972; Bruggemann et al., 1990).

Heavy metals (Pb, Ni, Cr, Co, As, Se)

Lead, nickel and chromium concentrations ranged from 10.3 to 67.3, 4.8 to 19.0 and 31.5 to 55.5 mg/kg, respectively in fly ash-amended soil and occurred within permissible limits (Page et al., 1979). Cobalt, arsenic and selenium ranged from 2.8 to 8.4, 3.8 to 4.8 and 2.4 to 3.5 mg/kg, respectively, and remained within permissible limits (Pendias and Pendias, 1984) with no distinct increase observed up to 30 % fly ash , which could be due to low concentrations of these elements in fly ash. Mobility of metals such as Pb, Cr and Ni and variation in available concentrations can be attributed to the strong association of these metals with organic matter, clays, precipitation as carbonates, hydroxides and phosphates (McBride, 1994; Ruby et al, 1999; Shen et al., 2002).

Different patterns in the concentrations of elements between plants and soil have also been found in other studies (Markert, 1987). Interactions between elements may arise from antagonistic and synergistic processes, which occasionally can involve the metabolism of more than two elements. The 20% fly ash rate was favourable for biomass production and maintenance of nutrient status in soil with all metals remaining within permissible limits (Page et al., 1979).

Microelements in leaves and stems of *Populus deltoides*

Plants capture and take up elements from a large soil volume (via roots and mycorrhizae) resulting in their differential bioaccumulation which is influenced by several factors such as atmospheric deposition on to leaf surfaces, seasonal physiology, the tissues under study, species-specific capacity for uptake, ion-channel pathways for translocation and compartmentalization (Bargagli, 1998; Greger, 1999; Demidchik et al., 2002). Elements such as Fe, Cu, Zn, and Mn are essential for plant nutrition and are required for the activity of various types of enzymes while As, Cd and Pb do not have any physiological function in plants. Zn can penetrate into the leaf while Pb is mostly adsorbed to the epicuticular lipids at the surface (Greger, 1999). The leaves and stems of *Populus deltoides* were analyzed for macroelements and heavy metals which varied as Ca>Mg>Fe>Na>Zn>Mn and corresponding metal concentration ranged from 4020 to 8970, 1617 to 4620, 459 to 2832, 310 to 618, 30.7 to 80.7 and 21.1 to 42.9 mg/kg respectively.

Bulk flow in the xylem from root to shoot is driven by transpiration from the shoot, which creates a negative pressure in the xylem that pulls up water and solutes (Taiz and Zeiger, 2002). Plants have a natural propensity to take up some metals for example Ca, Fe, Mn, Ni, Zn etc. which are essential mineral elements. The success of this process depends on several factors including metal availability for uptake into roots (bioavailability), and the plants ability to intercept, absorb, and accumulate metals in shoots (Ernst, 1996) ultimately depending on the interaction between soil, metal and plant.

The variation in the elemental concentration of leaves and stems could be attributed to diverse factors such as site conditions, age, position of leaves and season (Van den, 1984). Magnesium and calcium concentrations are generally reported to be higher in leaves of many tree species and the concentration shows seasonal variation (Kramer and Kozlowski, 1979). Pereira et al. (2000) found that nutrients in aboveground biomass of *Acacia* sp. were almost similar to what was observed for *Populus* in the present study. A number of studies have been reported regarding the presence of most of the nutrients in

the leaves (Pereira et al., 1997, 2000; Reis and Barros, 1990; Rezende et al., 1983; Sharma and Pande, 1989; Waring and Schleisinger, 1985).

Poplar was found to have a very good potential for metal uptake due to high biomass production besides fast growth, hardiness, and the ability to resprout when cut as a result of which it can be propagated as cuttings (Schnoor, et al. 1995). Poplar plants are efficient in redirecting water flow upward because they are deep-rooted and transpire at very high rates, creating a powerful upward flow (Dawson and Ehleringer, 1991; McCutcheon and Schnoor, 2003) thereby preventing downward leaching.

Effect of fly ash on microbiological properties of alkaline soil amended with ESP fly ash during nursery trial

Microbiological properties of soil amended with fly ash were studied in terms of dehydrogenase activity, bacterial counts, microbial biomass and rate of carbon dioxide evolution. Soil dehydrogenase activity varied from 0.20 to 2.75 $\mu\text{g TPFg}^{-1}$ soil day⁻¹ in fly ash amended soil with no distinct variation. Sensitivity towards natural and anthropogenic disturbances makes this enzyme an indicator of microbial activity in various types of soil and an integral parameter of soil quality assessment (Dick, 1997). Bacterial counts ranged from 0.38 $\times 10^6$ to 11.6 $\times 10^6$ cfu g⁻¹ soil with a distinct increase up to 10% fly ash which can be attributed to accumulation of nutrients from fly ash with time (Rippon and Wood, 1975).

Soil fertility is strongly affected by microbial biomass, which in the present study varied from 103.8 to 359.3 $\mu\text{g g}^{-1}$ soil with a distinct variation up to 10% fly ash, declining thereafter. The observed differences can be attributed to variation in pH, organic carbon and other chemical properties of fly ash amended soil (Moore et al., 2000). Microbial biomass comprises 1 to 3% of soil's total organic matter content and is both a source and sink of nutrients, controlling soil organic matter mineralization and nutrient availability (Jenkinson and Ladd, 1981; Smith and Paul, 1990; Friedel et al., 1996).

The study on rate of carbon dioxide evolution is one of the oldest and still most frequently used parameters for quantifying soil microbial activities in soil. A study was carried out to assess the effect of fly ash on rate of carbon dioxide evolution over a period

of four days. No distinct variation was observed in rate of carbon dioxide evolution in soil which ranged from 75.1 to 83.6 mg CO₂100g⁻¹ soil day⁻¹ without fly ash and soil plus fly ash mixtures with increasing fly ash percentage. After three days of incubation the rate of CO₂ evolution in soil mixed with 10% fly ash measured 83.6 mg CO₂100g⁻¹ soil day⁻¹. Addition of fly ash as an ameliorating agent at the optimum rate of 10% did not adversely affect the microenvironment of soil and addition up to 30% resulted in a consistent pattern of rate of carbon dioxide evolution. This can be attributed to the fact that microorganisms invariably adapt to the stressed conditions and show a gradual increase in respiration after an initial lag. The presence of organic matter in soil might have helped the microbial populations since organic materials reduce concentration of unfavorable compounds by sorption and provides compounds, which promote microbial proliferation and activity (Wong and Wong, 1986; Pitchel and Hayes, 1990).

A correlation and an increase in SDA, bacterial count and microbial biomass was observed corresponding to 10% fly ash amendment after the trial was completed which can be attributed to the leaf litter, organic matter etc. contributed by the growing plant along with release of several compounds by roots in the rhizosphere which increase the nutrient content of soil and hence provide a favourable micro-environment for microflora (Romheld and Marschner, 1986). 10% fly ash rate might have synergistically interacted with organic matter in terms of nutrients present in soil resulting in augmentation of nutrient supply creating a favourable microenvironment for microbial activities (Rippon and Wood, 1975; Page et al., 1979; Molliner and Street, 1982; Fang et al., 1998; Lai et al., 1999). Beyond 10% fly ash, decline in microbial activity could be due to a decrease in substrate availability owing to accumulation of persistent lignite-derived organic carbon compounds (Rumpel et al., 1998).

6. To study the effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* in established field plantation.

The effect of fly ash on the growth of *Acacia auriculiformis* and *Eucalyptus tereticornis* was monitored on an established field plantation on a twenty-acre area in village Durgaprasad, Distt. Dhenkanal (Orissa) in July, 1998 on partially degraded soil belonging to order Ultisol, having reddish brown lateritic characteristics with a clay texture. Agro forestry species improve the chemical properties of soil and to a certain extent the microbial cycling of nutrients along with an increase in organic carbon, phosphorous and potassium level in the soil (Kang et al., 1999).

pH and Electrical conductivity

The addition of fly ash to degraded soil at the plantation site did not alter the pH since the soil by nature was acidic with pH nearly same as that of the ESP ash used. The role played by the inherent buffering capacity of the soil might have helped in resisting pH changes resulting in pH up to 6.63 in rhizosphere soil of both tree species. Electrical conductivity increased in the rhizosphere soil with increasing fly ash percentage due to the presence of soluble salts (Wong and Wong, 1989) and ranged from 20 to 260 $\mu\text{S cm}^{-1}$ in rhizosphere soil of *Acacia* and *Eucalyptus* in soil amended with fly ash and soil mixed with chemical fertilizers.

Organic carbon and bacterial count

Highest organic carbon and bacterial counts were observed in soil amended with 12 % fly ash for both tree species over the four-year study period but was higher in *Acacia auriculiformis*. The overall organic carbon and bacterial count varied from 0.33 to 1.20 % and 0.05×10^6 to 13.6×10^6 cfu g^{-1} soil in both tree species with variation attributed to different carbon allocation strategies of each plant or tree species which results in a different pattern, rate, quality and quantity of organic carbon input to the soil (Logo and Brown, 1993). The population of nitrogen-fixing bacteria was very low and ranged from 0.01×10^6 to 4.5×10^6 cfu g^{-1} soil. The variation in bacterial counts could be due to variation in concentration and type of organic compounds released by the roots (Lynch and Bragg, 1985). In addition plants release a variety of photosynthesis-derived organic

compounds in the rhizosphere that can serve as carbon sources for bacteria and fungi (Bowen and Rovira, 1991) and around 20% of the carbon fixed by the plant is released from its roots (Olson et al., 2003). As a result microbial densities are 1-4 orders of magnitude higher in rhizosphere soil than in bulk soil, the so-called general rhizosphere effect (Salt et al., 1998). In turn the rhizosphere microbes can promote plant health by stimulating root growth, enhancing water and mineral uptake (Kapulnik, 1996).

Soil dehydrogenase activity

Soil dehydrogenase activity (SDA) was highest in rhizosphere soil amended with 6 to 12% fly ash which ranged from 0.19 to 0.63 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ in Acacia and 0.37 to 0.47 $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$ in Eucalyptus. SDA is an indicator of the oxidative metabolism in soil and thus of microbiological activity (Skujins, 1973), because being exclusively intracellular, it is linked to viable cells.

Enzyme activity in rhizosphere soil of Eucalyptus was less compared to Acacia, which can be attributed to lower pH as a result of which the organic matter decomposition process slows down. Organic matter plays an important protective role in maintaining soil enzymes in their active forms and enzymes are immobilized in a three dimensional network of clay and humus complexes and/or associated with a larger microbial population resulting from increase in soil organic matter (Deng and Tabatabai, 1997).

Presence of fly ash alone and mixed with chemical fertilizers was observed to have a positive effect on soil microbial activity at 10% amendment by supporting microbial processes and metabolic activities which is very critical since the microorganisms and their activities contribute in a major way towards maintenance of soil fertility, which in turn is beneficial for both soil and plants (Robert and Chenu, 1991). The positive influence of fly ash might be due to its nutrient content, which at an optimum concentration worked in the favour of soil microflora (Schutter and Fuhrmann, 2001).

The long-term benefits of trees on amended soil include binding of the soil with the help of massive root systems (Stomp et al., 1993) and establishment of an organic cover over the soil along with above-and below-ground biomass production. In addition transpiration of water by the trees reduces the overall flow of water down

through the soil, thus helping to reduce the amount of heavy metals, which are transferred to ground and surface water. The soil organic carbon level increases eventually, thus improving soil physical and chemical properties.

Physical parameters

Particle-size distribution

No distinct variation was observed in particle size distribution, which ranged from 71.1 to 89.6% sand, 1.0 to 14.8% silt and 7.4 to 20.4 % clay in rhizosphere soil of Acacia and Eucalyptus over the four-year study period amended with as much as 24% amendment but the presence of silt-size particles helps in improvement of soil texture (Chang et al., 1977). Texture is a basic soil property where proportion of each size group cannot be altered easily.

Water holding capacity

Water holding capacity ranged from 21.4 to 68.4% in the rhizosphere soil of the tree species with the higher range observed for Acacia compared to Eucalyptus, which might be due to its positive impact on soil stabilization which corroborates the earlier findings of increase in water holding capacity with fly ash addition in soil (Sharma et al., 1990; Scheltema, 1992; Marcer et al., 1995).

Hydraulic conductivity

Hydraulic conductivity, which is related to soil permeability ranged from 8.36 to 72.0 cm min⁻¹ and increased with rise in fly ash percentage which can be attributed to improved soil porosity and increased capillary pores (Campbell et al., 1983).

Bulk density

Bulk density ranged from 0.58 to 1.31g cm⁻³ in soil plus fly ash and soil mixed with fly ash and chemical fertilizers did not show any distinct variation over the four-year study period with increasing fly ash percentage. Bulk density of fly ash-amended soil was less than 1 to 1.8 g cm⁻³ reported for fly ash (Natusch and Wallace, 1974).

Chemical parameters

Essential elements (N, P, K, S)

Nitrogen and potassium ranged from 0.02 to 0.17 % and 1055 to 82500 mg/kg in rhizosphere soil of the two tree species and was higher in *Acacia auriculiformis*. On the other hand phosphorous and sulphur concentrations were higher in *Eucalyptus tereticornis* and ranged from 20 to 706 and 105 to 739 mg/kg. Addition of fly ash did not have any adverse effect on nitrogen concentration since no decrease was observed. The 12 to 18% fly ash rates were optimum for maintaining nutrient balance in soil over the four-year study period and all metals remained within permissible limits as reported for healthy cropland soil (Page et al., 1979).

Two-way ANOVA

Fly ash accounted for 5.14 and 2.78 % of total variance with a 78 and 91% chance of randomly observing the effect of fly ash on nitrogen in an experiment of this or bigger size in rhizosphere soil of *Acacia* and *Eucalyptus* (Table 46). In the case of phosphorous, percentage of total variance was 1.55 and 7.87 with a 97 and 62% chance of randomly observing the effect of fly ash on an experiment of this or bigger size in rhizosphere soil of *Acacia* and *Eucalyptus*. The percentage of total variance was 9.83 and 2.05 for the effect of fly ash on potassium in the rhizosphere soil of *Acacia* and *Eucalyptus* with 51 and 95% chance of randomly observing the effect of fly ash on an experiment of this or bigger size. In case of sulphur fly ash accounts for 7.53 and 12.7 % of the total variance with a 65 to 35% chance of randomly observing the effect of fly ash on an experiment of this or bigger size. Overall two-way ANOVA of nitrogen, phosphorous, potassium and sulphur (Table 46) showed that fly ash had no significant effect on the concentration of these nutrients with increasing percentage in soil.

Table 46. Two-way ANOVA of nitrogen, phosphorous, potassium and sulphur data in rhizosphere soil of *Acacia auriculiformis* (A.a) and *Eucalyptus tereticornis* (E.t) amended with 0-24% fly ash (v/v) without chemical fertilizers (T1) and with chemical fertilizers (T2).

		Fly ash		Treatment		Fly ash x Treatment	
		F	% Var	F	% Var	F	% Var
A.a	N	0.42 ^a	5.14	0.06 ^a	0.20	0.38 ^a	4.66
	P	0.12 ^a	1.55	0.22 ^a	0.74	0.06 ^a	0.89
	K	0.83 ^a	9.83	0.005 ^a	0.02	0.13 ^a	1.58
	S	1.14 ^a	7.53	0.02 ^a	0.07	0.04 ^a	0.60
<i>E.t</i>	N	0.24 ^a	2.78	1.48 ^a	4.21	0.67 ^a	7.73
	P	0.66 ^a	7.87	0.62 ^a	1.87	0.08 ^a	1.01
	K	0.16 ^a	2.05	1.11 ^a	3.48	0.006 ^a	0.78
	S	1.14 ^a	12.7	0.69 ^a	1.94	0.13 ^a	1.46

Degrees of freedom for Fly ash: 1; Treatment: 4; Fly ash x Treatment: 4;
P value: a (non-significant)

Nitrogen

Nitrogen concentration varied from 0.02 to 0.17% in rhizosphere soil of Acacia and Eucalyptus and did not show any significant variation with increasing fly ash percentage; however no drastic reduction was observed in soil with increased fly ash addition. Nitrogen increased slightly from 0.16% to 0.17% in the soil over the four-year study period. In rhizosphere soil of Acacia nitrogen concentration was slightly greater compared to Eucalyptus due to its nitrogen-fixing ability and overall concentration of nitrogen was greater than phosphorous and sulphur for both tree species. Nitrogen concentrations remained fairly consistent with increasing fly ash percentage with no significant variation over the four-year study period, which could be due to several reasons. Nitrogen in soil solution, particularly in the localized root zones to which the fertilizers have been applied, often is dominated by fertilizer-applied materials (Brady, 1995). Localized concentration of anhydrous ammonia and ammonium containing salts and urea (which hydrolyses to ammonia) stimulates fixation of ammonium ions by clays and organic matter levels are increased. High localized levels of ammonia result in the undesirable accumulation of nitrite ions and at times nitrogen fertilization followed by nitrification increases nitrogen losses from soil (Brady, 1995).

The population of nitrogen-fixing bacteria was very less compared to phosphate solubilizing bacteria in the rhizosphere soil of both tree species. The specificity between the nitrogen-fixing bacteria and the legume species which is the basis for classifying the nitrogen-fixer and host plant into seven cross inoculation groups might not have existed between Acacia and whatever sparse population of nitrogen-fixing bacteria were present. The absence of certain genera of bacteria such as *Azotobacter* and *Azospirillum* in the rhizosphere soil of the tree species, which increase the soil nitrogen level by using root exudates as a source of energy for nitrogen fixation, might be one of the reasons for low nitrogen levels (Brady, 1995).

Phosphorous

Phosphorous varied from 50 to 706 mg/kg in rhizosphere soil of Acacia and Eucalyptus and increased over the four-year study period which might be due to a synergistic effect of fly ash with chemical fertilizers and fly ash alone which had 1084 mg/kg phosphorous.

A decline in the phosphorous was observed in the rhizosphere soil by the year 2002 for both tree species, which can be attributed to interference by calcium and magnesium in the root-soil solution interface (Sikka and Kansal, 1995).

In acidic soil phosphorous though easily available as H_2PO_4^- meets with interference from iron, calcium, magnesium compounds (Brady, 1995). Availability of phosphorus is generally higher in clay soil since most of the compounds with which phosphorous reacts are in finer soil fractions. Phosphorus concentrations in ash are greater compared to soil (Page et al., 1979) but it is not in a readily available form due to interaction with Al, Fe and Ca. In due course the indigenous population of phosphate solubilizers can play an important role in increasing phosphorus availability (Gaind and Gaur, 1991) along with the use of phosphatic fertilizer diammonium phosphate (DAP) in soil in the present study. DAP contains approximately 48% P_2O_5 and provides more fertilizer phosphorous than any other material. Besides, many of the unavailable phosphates in this fertilizer become available under acidic conditions in soil, thereby increasing the available phosphorus content (Brady, 1995).

Potassium

Potassium is generally present in primary mineral and non-exchangeable forms and is present generally at higher concentration compared to any of the major nutrient element. Potassium ranged from 1300 to 82500 mg/kg in rhizosphere soil of both the tree species and increased significantly over the four-year study period. Potassium in fly ash was 1075 mg/kg which might have increased its concentration over the four-year study period due to the enhanced solvent action of carbonic acid and of stronger organic and inorganic acids, as well as by the presence of acid clays and humus. Available potassium exists in soil-solution form and as exchangeable potassium adsorbed on the soil colloidal surface. Potash salts such as muriate of potash used in the present study is water-soluble; hence; it increased the availability of potassium in the soil. Unlike nitrogen salts, most potassium fertilizers, even if employed in large amounts, have little or no effect on soil pH (Brady, 1995).

Secondary element (S)

Sulphur

Sulphur is held in several mineral forms in soil with the sulphide and sulphate minerals being most widespread, the sulphates most easily solubilized and SO_4^{2-} is readily available for uptake in soil. A noticeable effect of fly ash application is increase in sulphur due to dominance of SO_4^{2-} ions, which increases in proportion to fly ash added (Page et al., 1979). Sulphur increased over the four-year study period ranging from 105 to 739 mg/kg in the rhizosphere soil of both Acacia and Eucalyptus. This can be due to clay fraction of the plantation soil rich in iron where the clay fractions adsorb some of the sulphur and then later slowly release it by anion exchange reactions in an acidic environment. Sulphate ion is the form in which most plants absorb the so-called available sulphur from soil. (Brady, 1995).

Micronutrients (Fe, Zn, Mn)

Iron

Iron in Acacia and Eucalyptus ranged from 5315 to 87900 mg/kg in the rhizosphere soil with 12 to 18% fly ash as optimum for maintaining its level in the soil. Available iron increased significantly over the four-year study period in the soil for which a number of factors can be responsible. Plants possess highly specialized mechanisms to stimulate metal bioavailability in the rhizosphere and to enhance uptake into roots (Romheld and Marschner, 1986). Plants exude a class of organic compounds termed siderophores (mugineic and avenic acids) capable of enhancing the availability of soil Fe (Fushiya et al., 1982; Takagi et al., 1984). Some species facilitate Fe uptake by acidifying the rhizosphere via H^+ extrusion from roots. An acidic environment stimulates the reduction of ferric to ferrous iron, which is readily taken up by plants (Chaney et al., 1972; Bienfait et al., 1982). The iron in an aluminosilicate glass phase is the source of bioavailable Fe from coal fly ash (Vernath et al., 2000).

Zinc

Zinc in rhizosphere soil of Acacia and Eucalyptus ranged from 13.1 to 95.0 mg/kg and did not show a significant variation with increasing fly ash percentage and over the four-year study period. Zinc is tightly bound or fixed to certain clays and magnesium hinders its availability in soil which can also be due to presence of phosphatic fertilizers in soil (Brady, 1995).

Manganese

Manganese ranged from 175 to 5578 mg/kg in rhizosphere soil of both tree species and increased with increasing fly ash percentage. 12 to 18% fly ash maintained the zinc and manganese level in soil. An increase in manganese can be due to its high concentration of 739 mg/kg in fly ash, which might have resulted in increase in soil. Iron in rhizosphere soil was highest followed by manganese and zinc due to the inherent iron-oxide abundance in the Ultisols soil on which the plantation was established and high iron content in soil and fly ash corresponding to 29580 and 6762 mg/kg respectively, but it remained within permissible limits as reported in literature (Page et al., 1979).

Heavy metals (Pb, Ni, Cr)

Lead ranged from 11 to 97.2 mg/kg in the rhizosphere soil of *Acacia* and *Eucalyptus* without any distinct increase with increasing fly ash percentage. This can be due to the non-bioavailable nature of lead under the normal pH range of soil (Miller, 1996; Raskin et al., 1997). Total lead concentration in many soils may be high at a point in time but the available fraction is low due to its strong association with organic matter, Fe-Mn oxides, clays, precipitation as carbonates, hydroxides and phosphates (McBride, 1994; Ruby et al., 1999; Shen et al., 2002). Mobility of Pb is low due to its low solubility (Lindsay, 1979), which may be further reduced due to its interactions with the soil solid phase via sorption and ion-exchange (Dong et al., 2000). Nickel and chromium ranged from 12.3 to 116 mg/kg and 12.8 to 99.0 mg/kg in rhizosphere soil of *Acacia* and *Eucalyptus*, respectively, without any distinct variation with increasing fly ash percentage and over the four-year study period. The concentration of elements and their speciation varies with type of soil solution and the amount of moisture present in the soil (Fotovat et al., 1997).

Metal behaviour in acidic and alkaline soil is differentiated due to soil pH, which influences heavy metal adsorption, retention and movement (De Matos et al., 2001). In soil metals exist as a variety of chemical species in a dynamic equilibrium governed by soil physical, chemical and biological properties (Chaney, 1988).

Fly ash has the potential for use as a soil-amending agent on account of its heterogeneous chemical nature and its capacity to bring about changes in soil and plant chemical composition. These changes are a result of the role played by soil pH which affects

chemical solubilities and from soil enrichment with soluble salts and major and trace elements present in fly ash (Martens, 1971; Schnappinger et al., 1975; Martens and Beahm, 1976; Wallace et al., 1980).

Biometric parameters

Growth

Eucalyptus and Acacia have been studied for their metal uptake capacity based on their tolerance to high levels of metals, fast growth, high biomass production, high root-shoot ratio and profuse root system (Garbisu et al., 2002). Growth of Acacia and Eucalyptus was monitored by measuring height and collar diameter in the initial years followed by measurement of GBH.

Acacia auriculiformis

In *Acacia auriculiformis* with the addition of 18% fly ash and chemical fertilizers (F3T2) there was significant rise in the collar diameter up to 2.6 cm corresponding to an almost 19.2% increase. Over the four-year study period GBH in control (F0T1) without fly ash and chemical fertilizer increased by as much as 31.6% while in soil (F4T1) amended with 24% fly ash it increased by 25.3 % respectively. In fertilized control (F0T2) the GBH increased by 36.1% and in fertilizer containing soil mixed with fly ash at 24%, the GBH increased by 38.6%. A similar pattern of variation in GBH was observed in all treatments over the four-year study period with a significant rise with increasing fly ash percentage but the variation between the two treatments T1 and T2 was statistically insignificant. The height did not show a significant variation with increasing fly ash percentage but over the four-year study period an increase was observed. Acacias have been used for sand stabilization, mine site rehabilitation and improvement of soil through the fixation of atmospheric nitrogen. Plants belonging to the family Leguminosae have been demonstrated to have a high tolerance and survival in arid, infertile and metal-contaminated areas (Musil, 1993; Vajpayee et al., 2000; Cheung et al., 2000). *Acacia* sp. have been found to have a positive impact on dry land salinity and soil stabilization (Marcer et al., 1995; Scheltema, 1992).

Eucalyptus tereticornis

With the addition of 24% fly ash and chemical fertilizers (F4T2) there was a significant rise in the collar diameter up to 2.3 cm corresponding to almost 30.4 % increase after six months of plantation in *Eucalyptus tereticornis*. GBH was measured from the year 2002 onwards, which showed a significant increase in the soil without fertilizer and with chemical fertilizers over the four-year study period. GBH in soil mixed with 24% fly ash (F4T1) and mixed with chemical fertilizers (F4T2) showed an increase of 43.3 % and 27.8 % but the increase was not statistically significant. *Eucalyptus* species are adapted to grow in a range of conditions from tropical areas to temperate zones (Specht, 1970). The total biomass production of *Eucalyptus* is generally higher than native population of a particular region and its xerophytic character renders the genus resilient to withstand drought stress thereby indicating an ingenious and specialist strategy under harsh environmental conditions. The versatility of *Eucalyptus* lies in the fact that it completes its life cycle on sites of low nutrient status but also responds favourably to improved nutritional status (Pryor, 1976). *Eucalyptus* has inherent mechanisms to avoid drought-like conditions by higher photosynthetic rates, increased carbon uptake, deep rooting ability and osmotic manipulation to maintain turgor in leaves (Whitehead and Beadle, 2004).

Mean Annual Increment

Mean Annual Increment (MAI) in *Acacia auriculiformis* and *Eucalyptus tereticornis* ranged from 113 to 129 and 111.6 to 128.6 cm respectively. The overall effect of addition of chemical fertilizers for the MAI in height was greater in *Eucalyptus* as compared to *Acacia* and fly ash amendment between 6 to 12% acted synergistically with chemical fertilizers as a result of which no adverse effect on the MAI was observed. MAI was greater in fly ash amended soil compared to the control, indicating the positive role of fly ash as a source of nutrients for improved plant growth and biomass production. The contribution of fly ash as a soil-amending agent towards growth and biomass production would depend on the uptake of nutrients by the plants (Adhoelya., 1998 ; Cavaleri et al., 2004). The use of fly ash alone and co-mixed with fertilizers can act synergistically to provide nourishment to the plant or forestry species.

Fly ash contains basic mineral elements, which make it similar to the earth's crust. Its addition to soil results in agronomic benefits enabling raising a green cover and even growing of crops on nutrient-deficient soils, and can be successfully used to correct soil pH. Presence of high concentrations of elements like K, Mg, Fe, Zn and Ca in readily available ionic form increases their uptake by plants. Soils prone to wind or water erosion can be stabilized through fly ash amendment. The fine nature of fly ash is effective in increasing water holding capacity of sandy soils and removing compaction in clay soils. These improvements in physical and chemical nature coupled with a positive effect on microbial denizens make the problem soils productive. Not only that, use of fly ash for reclaiming threatened soils provides an economical way of disposal. Using weathered fly ash can effectively solve the problem of ground water contamination. Phytoremediation offers an effective way to prevent cycling of toxicants from fly ash and growing of multipurpose tree species on problem soils is an environmentally safe strategy for gainful utilization of fly ash. Nonetheless, new knowledge needs to be generated to further minimize soil and groundwater contamination and identify ways to efficiently exploit the fly ash as a soil ameliorating agent for waste land reclamation and biomass production.

Conclusions

1. Fly ash obtained from different sources showed marked heterogeneity and all the elements present in fly ash were within range as reported in the literature. Most of the secondary elements (Ca, Mg, Na) and micronutrients (Fe, Zn, Mn) were greater in acidic fly ash. Overall the order of all nutrients was Fe>Mn>Zn>Cr>Pb>Co, which implies that fly ash is rich in Fe and Mn and the texture of fly ash in general was loam sand. All nutrients present in soil used in nursery and field trials were also present within range as reported in the literature. Nitrogen was highest in the acidic and alkaline soil followed by phosphorous and potassium. The order of micronutrients was Fe>Mn>Zn in the acidic as well as alkaline soil, and greater release of heavy metals and micronutrients was observed in acidic soil which had a clay texture while the alkaline soil had a loamy sand texture.
2. A positive correlation between soil microbial activity in terms of rate of carbon dioxide evolution, soil microbial biomass, soil dehydrogenase activity and organic carbon was observed with 10 to 12% fly ash amendment in acidic and alkaline soil. Addition of glucose as an organic carbon source resulted in a concentration-dependent increase in soil dehydrogenase activity in soil amended with 5% fly ash. Fly ash can promote soil microbial activity and mixing with an organic substrate enhances its benefits, which assumes importance owing to eco-friendly disposal of fly ash.
3. Phosphate-solubilizing bacteria showed good adaptability in fly ash amended soils and better survival compared to free-living nitrogen fixing bacteria. The percentage solubilization of TCP was 78.5% by strain S1, 80.0% by strain S2, 86.1% by strain S3, 78.7% by strain S4 and 82.8 % by strain S5 that was highest for these five bacterial isolates. No free-living nitrogen-fixing bacteria were observed in soil containing 18-24% fly ash with treatments without fertilizer and with fertilizer. The population of phosphate-solubilizing bacteria in fly ash amended soil mixed with chemical fertilizer was higher in the presence of fly ash at 12%, attributed mainly to the synergistic effect of fly ash and fertilizers on the soil microenvironment.

4. Antibiotic profiling of selected strains showed that all strains were sensitive to kanamycin and chloramphenicol and were lac Z negative. Phosphate-solubilizing bacterial isolate S2 was tagged with *E.coli* lac Z⁺ strain and its ecological monitoring on selective media indicated that 10 % fly ash is good for the proliferation of microbes since its amendment provides micronutrients for growth. A similar positive correlation was observed for microbial biomass, which increased with increasing fly ash percentage up to 5-10%.
5. Unweathered electrostatic precipitator (ESP) fly ash from GGSTP (Guru Gobind Singh Super Thermal Power Plant), Ropar used for establishing nursery trial of *Populus deltoides*, was observed to show a positive effect on soil dehydrogenase activity, bacterial enumeration and microbial biomass at a concentration of 10%. Among the secondary elements the order of preferential uptake by the plants was Ca>Mg>Na and magnesium was higher than calcium in soil. An increase in nitrogen, phosphorous, potassium and sulphur was observed after the trial with concomitant increase in fly ash percentage. Micronutrients (Fe, Cu, Zn, Mn and Mo) and heavy metals (Cr, Co) were observed to occur within permissible limits in soil as a result of fly ash addition. No distinct variation in the bulk density and hydraulic conductivity of soil with and without fly ash was observed except the water holding capacity, which increased due to addition of fly ash. The texture of the soil was loamy sand, which remained unchanged with increased fly ash addition before the start of the trial and after the trial.
6. The growth in *Acacia auriculiformis* was enhanced by as much as 6.8 % while it increased significantly by 14.3% in *Eucalyptus tereticornis* and the increase in GBH ranged from 6.4 to 15.1 % in both the tree species in soil without chemical fertilizers and in soil admixed with both fly ash and chemical fertilizers. Flyash did not have any negative impact on the Mean Annual Increment of *Acacia auriculiformis* and *Eucalyptus tereticornis* which ranged from 113 to 129 cm and 111.6 to 128.6 cm and was higher in the presence of flyash. No significant variation was observed in nitrogen concentrations over the four-year study period and 12 to 18% fly ash maintained the level of essential nutrients in soil. Heavy metals lead, nickel and chromium remained within permissible limits in soil over the four-year study period

and with increasing fly ash percentage. No drastic change in the particle size distribution, bulk density and water holding capacity of fly ash amended soil was observed. However hydraulic conductivity in the soil increased by 45% in *Eucalyptus* compared to 21% of *Acacia* with increasing fly ash percentage. No distinct variation was observed in organic carbon content and soil dehydrogenase activity of *Acacia* and *Eucalyptus* with increasing fly ash percentage and over the four-year study period and these were higher in rhizospheric soil of *Acacia* compared to *Eucalyptus*. Fly ash amendment between 12 and 18% was optimum for use as a soil amending agent for improving chemical and physical properties in addition to acting in a synergistic manner with chemical fertilizers for maintaining the nutrient balance and soil fertility within range as reported in literature for common cropland soil.

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4. Suryan, S., **Jala, S.,** Goyal, D., Singh, J., Vijayan, V., Niding, A., Khanna, S. 2004. Impact of Fly ash addition in soil on heavy metal accumulation in *Eucalyptus tereticornis*” In *Biotechnological approaches for sustainable development*” (Ed.) Reddy, M.S and Khanna, S. Allied Publishers Pvt. Ltd. pp. 305-310.
5. **Jala, S.,** Goyal, D., 2005. Fly ash as a soil ameliorating agent in forestry plantations. Full length paper accepted for oral presentation and in FLYASH India 2005, International Congress to be held from December 4-7 , 2005, Ashok Hotel, New Delhi.

Conferences attended* / Abstracts published

1. ***Jala, S.** and Goyal, D. “Effect of industrial fly ash on soil microbial activity”. *Proc. in 43rd Annual conference of Association of Microbiologist of India*, held at CCSHAU Hisar, Dec. 11-13, 2002, pp 13 (**Awarded best Poster Prize**).
2. *Goyal, D., **Jala S.,** Singh, J., Sharma, A., Gupta, R., Vijayan, V., Niding, A., Khanna, S. Coal fly ash as a soil ameliorating agent. *Proc. In National Conference*

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ANNEXURE-I

Fig. 1 Dehydrogenase activity (SDA: $\mu\text{g TPF g}^{-1}\text{soil day}^{-1}$) in alkaline soil amended with different percentage of glucose (g) and fly ash (fa).

(1a) Dehydrogenase activity in soil amended with 1-4% glucose.

(1b) Dehydrogenase activity in soil amended with 5% fly ash and varying percentage of glucose.

(1c) Dehydrogenase activity in soil amended with 4% glucose and varying percentage (1-10%) of fly ash.

(1d) Enumeration of *E.coli* S-17 lac Z⁺ ($\times 10^6\text{cfu g}^{-1}\text{soil}$) in sterile and non-sterile soil amended with 0 to 30% fly ash over 12-day incubation of 28°C.

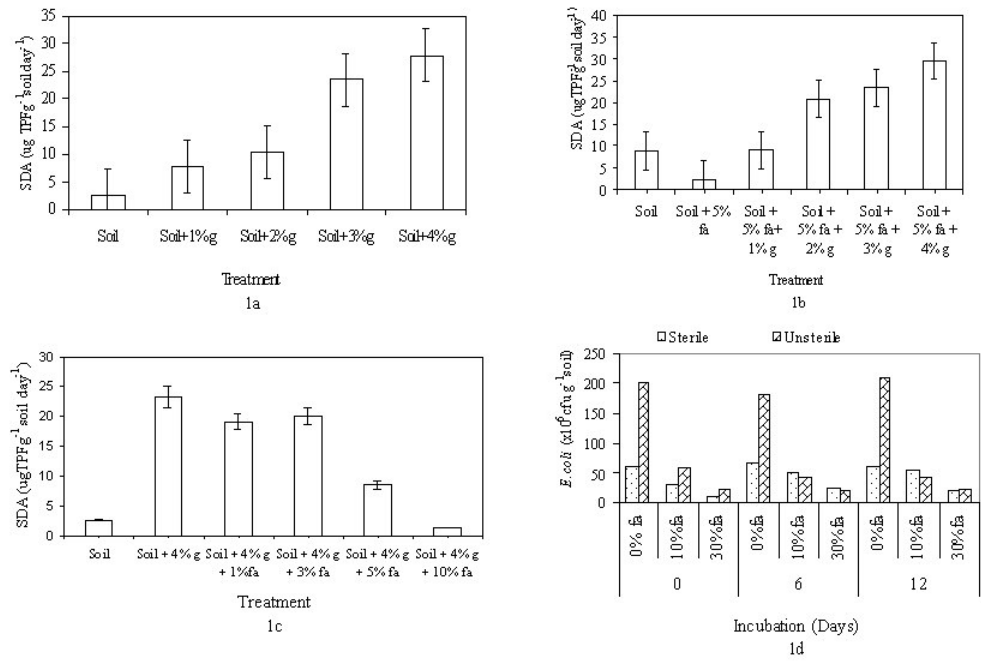


Fig. 1

Fig. 2 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline control soil without fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U : Sterile Uninoculated; Ns I : Non-sterile Inoculated; Ns U: Non-sterile Uninoculated).

- (2a) Bacterial counts (BC: $\times 10^4$ cfu g^{-1} soil) in control soil without fly ash and inoculated with phosphate-solubilizing bacterial isolate S2.
- (2b) Microbial biomass (MB : $\mu g g^{-1}$ soil) in control soil without fly ash and inoculated with phosphate-solubilizing bacterial isolate S2.
- (2c) Organic carbon (%) in control soil without fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (2d) Available phosphorus (ppm) in control soil without fly ash and inoculated with phosphate solubilizing bacterial isolate S2.

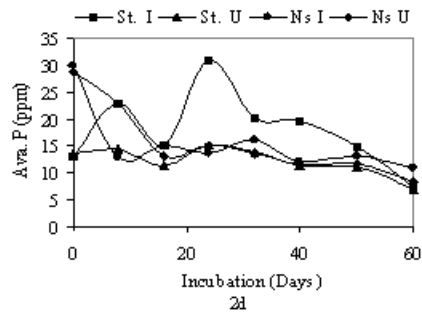
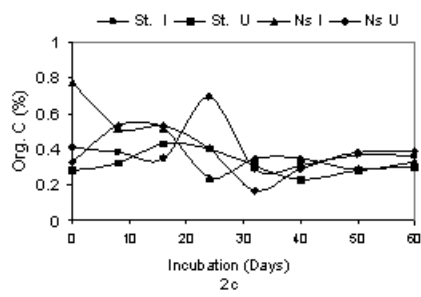
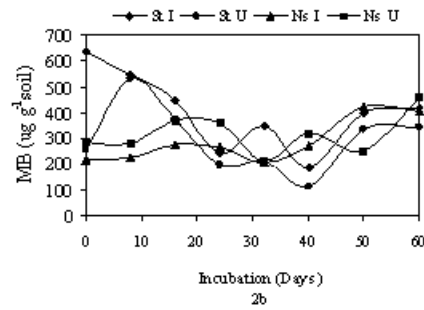
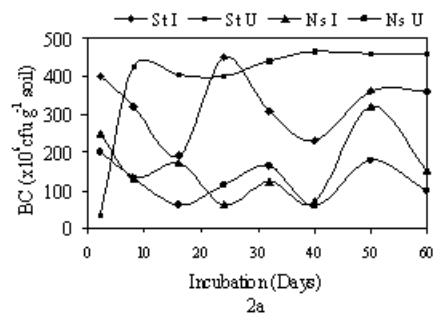


Fig. 2

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Fig. 3 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline soil amended with 10% fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U: Sterile Uninoculated; Ns I: Non-sterile Inoculated; Ns U: Non-sterile Uninoculated).

- (3a) Bacterial counts (BC: $\times 10^6$ cfu g^{-1} soil) in soil amended with 10% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (3b) Microbial biomass (MB : μg g^{-1} soil) in soil amended with 10% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (3c) Organic carbon (%) in soil amended with 10% fly ash and inoculated with phosphate solubilizing bacterial isolate S2 .
- (3d) Available phosphorus (ppm) in soil amended with 10% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.

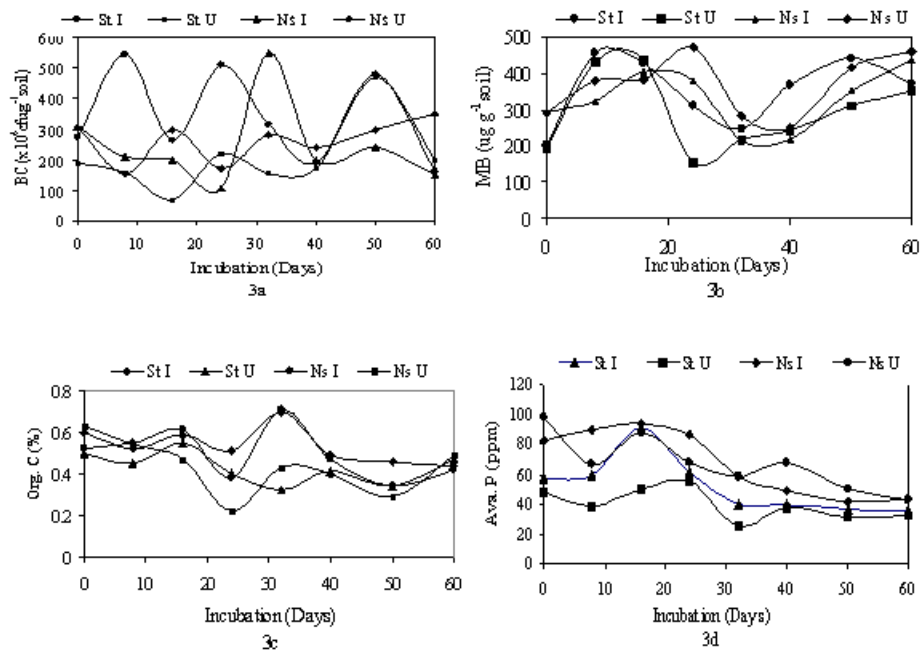


Fig. 3

Fig. 4 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline soil amended with 30% fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U: Sterile Uninoculated; Ns I : Non-sterile Inoculated; Ns U: Non-sterile Uninoculated).

- (4a) Bacterial counts (BC: $\times 10^6$ cfu g^{-1} soil) in soil amended with 30% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (4b) Microbial biomass (MB : μg g^{-1} soil) in soil amended with 30% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (4c) Organic carbon (%) in soil amended with 30% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.
- (4d) Available phosphorus (ppm) in soil amended with 30% fly ash and inoculated with phosphate solubilizing bacterial isolate S2.

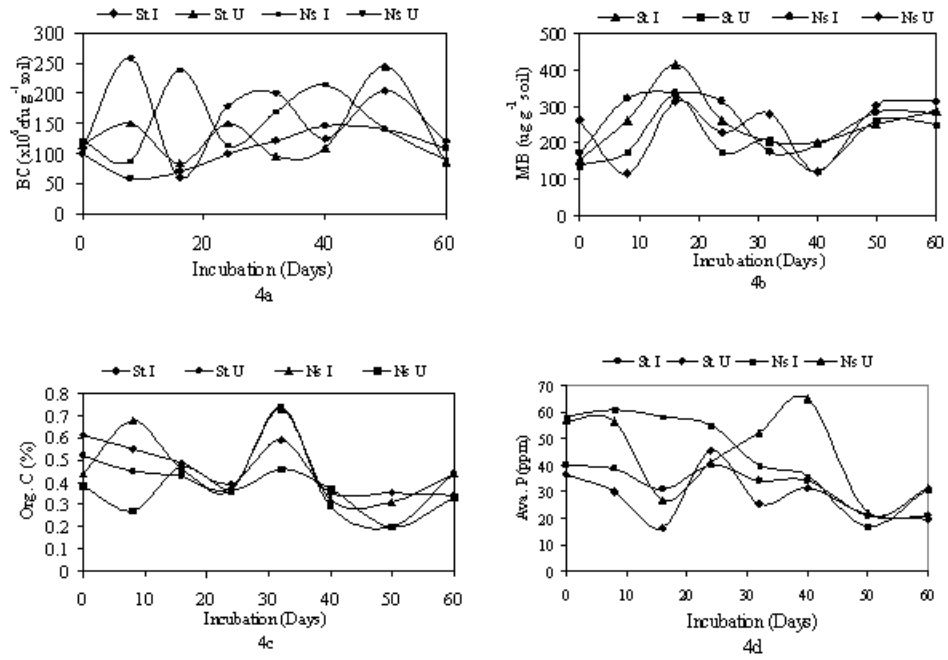


Fig. 4

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Fig. 5 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline control soil without fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U: Sterile Uninoculated; Ns I : Non-sterile Inoculated; Ns U, Non-sterile Uninoculated).

(5a) Bacterial counts (BC: $\times 10^4$ cfu g^{-1} soil) in control soil without fly ash and inoculated with transformant S2: pMIMB277.

(5b) Microbial biomass (MB : μg g^{-1} soil) in control soil without fly ash and inoculated with transformant S2: pMIMB277.

(5c) Organic carbon (%) in control soil without fly ash and inoculated with transformant S2: pMIMB277

(5d) Available phosphorus (ppm) in control soil without fly ash and inoculated with transformant S2: pMIMB277.

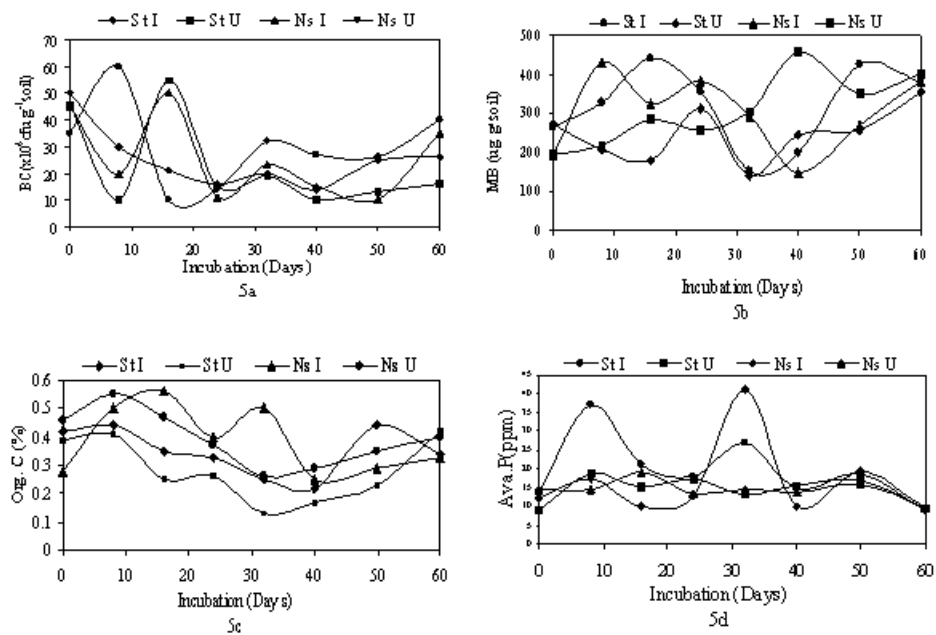


Fig. 5

Fig. 6 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline soil amended with 10% fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U: Sterile Uninoculated; Ns I: Non-sterile Inoculated; Ns U: Non-sterile Uninoculated).

- (6a) Bacterial counts (BC: $\times 10^4$ cfu g^{-1} soil) in soil amended with 10% fly ash and inoculated with transformant S2: pMIMB277.
- (6b) Microbial biomass (MB : μg g^{-1} soil) in soil amended with 10% fly ash and inoculated with transformant S2: pMIMB277.
- (6c) Organic carbon (%) in soil amended with 10% fly ash and inoculated with transformant S2: pMIMB277.
- (6d) Available phosphorus (ppm) in soil amended with 10% fly ash and inoculated with transformant S2: pMIMB277.

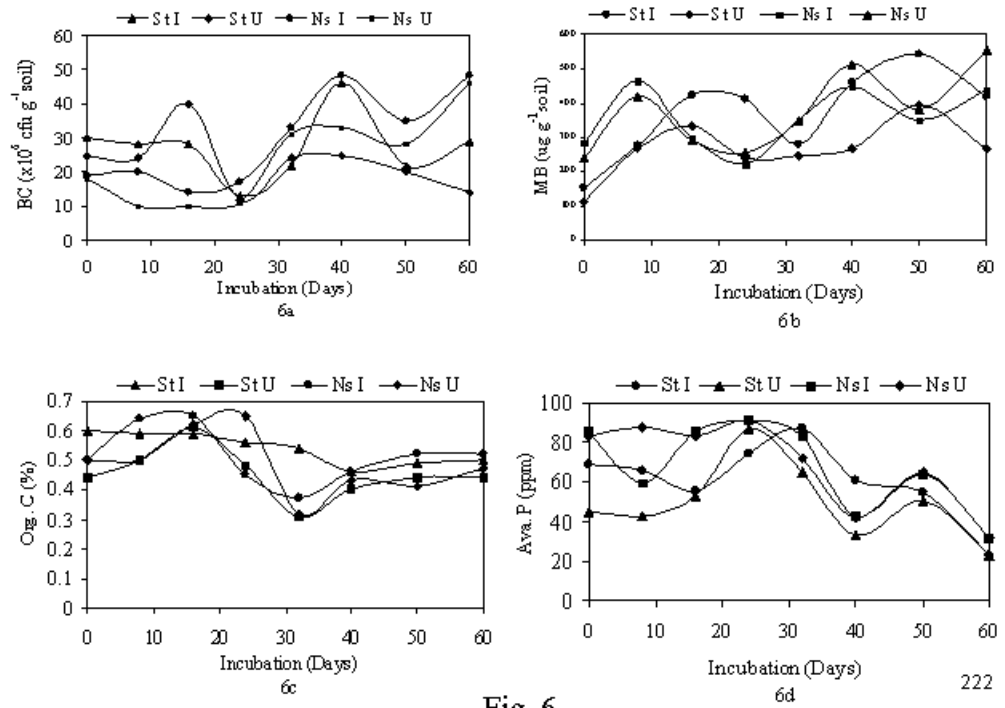


Fig. 6

Fig. 7 Bacterial counts, microbial biomass, organic carbon and available phosphorous in alkaline soil amended with 30% fly ash over a 60-day incubation period at 28°C (St I: Sterile Inoculated; St U: Sterile Uninoculated; Ns I : Non-sterile Inoculated; Ns U, Non-sterile Uninoculated).

- (7a) Bacterial counts (BC: $\times 10^4$ cfu g^{-1} soil) in soil amended with 30% fly ash and inoculated with transformant S2: pMIMB277.
- (7b) Microbial biomass (MB : μg g^{-1} soil) in soil amended with 30% fly ash and inoculated with transformant S2: pMIMB277.
- (7c) Organic carbon (%) in soil amended with 30% fly ash and inoculated with transformant S2: pMIMB277.
- (7d) Available phosphorus (ppm) in soil amended with 30% fly ash and inoculated with transformant S2: pMIMB277.

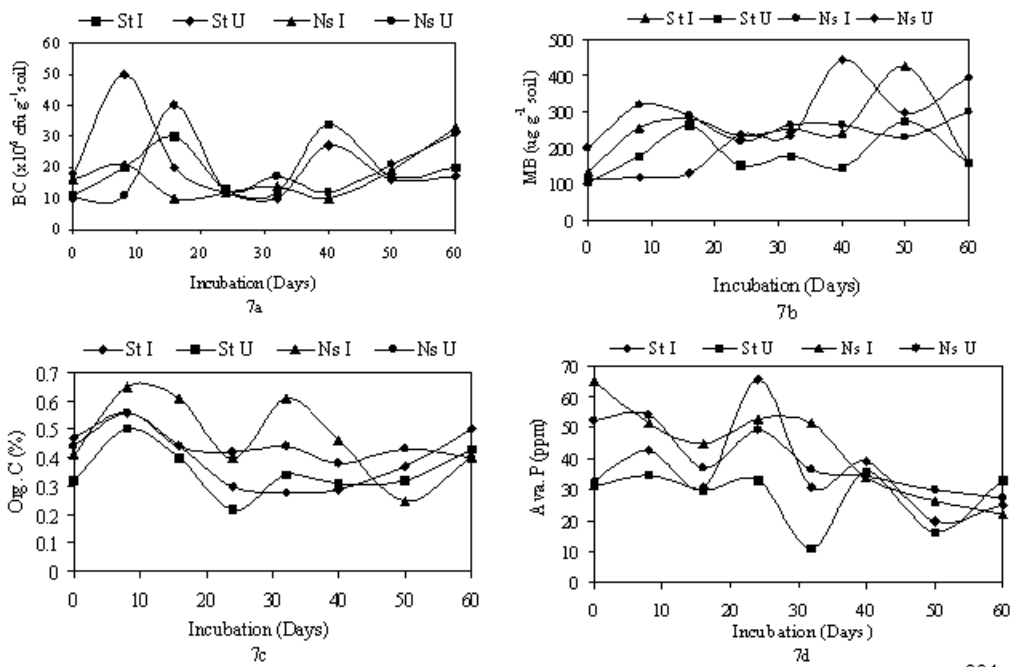


Fig. 7

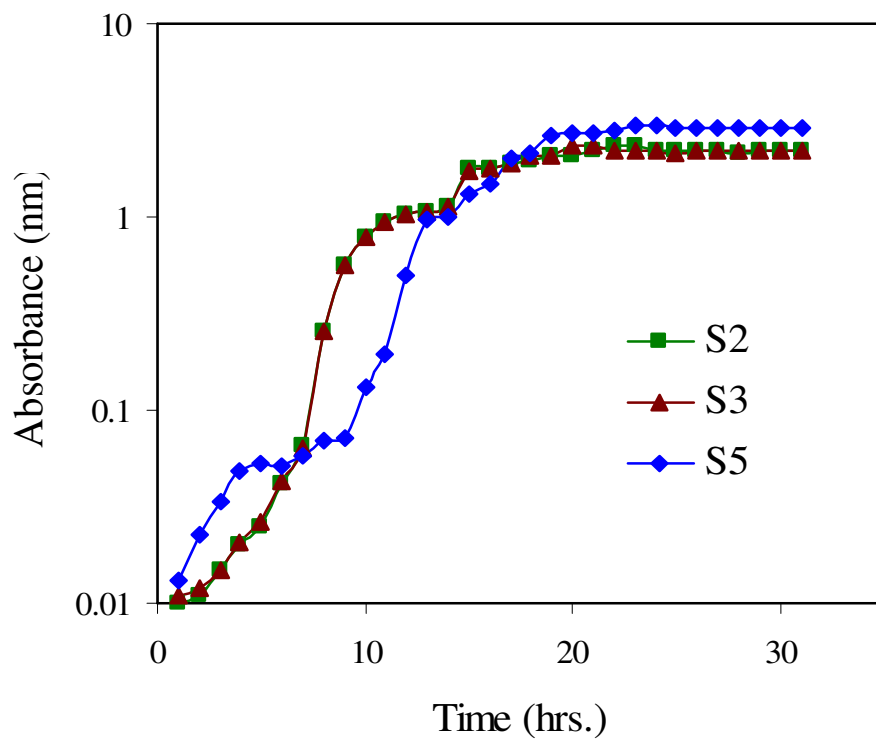


Fig. 8 Growth curve plotted as a function of time against absorbance for phosphate-solubilizing bacterial isolates S2, S3 and S5.

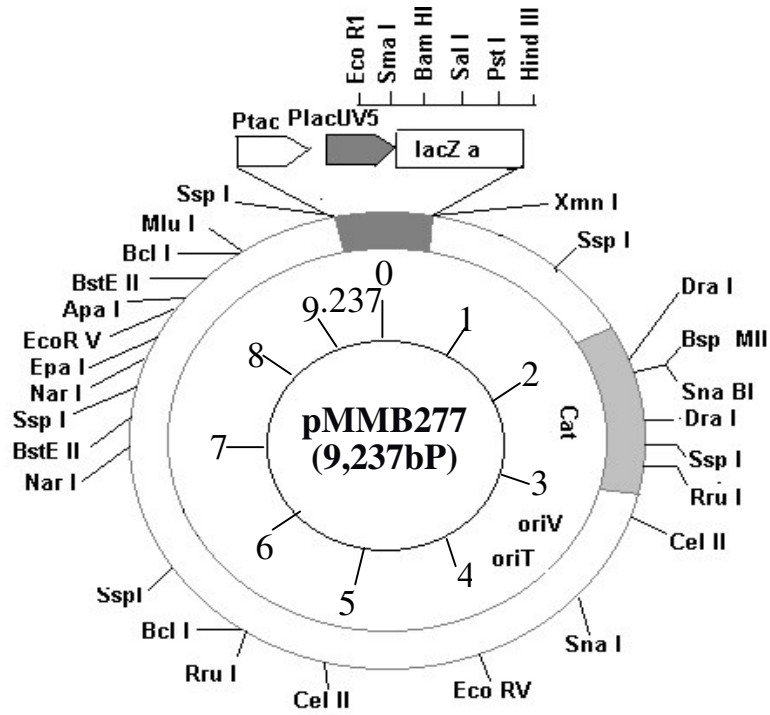


Fig. 9 Map of pMMB277 plasmid broad host range, artificial cloning vector (Gene, 1997).

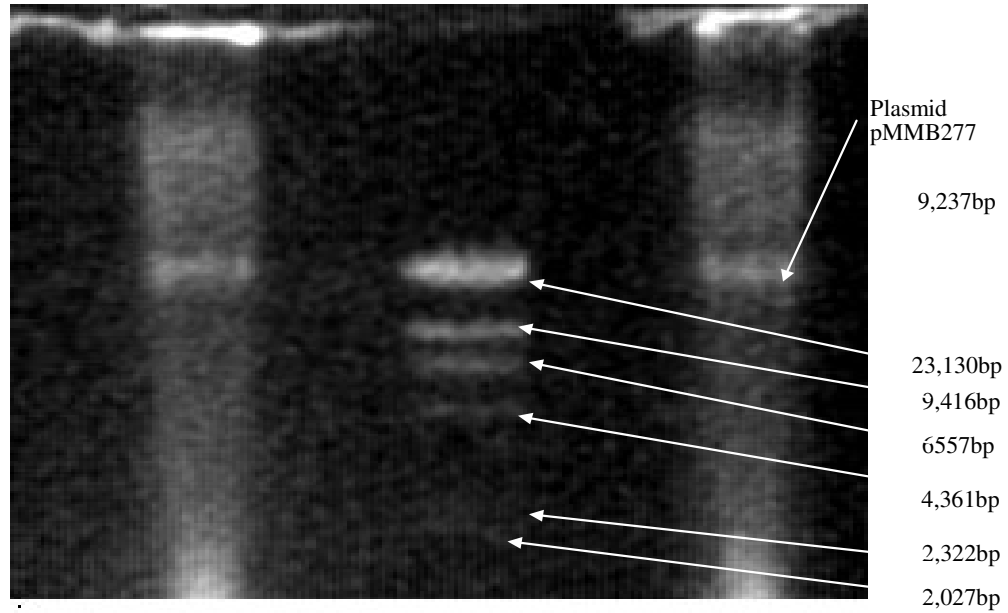


Fig. 10 Isolation and purification of wide host range expression vector plasmid pMMB277 on 0.7% agarose gel.
 Lane 1 &3 : Plasmid pMMB277
 Lane 2 : Lambda DNA marker cut by Hind III

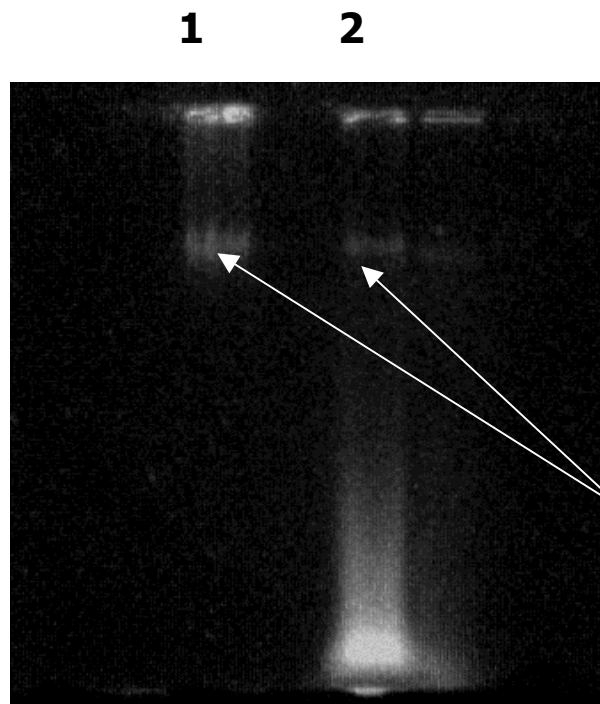


Fig. 11 Isolation of plasmid S2:pMMB277 (Mol.wt: 9,237bp) from S2 transformant on 0.7% agarose gel.
 Lane 1 : Plasmid pMMB277 isolated from S2: pMMB277 by Kado lysis.
 Lane 2 : Plasmid pMMB277 isolated from E.coli 2842 by alkali lysis.

Fig. 12 Bacterial counts, soil dehydrogenase activity, organic carbon and microbial biomass in alkaline soil amended with ESP fly ash from Ropar (*vvv*) @ 0, 5, 10, 20 and 30% before and after nursery trial of *Populus deltoides*.

(12a) Bacterial counts (BC : $\times 10^6$ cfu g^{-1} soil).

(12b) Soil dehydrogenase activity (SDA : μg TPF g^{-1} soil day $^{-1}$).

(12c) Organic carbon (%).

(12d) Microbial biomass (MB : μg g^{-1} soil).

Fig. 12 Bacterial counts, soil dehydrogenase activity, organic carbon and microbial biomass in alkaline soil amended with ESP fly ash from Ropar (*v/v*) @ 0, 5, 10, 20 and 30% before and after nursery trial of *Populus deltoides*.

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(12b) Soil dehydrogenase activity (SDA : μg TPF g^{-1} soil day^{-1}).

(12c) Organic carbon (%).

(12d) Microbial biomass (MB : μg g^{-1} soil).

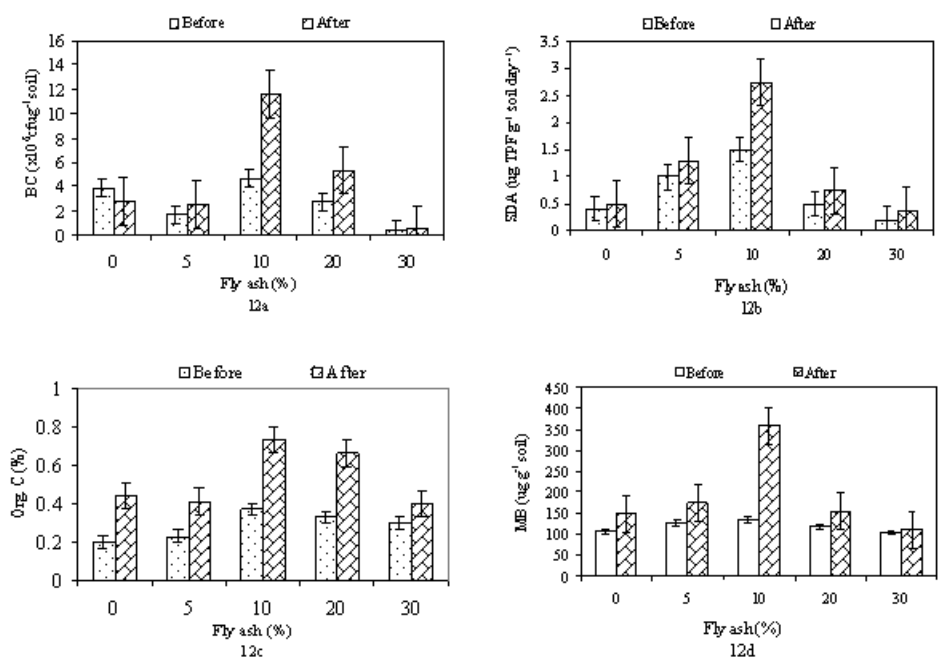


Fig. 12

Fig. 13 Rate of carbon-dioxide evolution ($\text{mg CO}_2/100 \text{ g}^{-1} \text{ soil day}^{-1}$) in alkaline soil amended with ESP fly ash from Ropar (v/v) @ 0, 5, 10, 20 and 30% (13a) before (13b) after nursery trial of *Populus deltoides*.

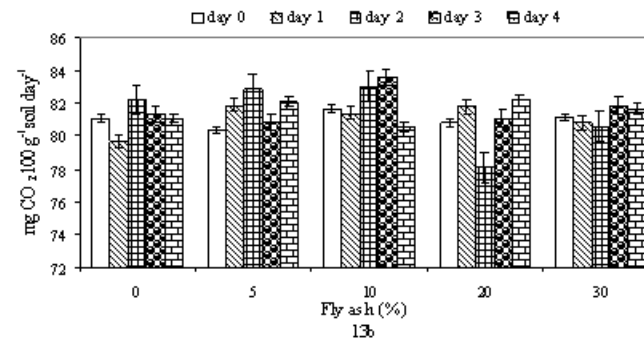
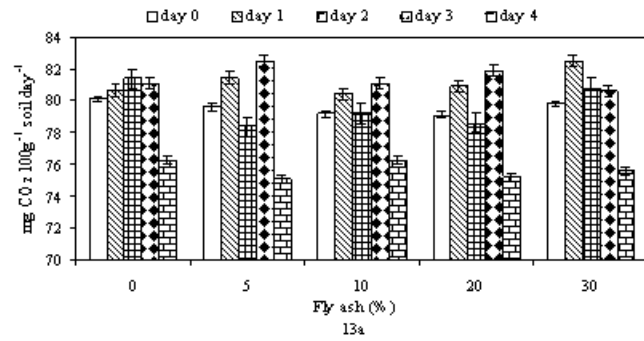


Fig. 13

Fig. 14 Organic carbon (%) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

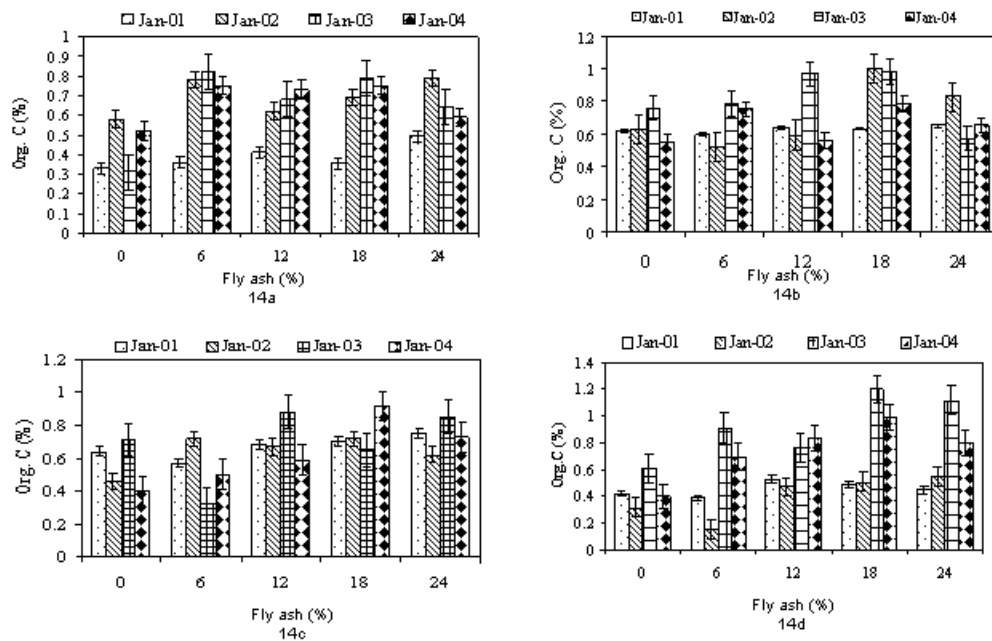


Fig. 14

Fig. 15 Bacterial counts (BC: $\times 10^4$ cfu g^{-1} soil) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24% (F4) at yearly intervals.

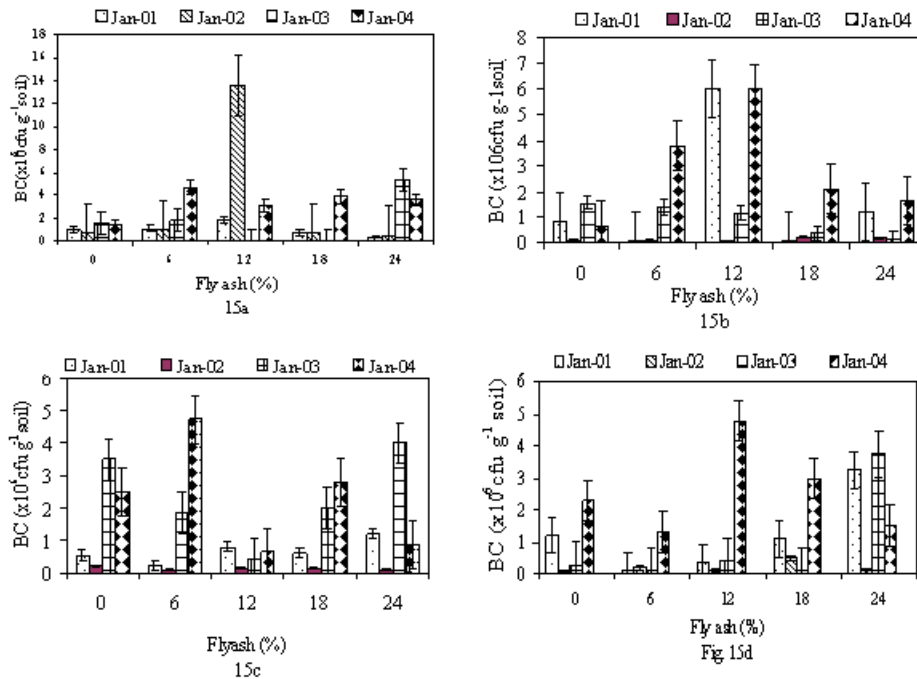


Fig. 15

235

Fig. 16 Soil dehydrogenase activity (SDA: $\mu\text{g TPF g}^{-1} \text{ soil day}^{-1}$) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24% (F4) at yearly intervals.

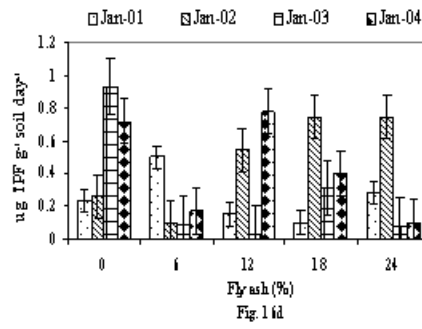
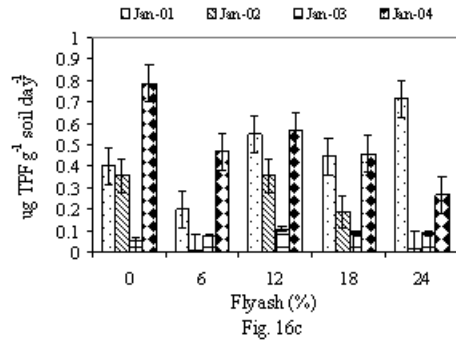
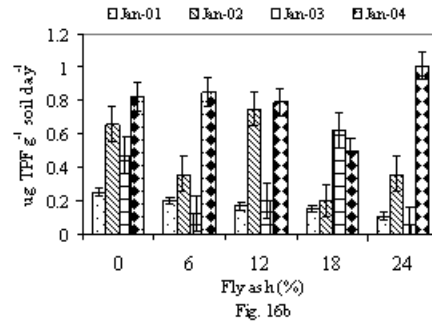
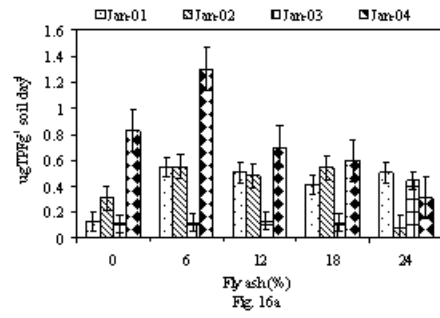


Fig. 16

Fig. 17 Particle-size distribution (PSD: %) and hydraulic conductivity (HC: cm min^{-1}) of rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) amended with different percentages of fly ash (*v/v*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals

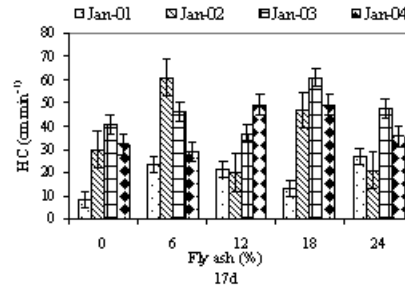
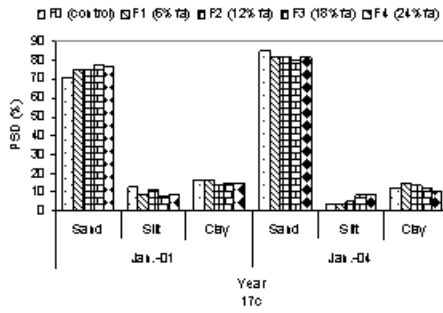
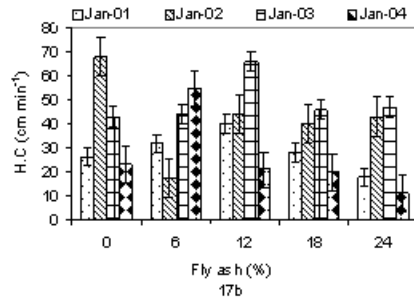
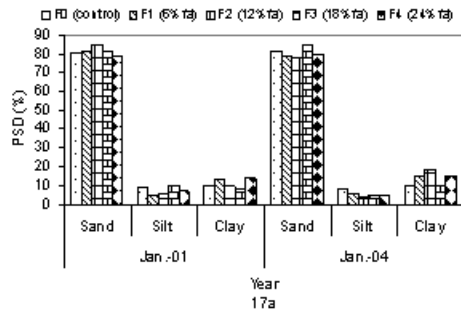


Fig. 17

Fig. 18 Water holding capacity (W.H.C: %) and bulk density (B.D: g cm^{-3}) of rhizosphere soil of *Acacia auriculiformis* (a, c) and *Eucalyptus tereticornis* (b, d) without chemical fertilizers (T1) amended with different percentages of fly ash (*v/v*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals

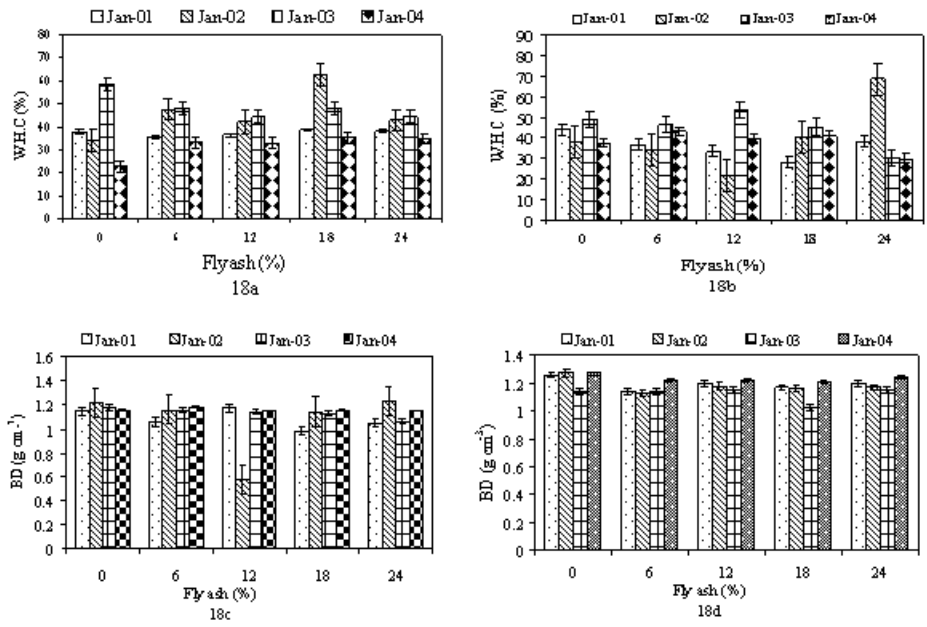


Fig. 18

Fig. 19 Available nitrogen (%) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24% (F4) at yearly intervals.

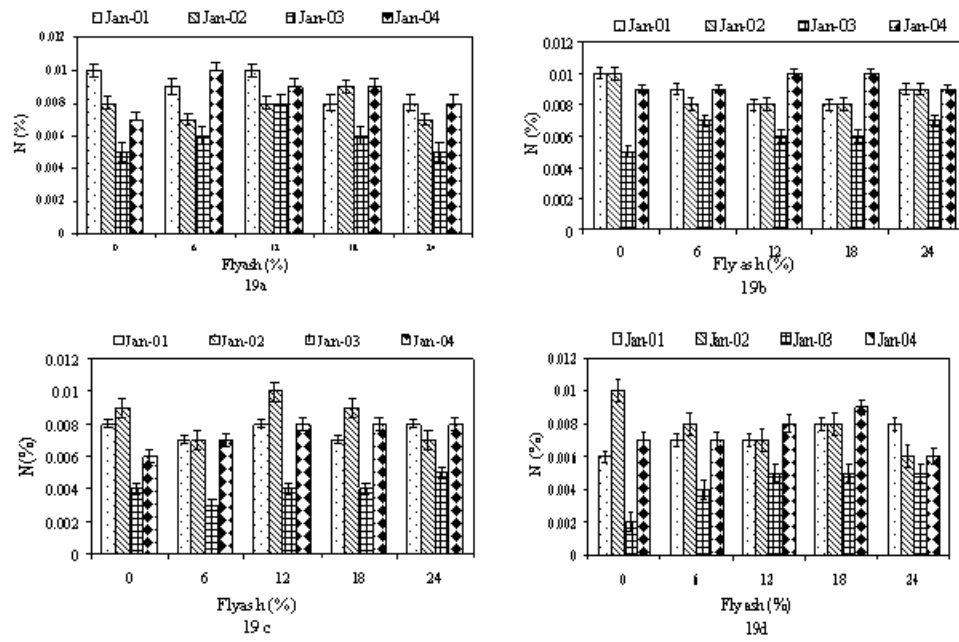


Fig. 19

Fig. 20 Available phosphorous mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

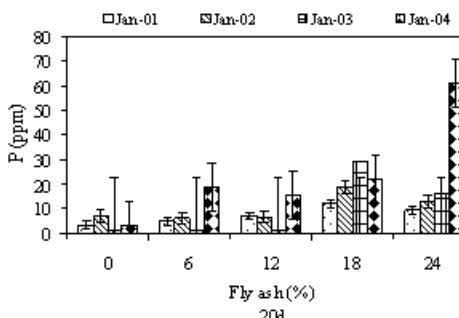
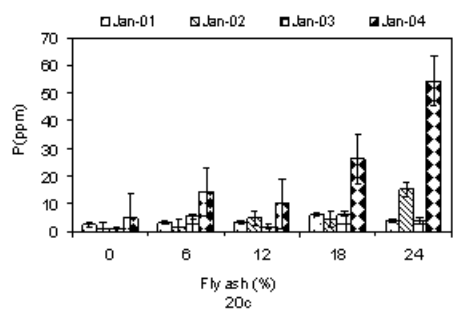
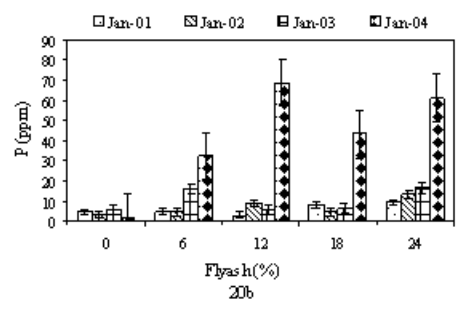
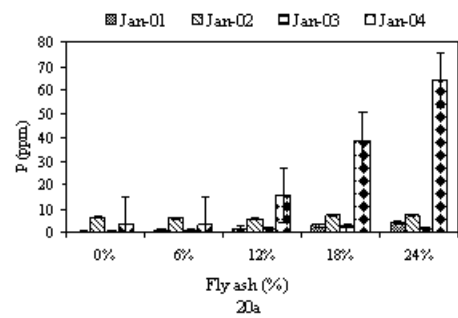


Fig. 20

245

Fig. 21 Available potassium mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (*v/v*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

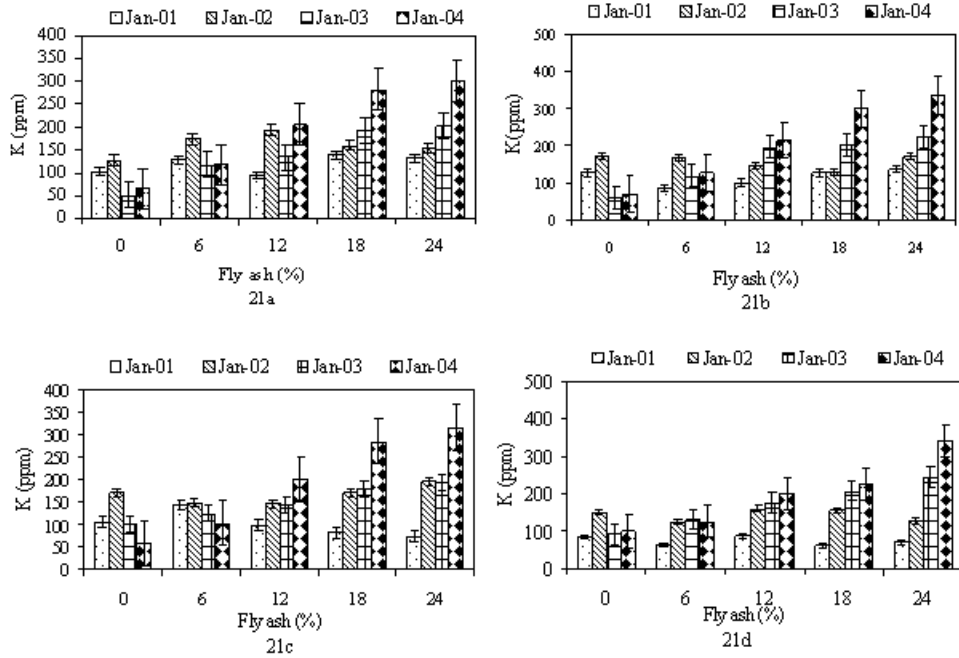


Fig. 21

Fig. 22 Available sulphur mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24% (F4) at yearly intervals.

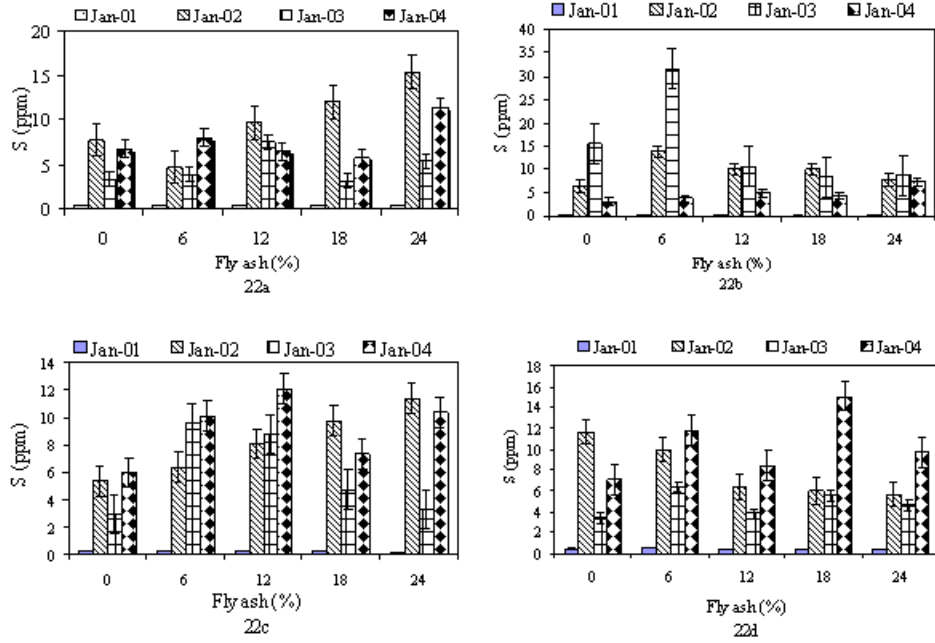


Fig. 22

Fig. 23 Total iron mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

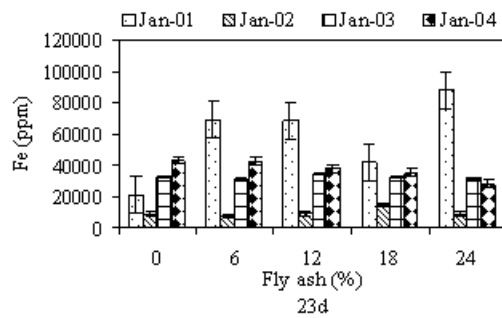
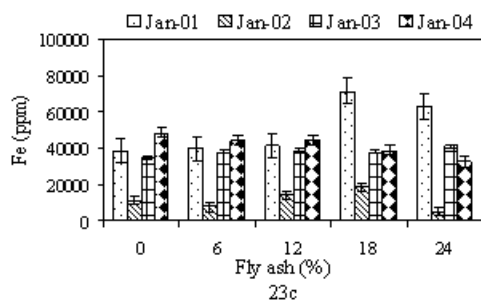
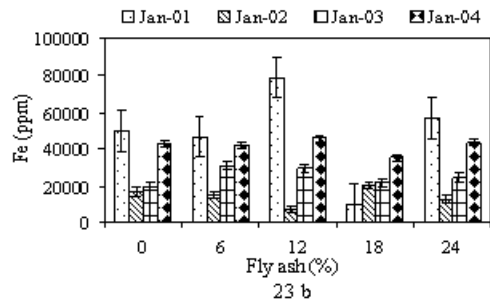
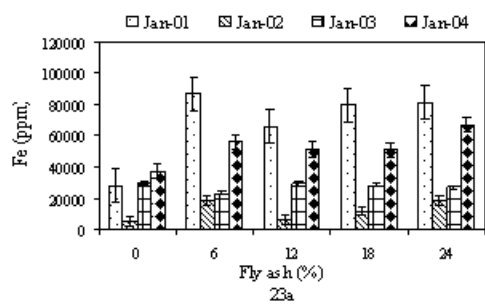


Fig. 23

Fig. 24 Total zinc mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (*wt*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

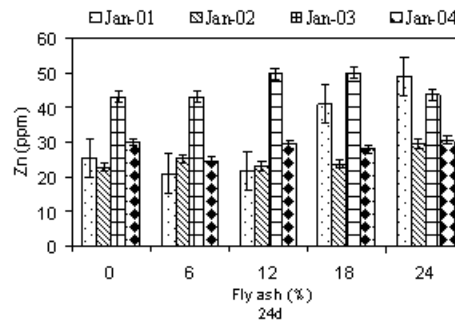
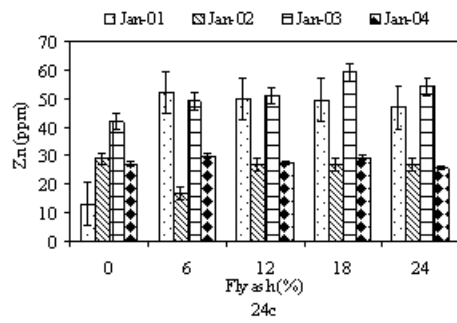
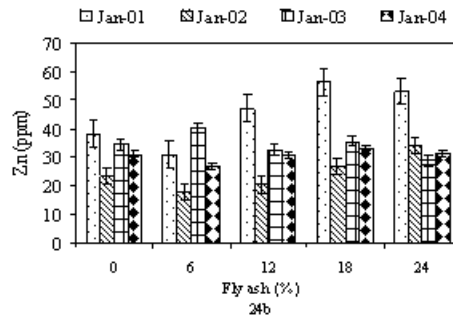
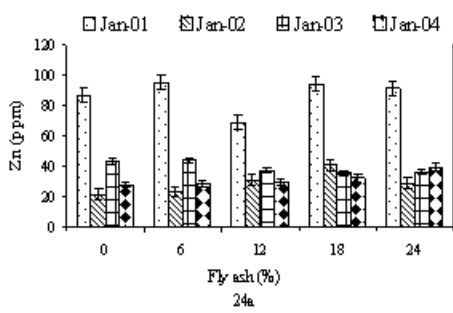


Fig. 24

253

Fig. 25 Total manganese mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (*v/v*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

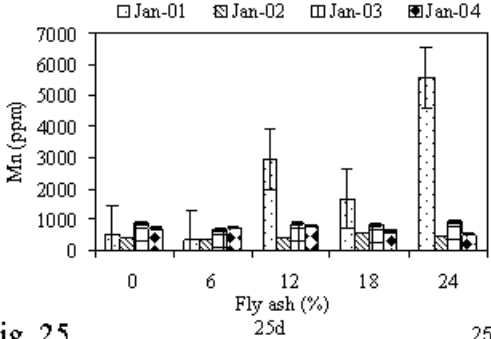
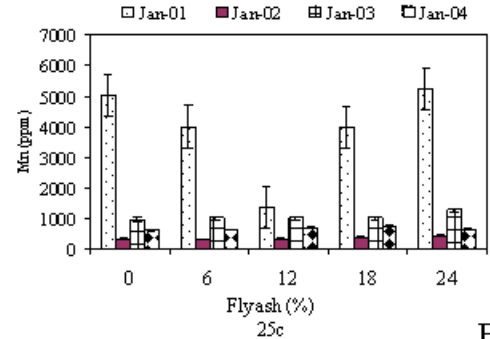
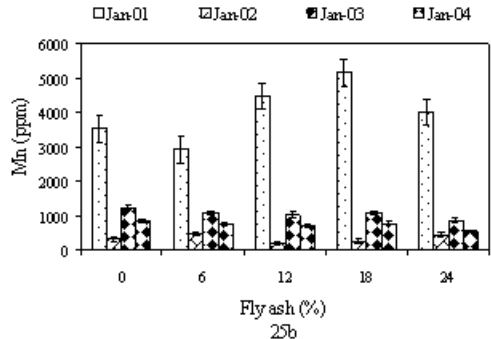
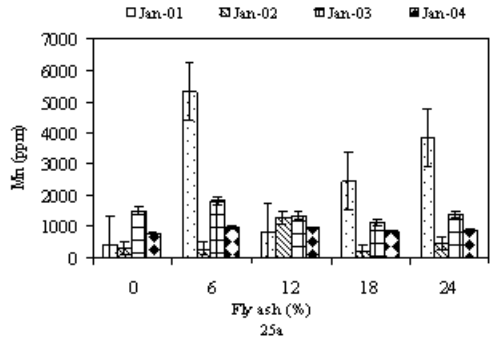


Fig. 25

Fig. 26 Total nickel mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (*v/v*) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

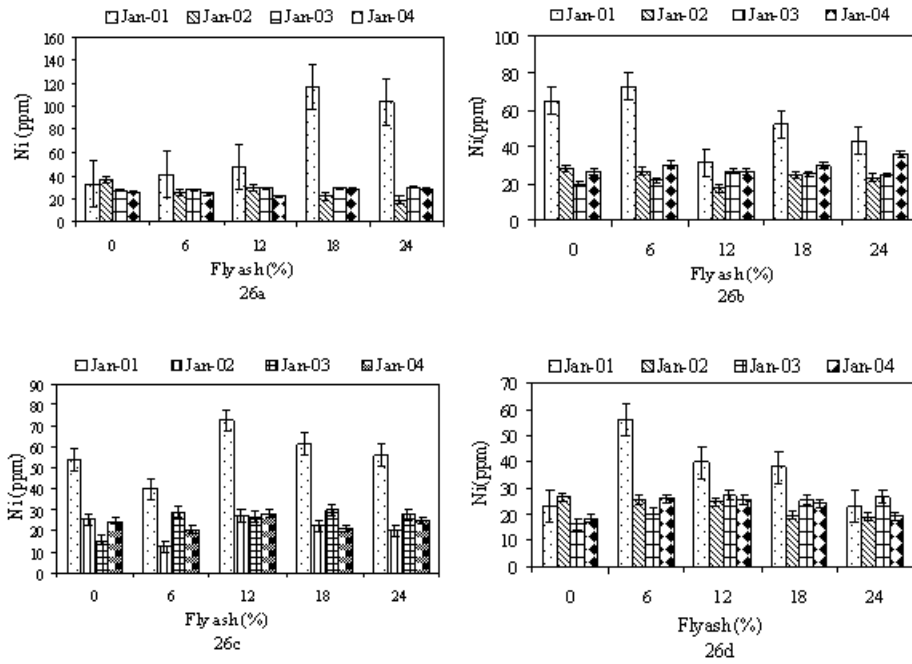


Fig. 26

Fig. 27 Total chromium mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

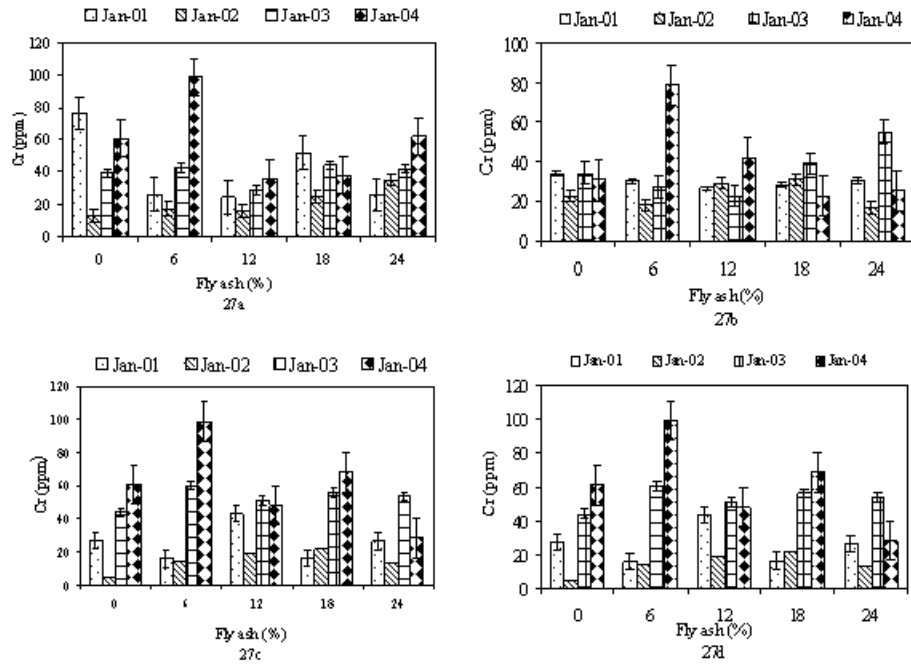


Fig. 27

Fig. 28 Total lead mg/kg (ppm) in rhizosphere soil of *Acacia auriculiformis* (a, b) and *Eucalyptus tereticornis* (c, d) without chemical fertilizers (T1) and fly ash plus chemical fertilizers (T2) amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) at yearly intervals.

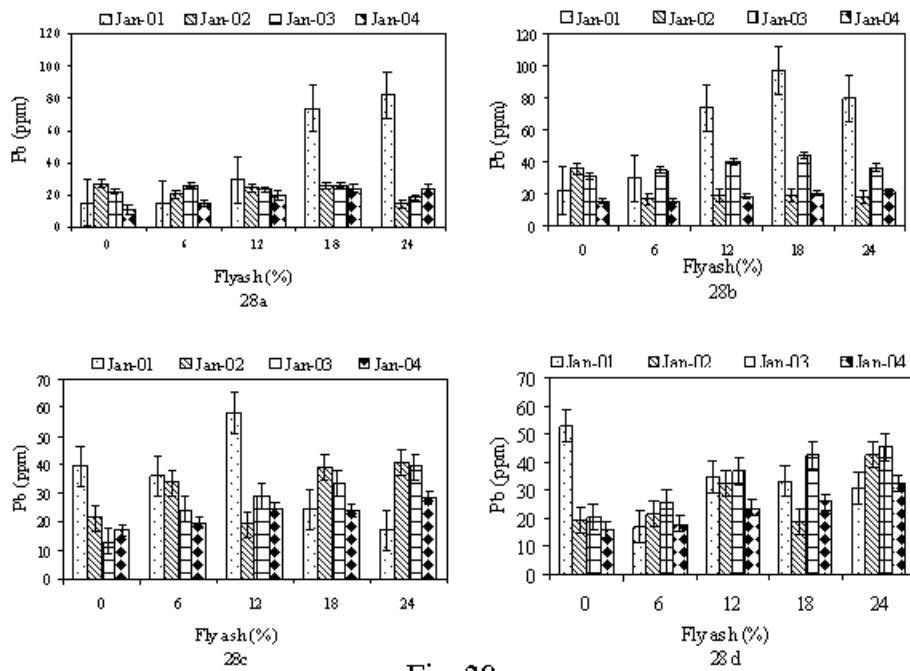


Fig. 28

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Fig. 29 Collar diameter (CD) and (GBH) Girth at breast height (a) Mean height (b) in cm of *Acacia auriculiformis* grown in soil amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) without chemical fertilizers (T1) and with chemical fertilizers (T2) at yearly intervals.

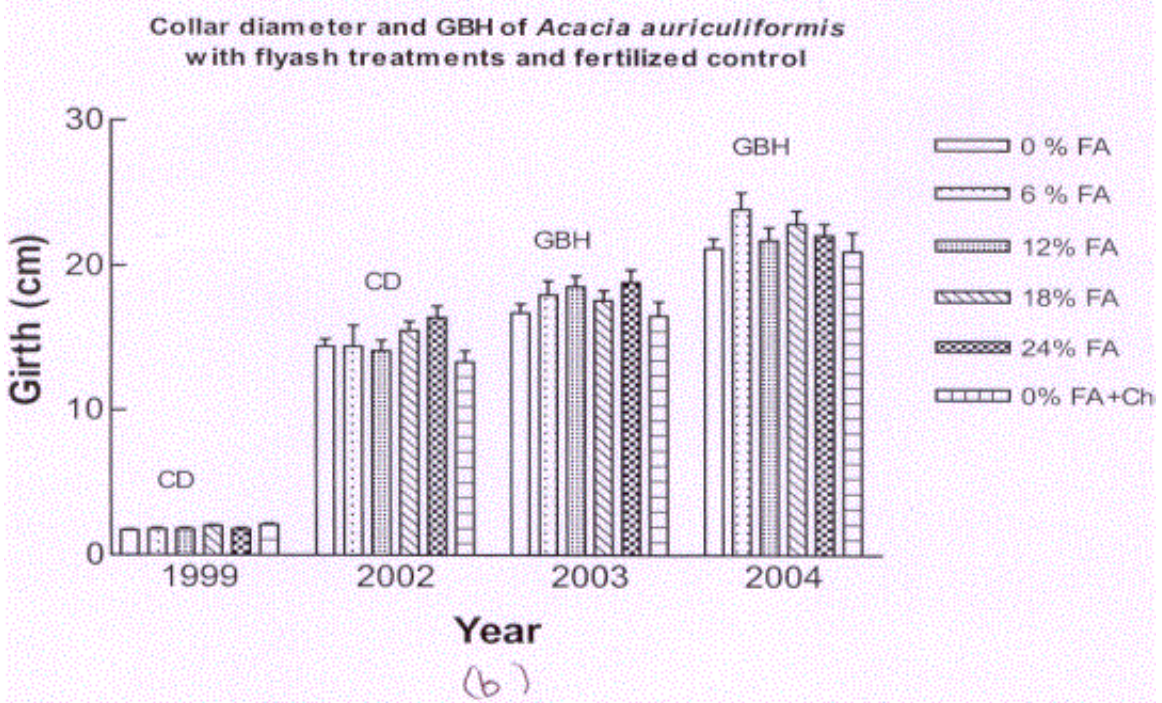
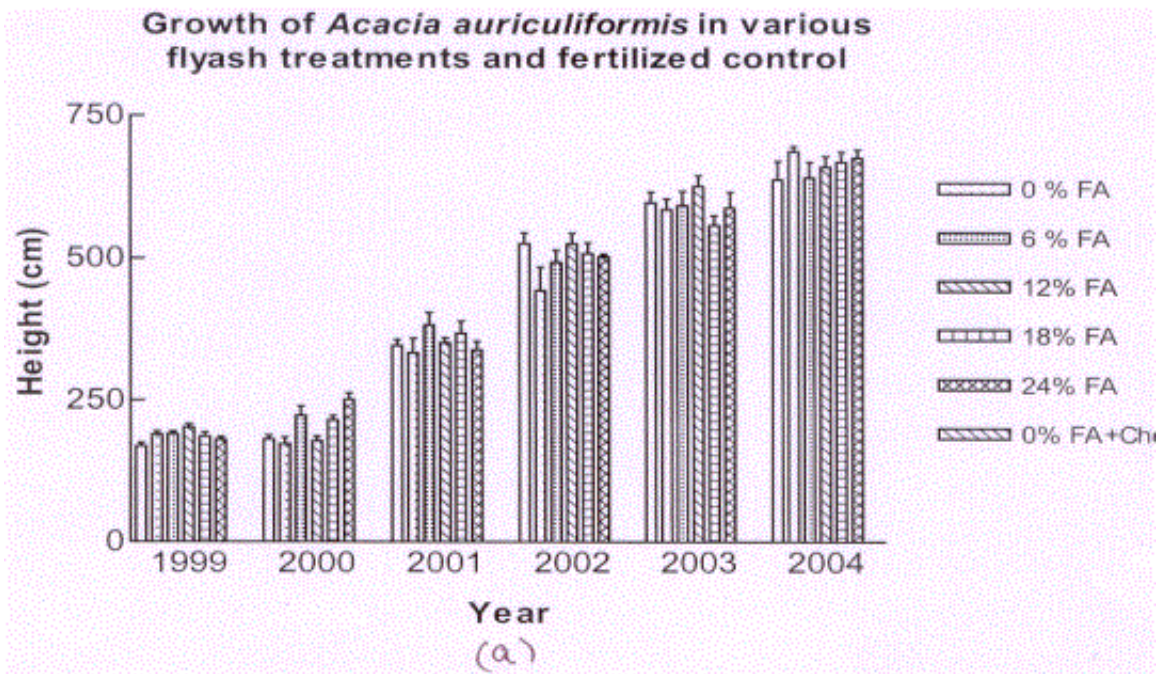
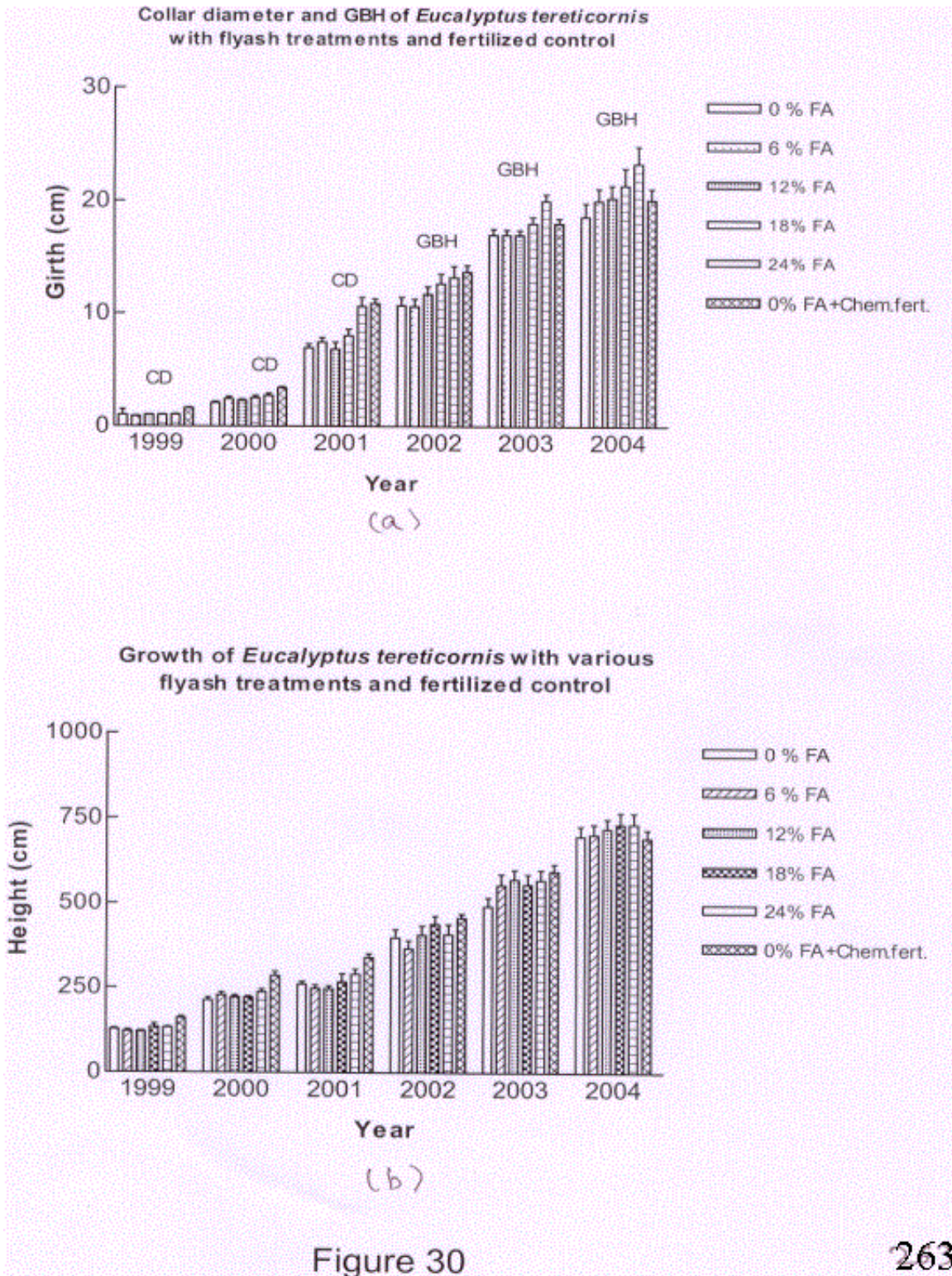
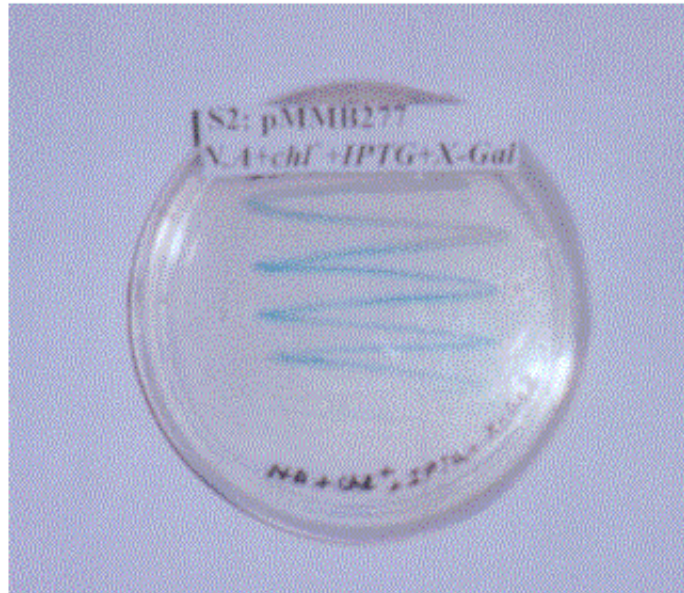


Figure 29

Fig. 30 Collar diameter (CD) and (GBH) Girth at breast height (a) Mean height (b) in cm of *Eucalyptus tereticornis* grown in soil amended with different percentages of fly ash (v/v) from 0% (F0), 6% (F1), 12% (F2), 18% (F3) and 24 % (F4) without chemical fertilizers (T1) and with chemical fertilizers (T2) at yearly intervals.

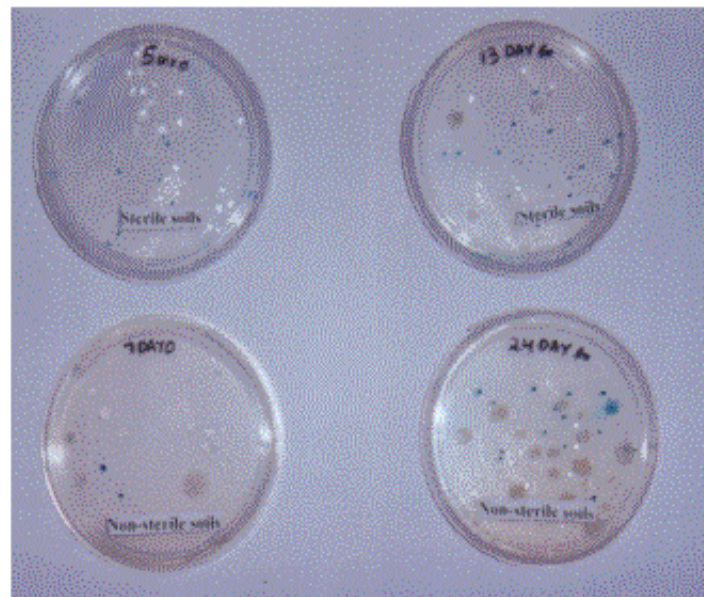


ANNEXURE-II



(a)

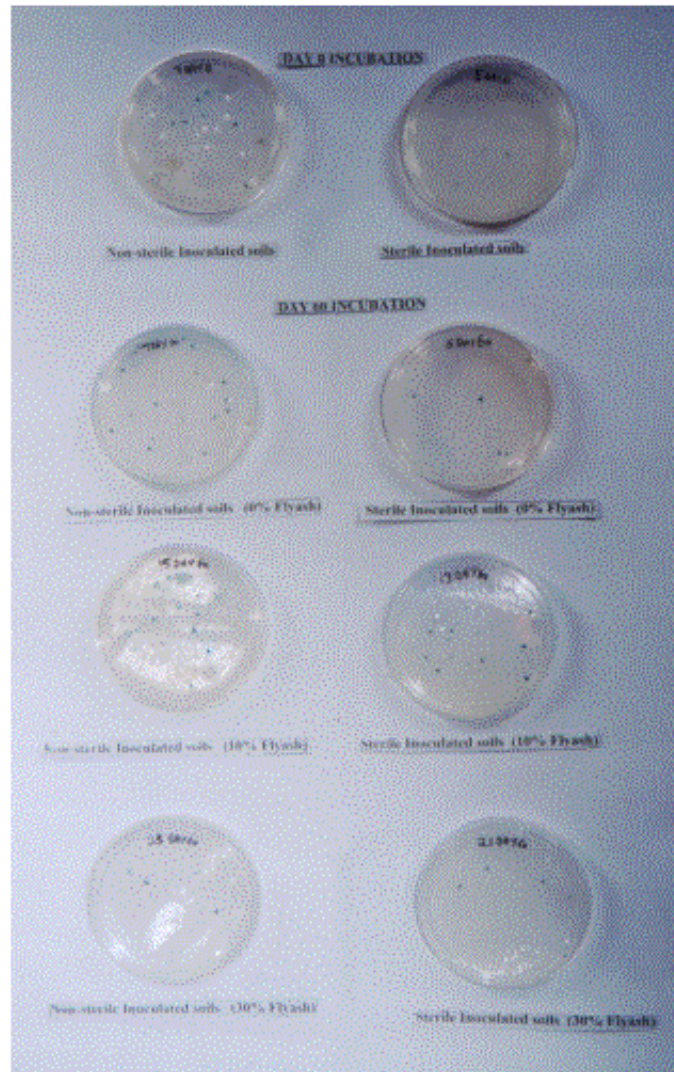
Transformant S2: pMIMB277 screened on X-gal+IPTG+ chloramphenicol ($10 \mu\text{g ml}^{-1}$) containing nutrient agar and checked for β -galactosidase expression



(b)

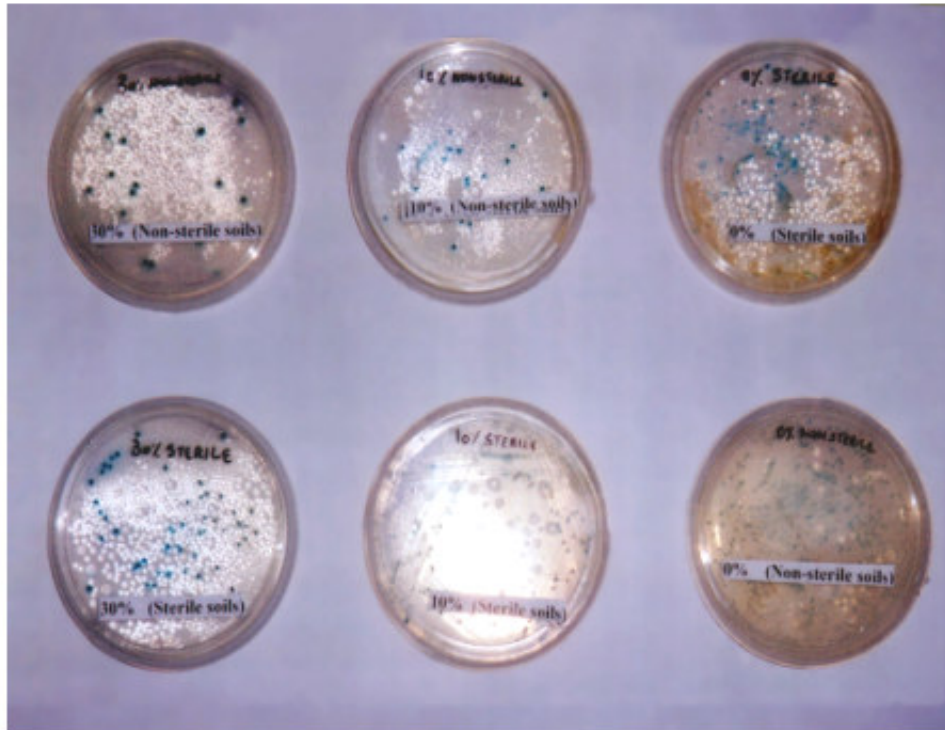
Enumeration inoculated S2: pMIMB277 on X-gal+IPTG+chloramphenicol ($10 \mu\text{g ml}^{-1}$) containing nutrient agar in sterile and non-sterile soil on day zero and day 60 of incubation

Plate 1



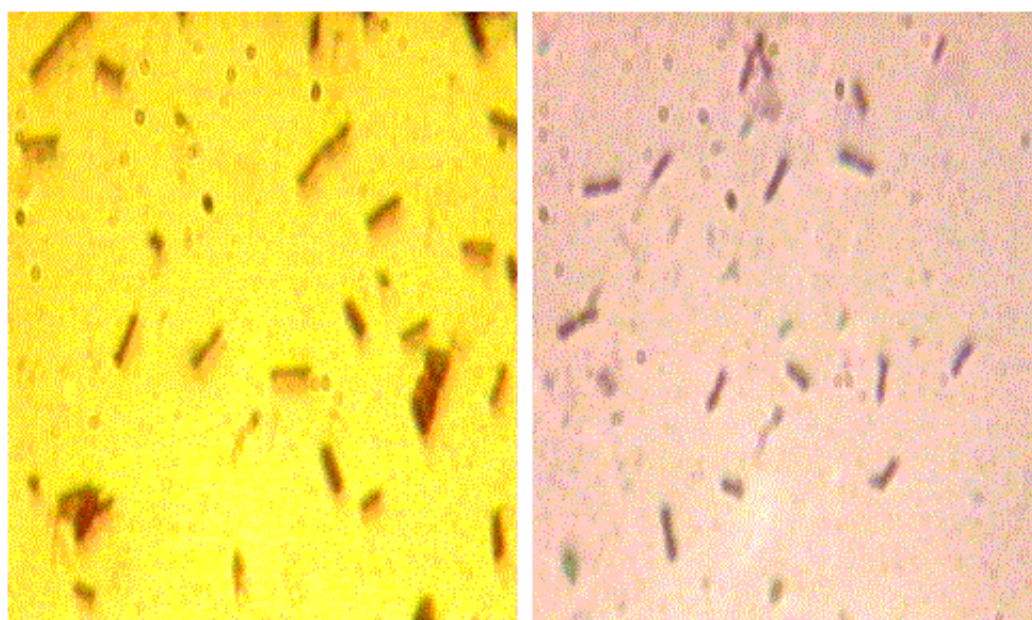
Enumeration of inoculated S2: pMIMB277 on X-gal+ IPTG+ chloramphenicol ($10 \mu\text{g ml}^{-1}$) containing nutrient agar from soil amended with 0-30% fly ash using sterile and non-sterile conditions

Plate 2



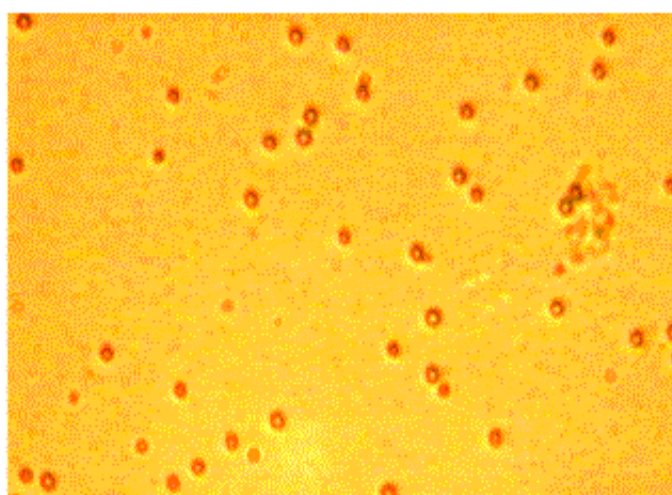
Enumeration of *E. coli* S-17 lac Z⁺ (blue colonies) on TY media containing kanamycin (50 μ g ml⁻¹) in sterile and non-sterile soil.

Plate 3



(a)

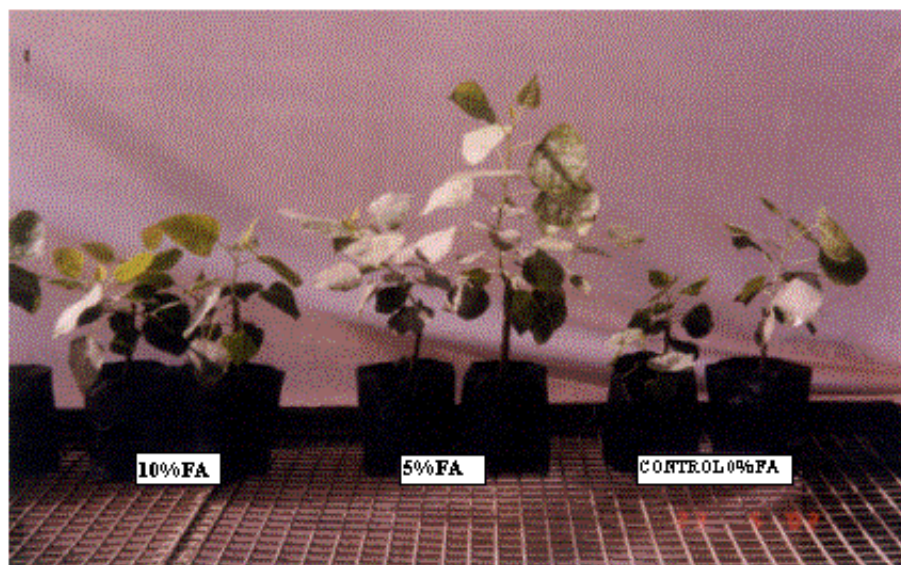
(b)



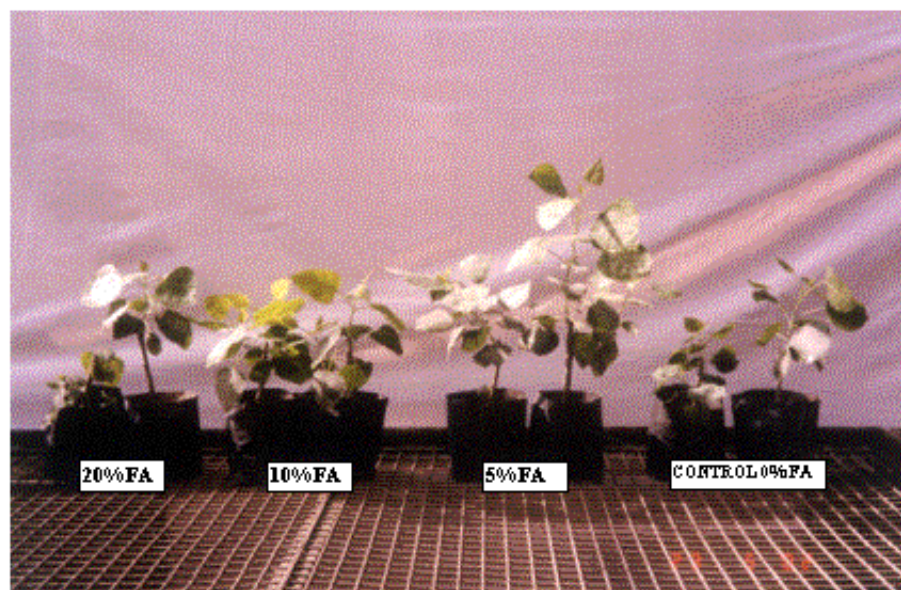
(c)

Photomicrographs of phosphate-solubilizing bacterial isolates
(a) S2 (Gram -ve) (b) S3 (Gram -ve) (c) S5 (Gram +ve)

Plate 4



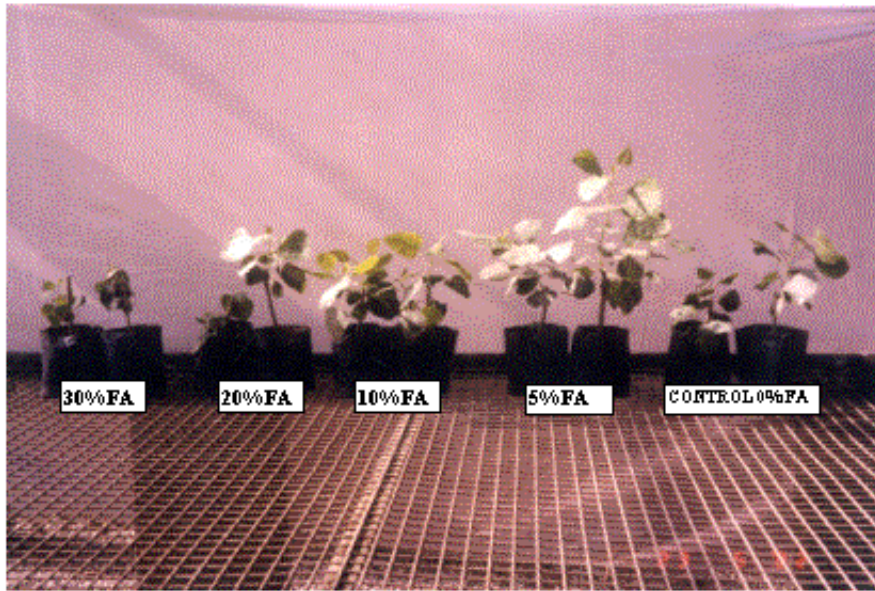
(a)



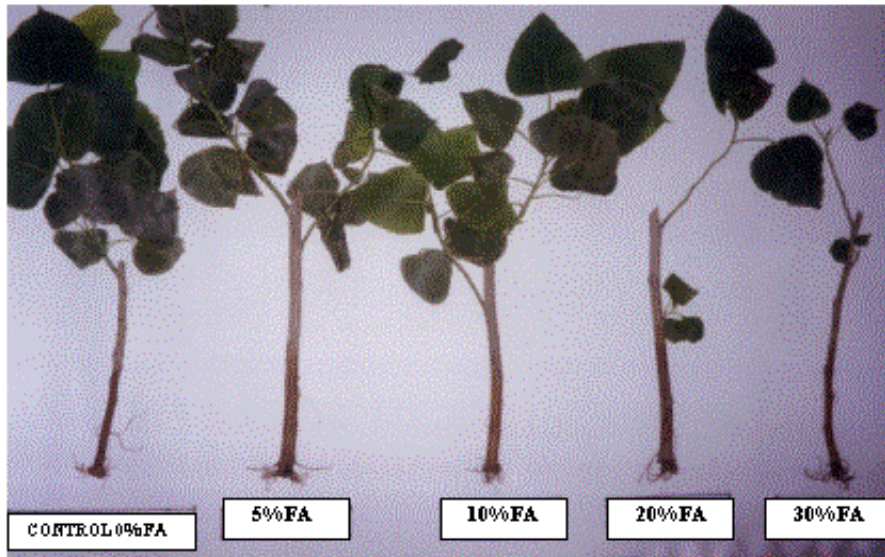
(b)

Populus deltoides (a-b) in alkaline soil amended with 0-20% ESP fly ash from Ropar during nursery trial.

Plate 5



(a)



(b)

Populus deltoides (a-b) in soil amended with 0-30% ESP fly ash after completion of nursery trial

Plate 6

