Microbial inoculants and industrial solid wastes as soil amendments in agroforestry

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

Doctor of Philosophy

In

Biotechnology & Environmental Sciences

Honey Aggarwal
(Regd no. 9041001)

Department of Biotechnology & Environmental Sciences
Thapar University, Patiala 147004
Punjab (India)

October, 2011

Contents

Certificate	vi
Candidate's Declaration	vii
Acknowledgement	viii-ix
Synopsis	x-xix
List of Abbreviations	xx-xxi
Index of Tables	xxii-xxiv
Index of Figures	xxv-xxvi
Index of Plates	xxvii

Chapter 1. Introduction **1-5**

Chapter 2. Review of Literature **6-37**

2.1 Agroforestry
2.1.1 Poplar (*Populus deltoides*) in Agroforestry
2.1.2 Toon (*Toona ciliata*) in Agroforestry
2.2 Soil Amendments
2.2.1 Microbial Inoculants
2.2.1.1 Nitrogen Fixers
2.2.1.2 Phosphate Solubilizers
2.2.2 Farm Yard Manure
2.2.3 Industrial Solid Waste
2.2.3.1 Integrated Iron & Steel Plant Slag
2.2.3.2 Phosphogypsum
2.2.3.3 Red Mud
2.2.3.4 Lime Mud
2.2.3.5 Waste Sludge and Residues
2.2.3.6 Coal Ash
2.2.3.6a Disposal of Fly ash
2.2.3.6b Use of Fly ash in Agroforestry
2.2.3.6c Use of fly ash and microbial consortium with organic manure
2.2.3.7 Distillery Waste
2.3 Remediation of heavy metals from soil
2.3.1 Phytoremediation
2.3.1.1 Phytoextraction
2.3.1.2 Rhizofiltration
2.3.1.3 Phytostabilization
2.3.1.4 Phytomining

Chapter 3. Materials and Methods 38-74

3.1 Analysis of soil and industrial solid waste
- 3.1.1 Collection of soil and industrial solid waste (FA & DW) and Farm yard manure (FYM)
- 3.1.2 Chemical Analysis
  - 3.1.2.1 pH
  - 3.1.2.2 Electrical Conductivity
  - 3.1.2.3 Organic Carbon
  - 3.1.2.4 Available Phosphorus (in alkaline soil)
  - 3.1.2.5 Available Phosphorus (in acidic soil)
  - 3.1.2.6 Available Nitrogen
  - 3.1.2.7 Total Nitrogen
  - 3.1.2.8 Available Sulphur
  - 3.1.2.9 Total Fe, Mn, Ni, Cr, Pb, Zn, Ca, Mg, Na, Cd, Co
  - 3.1.2.10 Ammonium-acetate extractable K
  - 3.1.2.11 DTPA-extractable Fe, Mn, Ni, Cr, Pb and Zn
- 3.1.3 Physical Analysis
  - 3.1.3.1 Water holding capacity
  - 3.1.3.2 Bulk density
  - 3.1.3.3 Particle size distribution
  - 3.1.3.4 Soil texture

3.2 Microbiological Analysis
- 3.2.1 Microbial Biomass
- 3.2.2 Enumeration of Bacteria
  - 3.2.2.1 Bacterial Enumeration on Nutrient Agar
  - 3.2.2.2 Enumeration of free living Nitrogen Fixing Bacteria
  - 3.2.2.3 Enumeration of Phosphate Solubilizing Bacteria
  - 3.2.2.4 Enumeration of *E.coli S-17 (lacZ$^+$), Pseudomonas striata (lacZ$^+$)* and *Azotobacter* CBD15 (*kan^R*)
- 3.2.3 Growth Curve
- 3.2.4 Antibiotic Profiling of Bacterial Strains
- 3.2.5 Plasmid Isolation
- 3.2.6 Gel Electrophoresis
- 3.2.7 Transformation of Bacterial Strains

3.3 Nursery Trials
- 3.3.1 Biometric Data Collection
- 3.3.2 Heavy metal uptake by root, stem and leaves of *P. deltoides a*nd *T.ciliata*

3.4 Statistical Analysis
- 3.4.1 Coefficient of Variation or Dispersion
- 3.4.2 Variance
- 3.4.3 Standard Deviation

3.4.4 Standard Error
3.4.5 Two-way Analysis of Variance

Chapter 4. Results **75-130**

4.1 To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Populus deltoides* and *Toona ciliata*
4.1.1 Physico-chemical characterization of soil, fly ash, distillery waste and farmyard manure
4.1.1.1 Major Elements
4.1.1.2 Secondary Elements
4.1.1.3 Micronutrients
4.1.1.4 Heavy Metals
4.1.2 Bacterial Enumeration of soil, fly ash, distillery waste and farmyard manure
4.1.3 Nursery Trial
4.1.3.1Percentage Survival
4.1.3.2Biometric Parameters
4.1.3.2a Plant Height
4.1.3.2b Collar Diameter
4.1.3.2c Total Dry Biomass

4.2 To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves
4.2.1 Physico-chemical characterization of soil in various treatments on day zero of nursery trial of *P. deltoides*
4.2.1.1 Total Organic Carbon
4.2.1.2 Major Elements
4.2.1.3 Secondary Elements
4.2.1.4 Micronutrients
4.2.1.5 Heavy Metals
4.2.2 Physico-chemical analysis of soil in various treatments on completion of nursery trial of *P. deltoides* (on day 180)
4.2.3 Analysis of major, secondary and micronutrients in soil and uptake by root, stem and leaves of *P. deltoides*
4.2.4 Analysis of heavy metals in soil and uptake by root, stem and leaves of *P. deltoides*
4.2.5 Reduction in concentration of heavy metals in soil after completion of nursery trial of *P. deltoides*
4.2.6 Physico-chemical analysis of soil in various treatments on completion of nursery trial of *T. ciliata* (on day 180)
4.2.6.1 Nutrition Status
4.2.6.2 Heavy Metals

4.2.7 Reduction in concentration of heavy metals in soil after completion of nursery trial of *T.ciliata* (on day 180)

4.3 To study the survival and establishment of molecular tagged microbes in soils amended with solid waste
4.3.1 Microbial Growth
4.3.2 Antibiotic Profiling
4.3.3 Bacterial Transformation
4.3.4 Growth of transformed *P. striata* (*lacZ*$^{+}$)
4.3.5 Survival and establishment of molecular tagged microbes in soils amended with solid waste in nursery trial

Chapter 5. Discussion **131-148**

5.1 To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Populus deltoides* and *Toona ciliata*
5.1.1 Physico-chemical characterization of soil, fly ash, distillery waste and farmyard manure
5.1.2 Percentage survival and Biometric Parameters (plant height, collar diameter and total dry biomass)
5.2 To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves
5.2.1 Interaction between fly ash, farmyard manure and microbial consortium and their effect on biometric parameters
5.2.2 Physico-chemical analysis of soil on completion of nursery trial
5.2.3 Heavy metals uptake by root, stem and leaves
5.2.4 Reduction in concentration of heavy metals in soil
5.3 To study the survival and establishment of molecular tagged microbes in soils amended with solid waste
5.3.1 Antibiotic Profiling and Bacterial Transformation
5.3.2 Survival and establishment of molecular tagged microbes in soils amended with solid waste in nursery trial

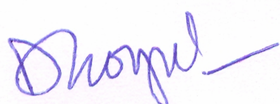
Chapter 6. Conclusions **149-151**

Chapter 7. Bibliography **152-188**

Annexure I: Plates 189-195
Annexure II: List of Research Publications and Conference Presentations 196-197

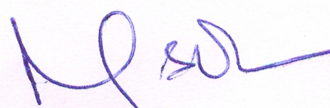
Certificate

Certified that the thesis "**Microbial inoculants and industrial solid wastes as soil amendments in agroforestry"** which is submitted by Ms. Honey Aggarwal, in fulfillment of the requirement for the award of the Degree of doctor of Philosophy in the Department of Biotechnology & Environmental Sciences, Thapar 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.



(Supervisor)

Dr. Dinesh Goyal
Professor
Department of Biotechnology
& Environmental Sciences
Thapar University
Patiala – 147004
Punjab (India)



(Head)

Dr. M. S. Reddy
Professor
Department of Biotechnology
& Environmental Sciences
Thapar University
Patiala – 147004
Punjab (India)

Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled "**Microbial inoculants and industrial solid wastes as soil amendments in agroforestry"** in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy, Department of Biotechnology & Environmental Sciences, Thapar University, Patiala, is an authentic record of my own work during the period from July 2004 to July 2010, under the supervision of Dr. Dinesh Goyal, Professor, Department of Biotechnology & Environmental Sciences, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Place: Patiala (Honey Aggarwal)

Date: 19.10.2011

Acknowledgement

I pay my gratitude to the Almighty for His everlasting blessings.

This thesis arose in part out of years of research that has been done since I came to Thapar University, Patiala. By that time, I have worked with a great number of people whose contribution in assorted ways to the research and the making of the thesis deserved special mention. It is a pleasure to convey my gratitude to them all in my humble acknowledgment.

In the first place I would like to record my gratitude to my supervisor ***Dr. Dinesh Goyal, Professor****, Department of Biotechnology & Environmental Science for his supervision, advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences through out the work. Above all and the most needed, he provided me unflinching encouragement and support in various ways. His truly scientist intuition has made him as a constant oasis of ideas and passions in science, which exceptionally inspire and enrich my growth as a student, a researcher and a scientist want to be. I am indebted to him more than he knows.*

I express my gratitude to ***Dr. Abhijit Mukherjee, Director****, Thapar University for all the facilities which have been immensely helpful for the completion of my work.*

I am highly thankful to ***Dr. Pramod K. Bajpai, Dean and Dr. Susheel Mittal, Ex-Dean (Research and Sponsored Projects)*** *Thapar University, Patiala for his encouragement and motivation.*

I am highly thankful to ***Dr. M. S. Reddy, Head,*** *Department of Biotechnology & Environmental Sciences for his encouragement and lab facilities for the course of my work.*

I am highly thankful to the member of my doctoral committee ***Dr. N. Das,*** *Department of Biotechnology & Environmental Sciences for his comments and suggestions during the course of my Ph.D.*

I wish to express my gratitude to all the faculty members of Department of Biotechnology & Environmental Sciences, Thapar University, Patiala.

I am thankful to the office and laboratory staff of the Department of Biotechnology & Environmental Sciences and Science & Technology Entrepreneur's Park, Thapar University, Patiala. I am also benefited by outstanding works from ***Mr. Ramesh Kumar Verma's*** *technical assistance with his particular skill in handling precisely delicate instruments and sampling.*

I was extraordinarily fortunate in having ***Dr. Anubha Kaushik*** *as my Professor in my master's degree at Guru Jambeshwar University. I could never have embarked and started all of this without her prior teachings and thus opened up unknown areas to me. Thank you.*

Taking this opportunity I wish to heartily thank my seniors and friends ***Dr. Sarabjeet Singh Ahluwalia, Mr. Saurabh, Ms. Vijay, Ms. Purnima, Ms. Himani and Mr. Nadeem*** *for their love and affection, constant encouragement they provided at all times and for always being there for me. Thanks are also due to all my hostel staff for their support during my stay at hostel.*

Where would I be without my family? My parents deserve special mention for their inseparable support and prayers. My Father, ***Mr. D. R. Aggarwal****, in the first place is the person who put the fundament my learning character, showing me the joy of intellectual pursuit ever since I was a child. My Mother,* ***Mrs. Kaushal Aggarwal****, is the one who sincerely raised me with her caring and gentle love.* ***Shikha*** *and* ***Himanshu****, thanks for being supportive and caring siblings. I thank my in-laws for their love and support throughout my work. Words fail me to express my appreciation to my husband* ***Mr. Deepak Sharma*** *whose dedication, love and persistent confidence in me, has taken the load off my shoulders.*

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Place: Patiala **(Honey Aggarwal)**

Date: 19.10.2011

Synopsis

Soils are central to the sustainability of our ecosystem which performs essential functions such as nutrient cycling to support plant growth, attenuation and transformation of potentially toxic compounds and maintenance of biodiversity. The application of solid waste to soil as a recycling option can only be sustained if there are demonstrable 'ecological benefits' justifiable in terms of enhanced biomass production, elevated organic carbon and stimulation of microbial activity and nutrient supply, provided threshold levels of heavy metals and pollutants does not exceed. Toxic heavy metals discharged into the environment through industrial wastes constitute one of the major causes of environmental pollution. Besides other uses of solid waste, its use in agroforestry has become an important thrust area. Agroforestry systems combine trees or shrubs with cropping, with the aim of developing a more productive and sustainable form of land-use. The ideal plant species to remediate a heavy metal contaminated soil would be high biomass producing crop that can both tolerate and accumulate the contaminants of interest. Short rotation woody crops such as poplars (*Populus* spp.), willows (*Salix* spp.) and safeda (*Eucalyptus* spp.) are the most common tree species used for phytoremediation because they grow rapidly, have deep roots and take up large quantities of water. *Toona ciliata* is used for various purposes as it has got high timber value, can also be used for food and fodder, aromatic oil, tannin and some medicines can also be extracted from the tree. *Populus deltoides* is one of the few hardwood species that has been planted and grown specifically for core stock in manufacturing furniture and for pulpwood. The timber is used principally for lumber, veneer, pulpwood, excelsior, and fuel.

Fly ash has a vast potential for use in agriculture, forestry and wasteland reclamation due to its excellent soil ameliorating properties. 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. 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. Its addition alters physical properties of soil such as texture, bulk density, water holding capacity and particle size distribution. Exploitation of metal uptake by plant biomass as a method of soil decontamination is limited by plant productivity and the concentration of metals achieved. The utilization and safe disposal of industrial solid waste can be looked upon for environmental benefits. The potential of *Toona ciliata* and *Populus deltoides* therefore for phytoremediation can be looked into alongwith biomass production, while maintaining soil health.

Azotobacter and *Pseudomonas sp.* are successful in getting established in the soil ecosystem due to their high adaptability in a wide variety of environments, their faster growth rate, and their biochemical versatility to metabolize a variety of natural and xenobiotic compounds. To study establishment of inoculum, it is necessary to have a sensitive and reliable method for specifically detecting and quantifying the inoculated strain in the field. Use of marker genes such as *lacZ* and antibiotic resistance genes has become an important tool in studies on microbial ecology. Molecular tagging of inoculant strains such as *Azotobacter* and *Pseudomonas striata* is essential for ecological monitoring and to assess their performance in the field.

Keeping all above factors in mind a comprehensive study was undertaken on "Microbial inoculants and industrial solid wastes as soil amendments in agroforestry" with following objectives:

Objectives

1. To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Toona ciliata* and *Populus deltoides*.
2. To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves.
3. To study the survival and establishment of molecular tagged microbes in soils amended with solid waste.

Approach adopted to meet above objectives

In the present study the effect of addition of fly ash @ 10% (v/v), distillery waste @ 05% (v/v), farm yard manure @ 20% (v/v) and microbial consortium of *Pseudomonas striata* and *Azotobacter* CBD15 @ 30 ml consortium / per pot in soil was investigated in terms of biomass production, microbial activity and nutrient status of soil. Soil was collected from agricultural field of Thapar Campus, electrostatic precipitator (ESP) fly ash was brought from GGS Superthermal Plant, Ropar (Punjab), distillery waste from Patiala distilleries, village Main, Patiala, farm yard manure was procured from local market, Patiala; both *Azotobacter* CBD15 and *Pseudomonas striata* were procured from Microbiological division, IARI, New Delhi. Soil and all soil amending agents (i.e., fly ash, distillery waste and farm yard manure) were characterized for physico-chemical properties. The phosphate solubilizing bacterium (*P. striata)* was transformed with the *lacZ* marker gene isolated from *E. coli* 2842 and inoculated in soil with and without soil amending agents to study its proliferation and to study survival multiplication or colonization of inoculum of labeled strain, the bacterial count and expression was determined. Pot experiments were conducted to study colonization by inoculated bacteria. Nursery experiments were carried out to see the effect of microbes and soil amendments of solid waste on the growth and biomass production of *Toona ciliata* and *Populus deltoides* and to study the microbial interactions, also to check the extent of heavy metal uptake by these tree species.

1. To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Toona ciliata* and *Populus deltoides*

Soil and all the soil amending materials were air dried, ground and passed through 2mm size sieve for physical analysis and 0.2mm size sieve for chemical analysis. The soil was mixed with different amending materials to make twelve treatments as shown below for *Populus deltoides* and *Toona ciliata*

T1- Soil (Control)	T2- Soil + MA
T3- Soil + FA	T4- Soil + FA + MA
T5- Soil + FA + DW	T6- Soil + FA + DW + MA
T7- Soil + FA + FYM	T8- Soil + FA + FYM + MA
T9- Soil + DW	T10- Soil + DW + MA
T11- Soil + FYM	T12- Soil + FYM + MA

(MA: Microbial amendment (*P. striata* and *Azotobacter sp.*) @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

A loopful of full grown cultures of both *P. striata* ($lacZ^+$) and *Azotobacter* CBD15 from the selective media plates were inoculated in Pikovskaya's broth and Jensen's broth for their mass cultivation in flasks, the flasks were incubated at 30 ± 2°C and 120 rpm for 48 hours. After 48 hours of growth 15 ml of each culture (leading to 30 ml of consortia) was added per pot to all the treatments.

In one experiment shoot cuttings of *Populus deltoides* G48 were grown singly in all the twelve treatments in 16 replications / treatment and in another seedlings of *Toona ciliata* were transplanted (one seedling / pot) from seed raised bed to all the different treatments from T1 to T12 in 12 replications / treatment. Irrigation from surface was used to maintain soil moisture at approximately field capacity. Plant height and collar diameter of the stem of both the tree species were recorded on day 180. Height was measured by elongating the plant and measuring to the top of the upper leaf with a ruler and collar diameter was measured with a digital vernier caliper. After the measurement the plants were harvested, roots and shoots were separated at the crown and then leaves were separated from the stem. The soil adhering to roots was removed by washing under a gentle stream of water under the tap. Roots, stem and leaves were dried in a hot air oven at 80°C for 48 hours, after that the dry weights of the plant parts were measured gravimetrically.

The percentage survival of *P. deltoides* was found to be around 75% and for *T. ciliata* it was around 59%. For both the tree species survival was maximum in treatments T8 and

T7 (soil amended with fly ash and farm yard manure). At day 180, the plant height of *P. deltoides* was maximum in treatment T8 amended with fly ash, farmyard manure and microbial consortium. The treatments having distillery waste as an amendment significantly decreased the height of *P. deltoides.* Treatments T4, T7, T11 and T12 significantly increased the height of plant as compared to the control (T1) treatment. For *Toona ciliata* the plant height was maximum in treatment T8, whereas, treatments T5 and T9 significantly decreased the plant height which were comparable to the T1 (control) treatment. Treatment T8 was the only treatment that showed significant impact on the height of plant of both the agroforestry tree species.

Treatment T8 had the maximum collar diameter in *P. deltoides* followed by treatment T12, leading to significant increase in the collar diameter of the plant followed by treatments T7 and T11, which were not statistically significant but were comparable to each other. The collar diameter of *T. ciliata* was maximum in treatment T8, followed by treatments T7, T4 and T2 respectively, the increase in these four treatments was comparable to each other. Collar diameter of *T. ciliata* decreased significantly in treatments T10 and T9 respectively, having distillery waste as one of the soil ameliorant.

Total dry biomass of *P. deltoides* was maximum in treatment T8, whereas, treatment T9 significantly decreased the total dry biomass of the plant, comparable to T1 (control soil). Treatments T2 – T6, T10 and T12 increased the dry biomass as compared to T1 and T9, but were statistically insignificant. The total dry biomass of *T. ciliata* was highest in treatment T8 followed by T3 and T7 respectively, The treatments T2, T4, T6 and T12 having microbial consortium as one of the soil ameliorant increased the production of total dry biomass.

2. To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves

The physico-chemical analysis of the soil and all the amending materials, viz., fly ash (FA) and farmyard manure (FYM) and distillery waste (DW) was carried out following standard protocols. pH and electrical conductivity (EC) were measured as per the protocol given by Jackson (1967), organic carbon was estimated as per Walkley and

Black (1934) method of estimation of organic carbon, available nitrogen, phosphorus, sulphur and ammonium acetate extractable potassium were estimated using Kjeldahl method given by Piper (1960), Olsen *et al*., (1954), Chesnin and Yien (1950) and Merwin and Peech (1951) respectively. Soil texture was determined using the hydrometer method as described by Buoyoucos (1962). The bulk density and water holding capacity were determined for all the substrates using the protocol given by Black (1965). For heavy metal analysis of soil, plant root, shoot and leaves, 1 g oven dried (70ºC) powderized and sieved sample (0.02 mm mesh size sieve) of each was digested by wet digestion method with concentrated HNO_3 and $HClO_4$ in the ratio 3:1 (Page, 1982). The samples were digested on a hot plate at a temperature corresponding to 100ºC for 3-4 hrs. Heating was done till it dried up completely and whitish brown dry mass was obtained. It was then cooled and the precipitate/digest mixture was extracted in acid water mixture (concentrated HCl: MilliQ water in the ratio 1:1), filtered through whatman filter paper No. 42 and the volume was made up to 50 ml. The filtrate was analyzed for metal content using Atomic Absorption Spectrophotometer (GBC 932 AA). The instrument was calibrated using standard solutions of Cr, Zn, Fe, Cd and Pb. The various metals along with their sensitivity limits ($\mu g\ ml^{-1}$) are as follows: Cr -0.05, Zn -0.008, Fe -0.05, Cd – 0.009, Pb - 0.06.

Heavy metal analysis in root, stem and leaves of *Populus deltoides* revealed that the stem of the plant had highest concentration of Pb (10.92 mg/kg) and Cd (3.40 mg/kg), whereas, leaves had maximum concentration of Cr (92.62 mg/kg), Zn (29.32 mg/kg) and roots of the plant had maximum uptake of Fe (1432.50 mg/kg) respectively. The heavy metal uptake by root, stem and leaves was more pronounced in treatment T2 (soil amended with microbial consortium) as compared to control soil (T1). The trend observed for highest concentration and metal uptake by different plant parts was leaves > stem > roots. In case of *Toona ciliata*, leaves of the plant showed highest accumulation of Zn (62.77 mg/kg), Cd (0.87 mg/kg) and Pb (19.47 mg/kg), whereas, roots of the plant had highest concentration of Cr (8.20 mg/kg) and Fe (1003.33 mg/kg) respectively. The trend observed for highest concentration and metal uptake by different plant parts was leaves > roots > stem. Treatments T2 and T4 having microbial inoculants as an amendment had shown potentially more reduction of heavy metal concentration in soil. Several lines of

evidence suggest that soil microorganisms possess mechanisms capable of altering environmental mobility of metal contaminants with subsequent effects on the potential for root uptake. In the present investigation, the mixed application of fly ash and farmyard manure had also shown promising results for carrying out reduction in heavy metal content in soil. Mixed application of fly ash and microbial consortium had shown potential for carrying out more reduction of heavy metals in soil as compared to control soil, which offered an effective way to prevent cycling of toxicants from fly ash and growing of multipurpose tree species, i.e., *Populus deltoides* and *Toona ciliata* on problem soils leading to an environmentally safe strategy for gainful utilization of fly ash. From the present study, it could be concluded that *P. deltoides* and *T. ciliata* resulted in decrease in metal concentration in the soil and was able to demonstrate the potential phytoextraction capacity.

3. To study the survival and establishment of molecular tagged microbes in soils amended with solid waste and to examine diversity of the bacterial community

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 μg ml^{-1}), kanamycin (50 μg ml^{-1}), nalidixic acid (50 μg ml^{-1}) and chloramphenicol (10μg ml^{-1}), respectively. *P. striata* showed resistance to Streptoycin and nalidixic acid, and was sensitive to chloramphenicol, kanamycin and rifampicin, whereas, *Azotobacter* CBD15 was resistant to all test antibiotics, and kanamycin resistant character was used for ecological monitoring.

Among these strains phosphate solubilizing bacterial isolate *P. striata* was transformed with *lac Z* and chloramphenicol marker carrying plasmid pMMB277 from E.coli MTCC 2842. The transformant *P. striata* pMMB277 was inoculated in soil @ 15 ml /pot. The ecological monitoring in terms of their population build-up was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient agar plates.

Bacterial community present in the rhizosphere of solid waste amended soils were isolated after enrichment on Pikovskaya's and Jensen's media plates. The bacterial communities based on the differences in the morphology of their colonies were randomly selected. Representative samples were drawn aseptically from each pot after harvesting and analyzed for total viable-cell counts, performed by spread-plating 0.1 ml of dilutions in sterile distilled water on nutrient agar plates. Colonies were enumerated after 48 hr of incubation at 37°C.

Blue coloured lactose positive and chloramphenicol resistant colonies of *P. striata* *($lacZ^{+}$)* were counted on Pikovskaya media plates containing 10µg/ml chloramphenicol and after being lightly spreaded with a solution of IPTG (Isopropyl-β-D-thiogalactopyranoside) (0.1g/ml in sterile water), X-Gal (20mg/ml in dimethyl formamide) and sterile water in ratio (8:30:40). 12-18% of the total culturable bacteria showed $lacZ^{+}$ character and were resistant to chloramphenicol, in comparison to 0.02 - 0.05 % total culturable bacteria showing both the traits as background microflora in soil, their phosphate solubilizing activity was further confirmed on NBRI-BPB media plates. The enumeration of inoculated bacteria on selective media indicated that with the addition of fly ash and farm yard manure there was a positive effect on soil microbial population and an optimum concentration of 10% is tolerable for microbes indicating that its amendment provides micronutrients for growth.

Salient Findings:

1. Treatment (T8) comprising of fly ash @ 10% (v/v), farmyard manure @ 20% (v/v) and microbial consortium @ 30ml / pot promoted plant growth and it had significant impact on the biometric parameters of *P. deltoides* with a p value of interaction between fly ash and microbial amendment for total dry biomass and plant height less than 0.001 and for collar diameter it was 0.0496, whereas, in *T. ciliata* it had significant impact only on plant height, however it increased the collar diameter and total dry biomass by 26% and 27% respectively.
2. The trend observed for highest concentration and metal uptake by different plant parts of *Populus deltoides* G48 was leaves > stem > roots and for *Toona ciliata* it was leaves > roots > stem.
3. Analysis of heavy metals in soil in different treatments before and after trial showed an overall reduction of heavy metals by *P. deltoides* and *T. ciliata*, which resulted in decrease in the metal concentration in the soil and was able to demonstrate the potential phytoextraction capacity of both the agroforestry tree species.
4. Mixed application of fly ash and microbial consortium had shown potential for carrying out more reduction of heavy metals in soil as compared to control soil, which offered an effective way to prevent cycling of toxicants from fly ash and growing of multipurpose tree species, i.e., *P. deltoides* and *T. ciliata* on problem soils leading to an environmentally safe strategy for gainful utilization of fly ash.
5. Phosphate-solubilizing bacterial strain *Pseudomonas striata* was tagged with *lac* Z^+ marker gene isolated from *E.coli* MTCC 2842 strain and its ecological monitoring on selective media indicated that 10% fly ash along with 20% farm yard manure is good for the proliferation of microbes since its amendment provides micronutrients for growth.
6. The combinations of reporter genes are suitable for tracking microorganisms in soil, because presence of both genes is potentially innocuous to the indigenous gene pool, assayed easily and rapidly. It was found convenient, however, to use an antibiotic

marker with some phenotypic marker, where the number of microorganisms becomes critically low in soil (10^2 CFU/g of soil).

7. Combined addition of fly ash, farm yard manure and microbial inoculants can be used as a good potting mixture for improving survival rates and plant growth in forestry nurseries.

List of Abbreviations

hr	Hour
rpm	Revolution per minute
g	Gram
mg	Milligram
µg	Microgram
l	Liter
$\mu S\ cm^{-1}$	Micro siemens per cm
ml	Milliliter
µl	Misrelate
$mg\ ml^{-1}$	Milligrams per milliliter
$\mu g\ ml^{-1}$	Micrograms per milliliter
%	Percentage
mg/kg (ppm)	Milligrams per kilogram (parts per million)
µg/kg (ppb)	Micrograms per kilogram (parts per billion)
mg/ha	Milligram per hectare
t/ha	tones per hectare
$cm\ min^{-1}$	Centimeter per minute
$\mu g\ g^{-1}$	Microgram per gram
$g\ l^{-1}$	Grams per liter
($x10^{6}$ CFU g^{-1} soil)	x 10^{6} colony forming unit per gram soil
V	Volume of the solution
mm	Millimeter
cm	Centimeter
N	Nitrogen
P	Phosphorous
K	Potassium
S	Sulphur
Ca	Calcium
Mg	Magnesium
Pb	Lead
Cr	Chromium
Zn	Zinc
Fe	Iron
Ni	Nickel
Mn	Manganese
As	Arsenic
Se	Selenium
Mo	Molybdenum
Co	Cobalt
Cu	Copper
U	Uranium
W	Weight in grams
FA	Fly ash

DW	Distillery waste
FYM	Farmyard manure
MA	Microbial amendment
EC	Electrical conductivity
WHC (%)	Water hlding capacity (%)
°C	Degree Celcius
P.striata	*Pseudomonas striata*
P.deltoides	*Populus deltoides*
T.ciliata	*Toon ciliata*
NA	Nutrient Agar
PKV	Pikovskaya's media
JM	Jensen's media
IPTG	Isopropyl-β-D-thiogalactopyranoside
X-gal	5-Bromo-4-chloro-3 indolyl-β-D-thiogalactopyranoside
Chl	Chloramphenicol
Kan	Kanamycin

Index of Tables

Table No.	Title	Page No.
1a	Microorganisms having promise to boost agricultural production	12
1b	Source and Quantum of generation of some major industrial waste	17
1c	Plants with the potential for phytoremediation of heavy metals and organic compounds	33
2a	Physical characterization of soil, fly ash, distillery waste and farmyard manure	75
2b	Chemical characterization of soil, fly ash, distillery waste and farmyard manure	78
2c	Microbiological characterization of soil, fly ash, distillery waste and farmyard manure	79
2d	Percentage survival of *P. deltoides* in nursery trial after a period of 180 days in 16 initial replicates	80
2e	Percentage survival of *T. ciliata* in nursery trial after a period of 180 days in 12 initial replicates	80
2f	Plant height (cm), collar diameter (cm) and total dry biomass (gm) of *Populus deltoides* grown in twelve different treatments over a period of 180 days	86
2g	Plant height (cm), collar diameter (cm) and total dry biomass (gm) of *Toona ciliata* grown in twelve different treatments over a period of 180 days	87
3a	Analysis of variance of biometric parameters of *Populus deltoides*	90
3b	Analysis of variance of biometric parameters of *Toona ciliata*	90
3c	Physico-chemical characterization of soil in different treatments on day zero of nursery trial of *P. deltoides*	91
3d	Organic carbon and major nutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*	95

3e	Chemical analysis of secondary nutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*	96
3f	Chemical analysis of micronutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*	97
3g (I)	Chemical analysis of heavy metals in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*	98
3g (II)	Chemical analysis of heavy metals in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*	99
3h	pH, EC, WHC (%) and bulk density of soil in different treatments after six months of nursery trial of *Populus deltoides*	100
3i	Organic carbon, available phosphorus and available sulphur in different treatments after six months of nursery trial of *Populus deltoides*	101
3j	Major and secondary elements (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial	103
3k	Micronutrients (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial	104
3l	Heavy metals (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial	106
3m	Heavy metal (mg/kg) analysis in soil before plantation and after harvesting of *Populus deltoides* and its percentage reduction in soil	107
3n	pH, EC, WHC (%) and bulk density of soil in different treatments after six months of nursery trial of *Toona ciliata*	108
3o	Organic carbon, available phosphorus and available sulphur in soil in different treatments after six months of nursery trial of *Toona ciliata*	109
3p	Major and secondary elements (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial	111

3q	Micronutrients (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial	112
3r	Heavy metals (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial.	114
3s	Heavy metal (mg/kg) analysis in soil before plantation and after harvesting of *Toona ciliata* and its percentage reduction in soil	115
4a	Absorbance of *Pseudomonas striata* at 600nm in Pikovskaya's broth, change in pH and available P	117
4b	No. of cells of *Azotobacter* CBD15 grown in Jensen's media	118
4c	Antibiotic profiling of *P. striata* and *Azotobacter* CBD15	119
4d	Absorbance of *transformed Pseudomonas striata (lacZ^{+})* at 600nm in Pikovskaya's broth, change in pH and available P	123
4e	Enumeration of phosphate solubilizing and nitrogen fixing bacteria and estimation of microbial biomass in rhizospheric soils of *Populus deltoides* and *Toona ciliata*	125
4f	Enumeration of tagged bacteria in rhizospheric soils of *Populus deltoides* on selective media after 180 days of nursery trial	126
4g	Enumeration of tagged bacteria in rhizospheric soils of *Toona ciliata* on selective media after 180 days of nursery trial	126

Index of Figures

Figure No.	Title	Page No.
1a	Height of *Populus deltoides* in various soil amendments over a period of 180 days	81
1b	Height of *Toona ciliata* in various soil amendments over a period of 180 days	82
1c	Collar diameter of *Populus deltoides* in various soil amendments over a period of 180 days	83
1d	Collar diameter of *Toona ciliata* in various soil amendments over a period of 180 days	83
1e	Total dry biomass of *Populus deltoides* in various soil amendments over a period of 180 days	84
1f	Total dry biomass of *Toona ciliata* in various soil amendments over a period of 180 days	85
2a	Growth of *Pseudomonas striata* in Pikovskaya's broth, change in pH and available P	117
2b	Growth of *Azotobacer* CBD15 grown in Jensen's broth	118
2c	Map of pMMB277 plasmid broad host range, artificial cloning vector (Gene, 1997)	120
2d	Agarose gel (0.7%) electrophoretogram of plasmid pMMB277 isolated by alkali lysis method and Lambda marker cut by Hind III	121
2e	Agarose gel (0.7%) electrophoretogram of purified plasmid	121
2f	Agarose gel (0.7%) electrophoretogram of plasmid pMMB277 isolated by alkali lysis method from *E.coli* 2842 and *Pseudomonas striata* transformed with pMMB27	122
2g	Growth of *P. striata (lacZ$^+$)* in Pikovskaya's broth, change in pH & available P	123

2h	Enumeration of tagged *P. striata lacZ*$^{+}$ in rhizospheric soil of *Populus deltoides* over a period of 180 days	127
2i	Enumeration of tagged *P. striata lacZ*$^{+}$ in rhizospheric soil of *Toona ciliata* over a period of 180 days	128
2j	Enumeration of *Azotobacter* CBD15 *(kan*^{R}*)* in rhizospheric soil of *P. deltoides*	129
2k	Enumeration of *Azotobacter* CBD15 *(kan*^{R}*)* in rhizospheric soil of *Toona ciliata*	129

Index of Plates

Plate No.	Title	Page No.
1	(a) *Pseudomonas striata* on NBRI-BPB media (b) *Azotobacter* CBD15 on Jensen's media (c) Transformed *P.striata ($lacZ^+$)* on Pikovskaya's media + chloramphenicol + IPTG + Xgal (d) and (e) *P.striata ($lacZ^+$)* on NBRI-BPB media	189
2	*Pseudomonas striata ($lacZ^+$)* (a - f) Enumeration of inoculated *P.striata ($lacZ^+$)* on Pikovskaya's media containing chloramphenicol + IPTG + X-gal from different soil treatments ameneded with fly ash, farmyard manure, distillery waste and microbial inoculants	190
3	*Populus deltoides* (a) Nursery trial of *Populus deltoides* in 12 different treatments (b-d) *Populus deltoides* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial	191
4	*Populus deltoides* (a-d) *Populus deltoides* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial	192
5	*Toona ciliata* (a-b) Nursery trial of *Toona ciliata* in 12 different treatments (c-d) *Toona ciliata* in alkaline soil amended with microbial inoculants and fly ash during nursery trial	193
6	*Toona ciliata* (a-d) *Toona ciliata* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial	194
7	*Toona ciliata* (a-b) *Toona ciliata* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial (c) Roots of *Toona ciliata* in treatments amended with fly ash and microbial inoculant (T4) alongwith its control and (d) in treatments amended with fly ash, farmyard manure and microbial inoculant (T4) alongwith its control	195

Chapter 1
Introduction

The desire of an ever-increasing population for improving their living standards necessitated the conversion of agricultural land for non-agricultural applications, intensive cropping with the indiscriminate application of fertilizers and pesticides resulting in inadvertent reduction in the forest cover. Implementation of these man-made strategies led to frequent floods, erosion and degradation of soil, cumulatively deteriorating the quality of air, soil, water streams and overall ecological balance. With the onset of industrial revolution, pollution of the environment with toxic metals has increased dramatically. Mining, manufacturing and use of synthetic products (e.g., pesticides, paints, batteries, industrial waste and land application of industrial or domestic sludge) has resulted in heavy metal contamination of urban and agricultural soils. To address these ground realities, application of an integrated plant nutrition system (IPNS) appears to be an effective approach to provide maximum forestry output per unit area, per unit time in a cost-effective and sustainable manner (Tandon, 1992). Simultaneously protecting the environment from hazardous waste generation and disposal is a major concern in today's heavily industrialized world (Dermatas and Meng, 2003). Agroforestry systems combine trees or shrubs with cropping, with the aim of developing a more productive and sustainable form of land-use (Van and Ong, 1999).

In India about 70% of the electricity is generated by coal-based thermal power plants, which produces more than 100 mt fly ash per annum as a by-product (Jamwal, 2003) that creates the problem of its eco-friendly disposal. Fly ash a resultant of combustion of coal at high temperature has been regarded as a problematic solid waste all over the world (Jala and Goyal, 2006). 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 precipitator (ESP), flue gas desulphurization (FGD) system and bag houses (Mattigod *et al.,* 1990). Chemically, 90-99% of fly ash is comprised 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). Disposal and utilization of fly ash needs

careful assessment to prevent conversion of arable land into landfills and accumulation of toxic metals in soil (Petruzzelli, 1989). Restoration and utilization of fly ash dumps for biomass production will be an adjunct to these efforts. In conjunction with organic manure and microbial inoculants, fly ash can enhance plant biomass production from degraded soils (Jala and Goyal, 2006). 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. Increased microbial activity was reported for ash amended soils containing sewage sludge (Pitchel, 1990; Pitchel and Hayes, 1990). Fly ash typically consists of a variety of trace and heavy metal elements, some essential and other toxic to both plants and animals. They are present in the form of oxides, silicates, sulphates, phosphates etc. (Page *et al.,* 1979). Fly ash is a useful ameliorant that may improve the physical, chemical and biological properties of problem soils and is a source of readily available plant macro and micro nutrients (Jala and Goyal, 2006). Several workers have reported the use of fly ash as a soil amendment for field crops (Elseewi *et al.,* 1978; Adriano *et. al.,* 1978, 1980; Ciravolo and Adriano, 1979; Molliner and Street, 1982; Aitken *et al.*, 1984). 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 *et al.*, 1984; Sharma *et al.,* 1990). Fly ash– sludge mixtures containing 10% ash had positive effect on soil microorganisms in terms of enzyme activity, N and P cycling and reduction in the availability of heavy metals (Lai *et al.,* 1999). Fly ash and its different combinations with soil (w/w) were tested for use as a carrier for diazotrophs (*Azotobacter chroococcum, Azospirillum brasilense*) and phosphobacteria (*Bacillus circulans, Pseudomonas striata)* (Gaind and Gaur, 2003) which showed their maximum viability in fly ash alone or soil:fly ash (1:1) combination.

A large portion of biosphere is contaminated by heavy metals as a result of human activities. Conventional solutions such as disposal of contaminated soil in landfills, which relies heavily on 'dig and dump' or encapsulation, neither of which takes into consideration the issue of decontamination of the soil, account for a large proportion of the remediation operations at present (Pulford and Watson, 2003). Remediation techniques like immobilization or extraction by physico-chemical techniques are expensive and are often appropriate only for small areas. On the other hand, for the

decontamination of polluted sites phytoremediation seems attractive as it offers site restoration, partial decontamination, maintenance of biological activity and biorecovery of metals (Baker *et al*., 1991, 1994; Gardea-Torresday *et al*., 2005). It has attracted attention for its low cost of implementation and environmental benefits. Phytoremediation is the use of green plants as well as associated rhizospheric microbes to remove pollutants from the environment or to render them harmless (Raskin *et al*., 1994; Salt *et al*., 1998). It is easier to manage because it is an autotrophic system of large biomass that requires little nutrient input (Evans and Furlong, 2003). Moreover, plants offer protection against water and wind erosion and in preventing spreading of contaminants (Pulford and Watson, 2003).

The success of phytoremediation as an environmental cleanup technology depends upon number of factors including the extent of soil contamination, metal availability for uptake into roots and plant ability to intercept, absorb and accumulate metals in shoots (Ernst, 1996). Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain or immobilize contaminants from soil and water (USEPA, 2000). In this approach, plants capable of accumulating high levels of metals are grown in contaminated soil, at maturity, metal enriched aboveground biomass is harvested and a fraction of soil metal contamination gets removed (Lasat, 2002). A number of Agroforestry tree species like *Eucalyptus* hybrid, *Acacia, Populus deltoides* etc. were found to grow and establish well on fly ash overburdens (Adholeya *et al.,* 1998). Poplar (*Populus sp.*) as one of the most often used tree species in phytoremediation, play a significant role in remediation of contaminated sites, their advantages refer to high biomass production, rapid growth, easy vegetative propagation and high transpiration rate. Some greenhouse and field researchers showed potential of Poplar for phytoextraction of some heavy metals such as As, Cd, Ni and Zn (USEPA, 2000).

The ideal plant species to remediate a heavy metal contaminated soil would be high biomass producing crop that can both tolerate and accumulate the contaminants of interest. It also led to more uniform growth and a greater shoot number than in control pots. Short rotation woody crops such as poplars (*Populus* sp.), willows (*Salix* sp.) and safeda (*Eucalyptus* sp.) are the most common tree species used for phytoremediation because they grow rapidly, have deep roots and take up large quantities of water

(Isebrands and Karnosky, 2001; Kopp *et al.,* 2001; Rockwood *et al.,* 2004 ; Licht and Isebrands, 2005; Mirck *et al.,* 2005). Biowaste/organic solid waste can be used as organic manure in agriculture. It improves physical, chemical and biological properties of the soil, improves texture, aeration, water-holding capacity. *Rhizobium, Azotobacter, Azospirillum, Blue green algae, Pseuodomonas striata* can be used as biofertilizers to increase the crop production. The establishment of large numbers of metabolically active populations of beneficial soil microbes is critical for the success of several environmental remediation and agricultural practices (Metting, 1992). One's concern is the ability to monitor released microorganisms in fly ash amended soils, so conventionally, specific phenotypic markers are used which allow selective recovery or enumeration of bacteria (Jansson, 2003). Other approaches include immunological techniques, DNA probing and PCR (Schneegurt and Kulpa, 1998). An alternative to tracking marker genes at the DNA level is to track the phenotype of the marker gene. A number of useful marker genes have recently been described (Atlas, 1992; Gustafsson and Jansson, 1993; Kluepfel, 1993; Prosser, 1994). Genes encoding metabolic enzymes have also been used as non-selective markers. These include *xylE* (encoding catechol 2,3 oxygenase), *lacZY* (encoding fi-galactosidase and lactose permease) and *gusA* (encoding B-glucuronidase [*gus*]). The *xylE* gene product can be detected by the formation of a yellow colored catabolite (2-hydroxymuconic semialdehyde) from catechol. Increasingly, it is becoming apparent that the best solution for tracking a GEM in the environment is to use either several markers instantaneously or multiple detection methods. Sometimes single markers or certain combinations of markers are not selective enough, such as *lacZY* used either alone or together with antibiotic selection (Masson *et al.,* 1993; Wilson and Lindow, 1993). Even so, use of antibiotic selection, in combination with bioluminescence (Masson *et al.,* 1993; Flemming *et al.,* 1994), *xylE* (Leij *et al.,* 1994) or *gusA* (Wilson *et al.,* 1992), has been found to be very effective and useful for selection of low numbers of tagged cells. Methods based on tracking different types of molecule specific to the GEM, such as protein and DNA (Flemming *et al.,* 1994) also allow more versatile and thorough monitoring.

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

Objectives

1. To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Toona ciliata* and *Populus deltoides*
2. To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves
3. To study the survival and establishment of molecular tagged microbes in soils amended with solid waste

In the present study the effect of addition of fly ash @ 10% (v/v), distillery waste @ 5% (v/v), farm yard manure @ 20% (v/v) and microbial consortium of *Pseudomonas striata* (*lacZ*$^{+}$) and *Azotobacter* CBD15 (*kan*^{R}) @ 30 ml consortium/per pot in soil was investigated in terms of biomass production, microbial activity and nutrient status of soil. Soil was collected from agricultural field of Thapar Campus, electrostatic precipitator (ESP) fly ash was brought from GGS Superthermal Plant, Ropar (Punjab), distillery waste from Patiala distilleries, village Main, Patiala, farm yard manure was procured from local market, Patiala; both *Azotobacter* CBD15 and *Pseudomonas striata* were procured from Division of Microbiology, IARI, New Delhi.

Soil and all soil amending agents (i.e., fly ash, distillery waste and farm yard manure) were characterized for physico-chemical properties. The phosphate solubilizing bacterium (*Pseudomonas striata)* was transformed with the *lacZ* marker gene isolated from *E. coli* 2842 and inoculated in soil with and without soil amending agents to study its proliferation and survival in nursery experiments to see the effect of microbes and solid wate amendment on the growth and biomass production of *Toona ciliata* and *Populus deltoides.* The soil and plant biomass were analysed for heavy metal content to study heavy metal uptake by nursery seedlings of two different tree species.

Chapter 2
Review of Literature

2.1 Agroforestry

To reduce deforestation and meet the growing demand for timber, fuelwood and fodder, ecologically and economically sustainable changes in land use are urgently needed. Promotion of agroforestry by wood-based industries to reduce dependence on forests is perceived to be a right step in this direction. Several studies show that poplar-based agroforestry is economically viable and more profitable than many of the crop rotations Jain and Singh, 2000). It is capable of providing continuous employment on farms and preserving ecological system as well. However, there exists plenty of scope for improving the output and income from poplar-based agroforestry through intensive management practices and application of optimum level of inputs (Atta-Krah *et al*. 2004).

Agroforestry is a collective name for land use systems and practices in which woody perennials are deliberately integrated with crops and/or animals on the same land management unit. The integration can be either in a spatial mixture or in a temporal sequence. There are normally both ecological and economic interactions between woody and non-woody components in agroforestry (World Agroforestry Centre, 1993). In agroforestry systems, trees or shrubs are intentionally used within agricultural systems, or non-timber forest products are cultured in forest settings. Knowledge, careful selection of species and good management of trees and crops are needed to optimize the production and positive effects within the system and to minimize negative competitive effects.

In some areas, a narrow definition of agroforestry might simply be: trees on farms. Hence, agroforestry, farm forestry and family forestry can be broadly understood as the commitment of farmers, alone or in partnerships, towards the establishment and management of forests on their land (Beetz, 2002). Where many landholders are involved and the result is a diversity of activity that reflects the diversity of aspirations and interests within the community. Agroforestry systems can be advantageous over conventional agricultural and forest production methods through increased productivity,

economic benefits, social outcomes and the ecological goods and services provided. Biodiversity in agroforestry systems is typically higher than in conventional agricultural systems. Agroforestry incorporates at least several plant species into a given land area and creates a more complex habitat that can support a wider variety of birds, insects, and other animals. Agroforestry also has the potential to help reduce climate change since trees take up and store carbon at a faster rate than crop.

Trees are increasingly grown on-farm to supply wood and biomass needs, in both temperate and tropical climates, as well as in developed and developing countries. Over the last several decades, within the irrigated rice-wheat growing lands of northern India, a considerable number of fast growing poplar trees have been planted on tens of thousands of small farms. This trend is driven by economic incentives (as wood production is often more valuable or less labor intensive) and by national policies (as they aim to limit further deforestation in regional forests) (Douglas and Hart, 1985). The contribution of poplar trees to the local economy and farmer livelihoods is quite significant and well established. Across northern India, from Punjab through Haryana and Uttar Pradesh to West Bengal, poplar (*Populus deltoides*) trees, which were introduced in the early 1970s to supply wood to a local match factory, have been widely adopted. These trees are planted on irrigated land traditionally used for cereal production in a rice/ wheat rotation. It has been proposed that including a tree component within the farming system, either on bunds and boundaries (sequentially with crops) or intercropped in an agroforestry type configuration, can lead to increased land productivity while diversifying the farming enterprise (Atta-Krah *et al*., 2004; Huxley, 1999; Young, 1989), and increase economic security for small farmers (Russell and Franzel, 2004). In light of prevailing low grain prices, due in part to successive abundant harvests from these intensively cultivated lands, poplar agroforestry has become increasingly attractive to farmers.

2.1.1 Poplar (Populus deltoides) in Agroforestry

In developing countries, forests are the main source of timber, fuelwood and livelihood for a large number of villagers and forest dwellers. With the growing concern for the rapid depletion of forests, several countries have encouraged wood-based industries to

develope tree plantations for meeting the needs of the local people and wood-based industries, and conserving the forest wealth and environment. Examples include the Paper Industries Corporation of the Philippines (PICOP), Alto Parana S. A.'s Pulp Mill in Argentina and Timsales Limited in Kenya (FAO, 1986). Although India has invested in tree plantations since the mid 1970s, contract tree farming, in which both wood-based industries and farmers are directly involved in commercial plantations, is a recent development. Started by Western Indian Match Company (now known as Wimco Ltd.) in 1984 in the northern region of India, tree contract farming has also been initiated by the ITC-run Bhadrachalam Paper Mills in Andhra Pradesh in southern India. Beginning in the late 1960s, Wimco faced an acute short supply of match-wood and was not able to meet its demand (Bhalla, 1989). Therefore, Wimco was forced to explore other avenues for its raw material which resulted in the development and promotion of poplar-based agroforestry in the northern region.

Poplar (*Populus deltoides*), a fast growing and short duration forest crop, is grown in irrigated and fertile lands. As a result, large chunks of irrigated and fertile lands are being brought under poplar-based agroforestry. The allocation of land and water for irrigated timber production, particularly in those areas where the two are scarce, conflicts with agricultural production. Kanshik (1961) suggested that when cultivable land and water are required for agriculture, the best course is to integrate forestry with agriculture. However, irrigated plantations are sensible only when economic returns are attractive, irrespective of the need of the product (Lerche and Khan, 1967). Furthermore, poplar-based agroforestry has become popular among farmers in the northern region of India owing to its promotion by Wimco.

For more than six decades, the poplar (*Populus deltoides*) has been an important tree in northern India, especially in the lowland 'Terai' areas at the base of the Himalayas. In 1969, four clones of *P. deltoides* were received from Australia, which propagated rapidly ensuring attractive returns to the leaseholders (Chaturvedi, 1982). Poplar is the most common forest crop in the Terai region and some parts of the Gangetic plain in the northern region. Among exotic species of poplar, 'G-3' and 'G-48' which are harvestable in six to eight years, are widely cultivated in these areas. Poplar is widely cultivated along with agricultural crops for the production of wood and fuelwood in block

plantations. Poplars are also planted in rows and on farm boundaries (Tejwani, 1994; Sharma, 2001).

2.1.2 Toon (Toona ciliata) in Agroforestry

It has many vernacular names: Toon, Indian mahogany, Australian red cedar. *Toona ciliata* originates from tropical Asia and tropical Australia, but is now much cultivated throughout the tropics for its timber and as an ornamental or wayside tree.

Toon is a large deciduous tree generally with a wide spreading and handsome crown attaining a height of 45 m and a stem diameter of 2 m. In cities may be of a much smaller stature. Also known as Red Cedar, Toon is famous for its fragrant red wood that is much sought after for use in furniture making, medicines, building and ornamental woodwork. The soft wood is easily worked and polished to a rich red that is enhanced with age. Flowers are white, fragrant, in a large pyramidal panicle at the end of the branchlets. Individual flower is about 5 mm long. Flowering period is in spring. Leaves are alternate, pinnate, consisting of five to seventeen leaflets. Leaflets opposite or irregularly alternate, ovate-lanceolate, 4-13 cm long, often drawn out to a long point at the tip, unequal at the base, green both surfaces, paler beneath, red and downy when young.

Australian red cedar (*Toona ciliata*) is possibly Australia's most celebrated native timber. Soon after European settlement the cedar cutters began scouring the rainforests up and down the east coast from south of Sydney to the Atherton Tablelands in search of cedar logs. Red Cedar has a light, fine-grained timber with a beautiful even deep-red colour. The heartwood is naturally durable, dries with splitting and is easy to work. In its natural tropical environment Red Cedar is actually deciduous losing it leaves in winter to avoid the 'dry season'. It thrives on deep well drains basalt soils or along rivers and creeks in areas of high summer rainfall.

The ethanol extract of *Toona ciliata* Roemer (heart wood) was evaluated for its anti-ulcer activity against aspirin plus pylorous ligation induced gastric ulcer (antisecretory), Ethanol extract of *Toona ciliata* showed significant reduction in gastric volume, free acidity, total acidity and ulcer index. The plant extract also showed gastro protective activity (52.94%), whereas standard drug sucralfate showed 94.85%. *Toona ciliata*

extract showed protection index 43.0% in water immersion stress induced ulcer (Malairajan, 2007).

Toona ciliata is useful for many purposes. It has been recommended in Australia for planting because of its high-value timber, in Mexico as a shade tree in coffee plantations and in India as a multipurpose tree in agroforestry systems. The attractive multipurpose wood and the fast growth make *Toona ciliata* attractive for planting. In its natural area of distribution *Toona ciliata* occurs in primary as well as secondary forest, often along rivers and in valleys, up to 1500 m altitude, in areas with 800–1800 mm annual rainfall. It is capable of regeneration in full sunlight. It prefers well-drained sites on deep, fertile soils, and does not grow well in sandy localities. It tolerates some drought if the tree is well established. It is frost hardy. In many regions within the natural area of distribution of *Toona ciliata*, the timber is highly prized and has been much overexploited, particularly in Australia where it once was the most important native timber. Nowadays it is exploited in many areas in South-East Asia, e.g. in Myanmar, India and China.

2.2 Soil Amendments

2.2.1 Microbial Inoculants

In developing country like India, use of chemical fertilizers is economically not feasible in agroforestry. The high price tag of these conventional fertilizers deters its possible use in tree plantation. Environmental and health hazards associated with chemical fertilizer further make their possible use difficult. On the other hand success in agroforestry requires various inputs leading to proper establishment and growth of tree species. Selection of low demanding and fast growing tree species, fertilization, disease and pest management and proper protection of plants are some vital factors. Agro forest ecosystem after a few years becomes self sustainable because it follows a "feedback regulating system". The nutrient budget in such ecosystem is polycyclic in nature. Weathering of litter and biological cycle are some important processes of nutrient management. The inoculation of *Rhizobium*, VAM and *Rhizobium* + VAM significantly improved growth parameters in *Acacia nilotica* (Singh and Dwivedi, 1996; Mishra, 2004).

Indian agriculture has shifted from traditional to intensive farming with indiscriminate use of chemical fertilizers and pesticides, which in turn has made our soils largely non-productive and contaminated the ground water. Indian soils are also becoming poor in organic matter and there is a growing concern for continuing regular application of organic manure and recycling crop residues to sustain productivity and high responses to NPK fertilizer. Forest areas have been adversely affected with large-scale deforestation activities, which have considerably reduced the forest cover. The impending energy crisis due to fast depleting mineral oil reserves has necessitated the search for renewable energy resources to ease the pressure on fuel energy. Chemical fertilizers always falling short by 8-10 million tons are staggering, considering country's future demand for food. Incorporation of biofertilizers or microbial inoculants in combination with organic manure can reduce the detrimental effects of the current agricultural practices in an eco-friendly manner. Biofertilizers are best described as microorganisms, which add, conserve and mobilize the crop nutrients in the soil. Use of biofertilizers such as *Rhizobium, Azospirillum, Azotobacter,* blue green algae, *Azolla,* mycorrhiza and phosphate solubilizers can facilitate management of crop nutrients leading to long term sustainability in crop production. The most striking relationship that these have with plants is symbiosis, in which the partners derive benefits from each other. Their addition also increases the fertilizer use efficiency of the crop plants. With ever increasing population, it is necessary to increase crop productivity per unit area without causing further deterioration in soil health and maintaining micronutrient balance (Gaur, 1990; Meyers, 1994).

Later half of the last century has witnessed remarkable development in the field of biofertilizers. The efforts have created a *cafetaria* of biofertilizers and revealed variety of attributes of these microbes implicating them in nitrogen fixation, phosphate solubilization, addition of organic matter, secretion of growth factors, improving the physical and chemical properties of the soil, better utilization of the chemical fertilizers, bioconcentration of nutrients in the rhizosphere and acceleration of the process of composting (Table 1).

Table 1a. Microorganisms having promise to boost agricultural production (Goyal, 2001)

Function	Organism	Crop
Nitrogen fixation	*Rhizobium meliloti, R. leguminosarum, R. trifolii, R. lupini , R. japonicum,*	Arhar, Pea, Lentil, Gram, green gram, Rajmah, Cowpea, Berseem, leucerne, Groundnut, Soybean,
	Azotobacter chroococcum, A. vinelandii,	Wheat, Barley
	A. beijjerinkii,	Maize, Cotton, Paddy
	Acetobacter diazotrophicus	Sugarcane
	Azospirillum lipoferum, A. amazonense,	Maize, Sugarcane,
	A. seropedica, A. americanum,	Jowar, Paddy, Pearl millet
	Cyanobacteria (blue green algae) *Anabaena, Nostoc, Aulosira, Calothrix, Tolypothrix, Azolla-Anabaena azollae*	Paddy
Phosphate solubilization	*Bacillus polymyxa , B. megaterium, Pseudomonas striata, Aspergillus awamori,* *Penicillium digitatum*	All crops
Nutrient translocation	Endomycorrhizae *: Glomus, Gigaspora, Acaulospora*	Forest tree species
	Ectomycorrhizae *: Laccaria, Pisolithus*	Forest tree species
Organic matter decomposition	Cellulolytic: Fungi : *Trichoderma, Chaetomium Aspergillus* Bacteria : *Bacillus, Cellulomonas, Cytophaga* Lignolytic : Fungi : *Clavaria, Cephalosporium,*	
Growth accelerators	Bacteria : *Pseudomonas, Flavobacterium* Plant growth promoting Rhizobacteria (PGPR), *Pseudomonas, Xanthomonas,*	All crops

Use of beneficial micro-organisms such as nitrogen fixing bacteria and phosphate solubilizing bacteria, is an integral component of cultivation practices for various crops because of their eco-friendly and cost effective technology. It is always better to use bio-incoulants as they provide enhanced yields in good rainfall years and they are cheap and eco-friendly besides the microbial build-up which is always important for various microbial transformations.

2.2.1.1 Nitrogen fixers

Rhizobium and *Azotobacter* can be produced in conical flasks mounted on to a shaking platform offering an excellent way of producing bacterial biofertilizers on a small scale. The media used for their growth contain organic substrates like glucose, mannitol and yeast extract and inorganic salts like calcium carbonate, magnesium sulphate and potassium orthophosphate. Axenic mother cultures grown on agar slants are used to inoculate appropriate medium and put on the shaking platform. After the growth, individual flasks are pooled aseptically and mixed with carriers such as sterilized peat, lignite or fortified charcoal powder and dispensed into polythene bags and sealed. For large-scale production, fermentors with complete automation for control of temperature, nutrient level and pH are used. The mixing of the culture and carrier material is done in bulk and dispensed in presterilized containers (Goyal *et al.,* 2001).

Incorporation of efficient plant-specific species of *Rhizobium* right from sowing/seedling stage in nursery and subsequently in fields is highly desirable. Optimal results are obtained by the bacterization of seeds with the bacterial cultures, using 15% jaggery solution prior to their sowing in the root trainer cups or adding cell suspension prior to germination at nursery level. To ensure their viability in view of limited shelf-life, it is desirable to produce desired quantities of fresh inoculum at site. However, this is feasible and cost-effective only where large scale nursery operations are done for commercial plantation. To make such inoculations effective, they are inoculated in numerical superiority ($1x10^8$ cells/ml/plant) over the indigenous flora. Once their compatibility with local micro-flora is established, they continue to show their effect, unless the soil is exposed to wild fire, innundation due to flood or excessive use of pesticides. It is estimated that N_2-fixers fixes 20-30 kg of N_2 per hectare per annum, which is equivalent

to the addition of 40-60 kg of urea. Improvement in various growth attributing characters like germination, chlorophyll content and dry matter was attained in vegetables like carrot, radish and chillies due to inoculation with *A. chroococcum.* This increase was attributed due to its ability to produce growth promoting substances (Khullar and Chahal, 1977; Rao and sharma, 1981). Verma and Shende (1993) reported that vegetable crops in general and potato, onion, tomato and brinjal in particular respond well to *Azotobacter* inoculation (El-Shaushouny *et al.,* 1989). Nitrogen fixing blue green algae is also used to add biologically fixed nitrogen in rice crop. These photoautotrophic organisms are multiplied in open air raceways in media containing inorganic salts like magnesium sulphate, dipotassium orthophosphate, single super phosphate and calcium chloride. Raceways are shallow ponds incompletely divided by a central wall and fitted with an electrically driven paddle wheel. Rotation of the paddle wheel continuously agitates the culture and ensures uniform exposure of the cells to nutrients and light. The harvested algal biomass is thoroughly mixed with pre-soaked multani mitti (fueller's earth) in equal quantity and the paste is sun dried in trays lined with polythene sheet, sealed in polythene bags and stored at ambient conditions. The titre value of the finished product must be $10^4 g^{-1}$ and the shelf life of 3 years. This is mixed with sieved farm soil and broadcasted on standing water after rice transplantation (Goyal *et al.,* 1997).

2.2.1.2 Phosphate solubilizers

Phosphorous (P) plays a crucial role in increasing crop yields. Unfortunately only 30 % of the applied P is used by the plants and the remaining gets converted to sparingly soluble/insoluble compounds such as tricalcium phosphate and phosphates of iron, aluminium and magnesium. Both bacteria and fungi have the ability to solubilise these compounds by secretion of aliphatic and aromatic acids and enzymes such as phytases and phospholipases. These microbes are referred to as phosphate solubilising microorganisms (PSM) (Table 1). Different mycorrhizae also solubilize insoluble phosphorous and finally help in its mobilization. Application of mycorrhizae as biofertilizer has gained momentum in forestry sector as they are known to confer several attributes to the plants including enhancement in growth of containerized seedlings and their subsequent better establishment and survival in field.

The use of PSM for effective utilization of P is yet to catch up in a big way. Inoculations of PSM have shown positive response in crops such as rice, wheat, barley, tobacco, sugarcane, potato and other vegetable and forage crops. PSM increases the efficiency of ground rock phosphate, basic slag, bone meal and superphosphate applied to neutral and alkaline soils. Their inoculation at nursery stage affords stabilization with the root system so as to be compatible with the local micro-flora upon transplantation in the field (Gaur, 1985). Integrated use of suitable microbial cultures alongwith low grade rock phosphates can add 30-35 kg P_2O_5 per hectare. India is perennially dependent upon large scale import of phosphatic fertilizers and rock phosphate, application of such microorganisms will have tremendous financial implications (Illmer and Schinner, 1992; 1995).

Tiwari *et al.,* (1988) found that *Azotobacter* and phosphobacteria in the rhizospheric soils were more when plants were inoculated with consortium of these organisms in comparison of individual inoculation. Addition of 2% farmyard manure to the richer soil enhanced this effect. Plant growth was more when seedlings were inoculated with both *Azotobacter* and the phosphobacteria (Tiwari *et al.,* 1988). These microorganisms are successful in getting established in the soil ecosystem due to their high adaptability in a wide variety of environments, their faster growth rate, and their biochemical versatility to metabolize a variety of natural and xenobiotic compounds (Kundu and Gaur, 1980).

2.2.2 Farm Yard Manure

Application of farm yard manure (FYM) for improving the availability of nutrients and physical properties is an age old practice in Indian agriculture. But with the introduction of chemical fertilizers and slow response of organic manures, its use is reduced. Earlier studies conducted on various crops at different locations indicated that the application of FYM in many crops enhanced the grain yields through the improvement in the availability of nutrients caused by enhanced soil biological productivity. Laboratory analysis confirmed that considerable amounts of macronutrients and small amounts of micronutrients were supplied by FYM. A study was initiated in 1997 to introduce the culture of supplementing low rates of NP fertilizers with farmyard manure (FYM) in the maize based farming system of western Oromia. Statistical analysis revealed that the N/P fertilizers and FYM significantly ($p < 0.05$) increased grain yield in all locations except

for Walda in 1997. Interactions of FYM and NP fertilizer rates were significant ($p \leq 0.05$) at all locations except for Shoboka. The application of FYM alone at rates of 4, 8, and 12 t ha^{-1} produced average grain yields of 5.76, 5.61 and 5.93 t ha^{-1}, respectively, compared to 3.53 t ha^{-1} for the control treatment in 1997. There were significant residual effects of FYM and NP fertilizers applied in 1997 on maize grain yields in 1998 (Negassa *et al.,* 2005).

2.2.3 Industrial solid waste

Assessment of industrial solid waste management problem greatly varies depending on the nature of the industry, their location and mode of disposal of waste. Further, for arriving at an appropriate solution for better management of industrial solid waste, assessment of nature of waste generated is also essential. Industries are required to collect and dispose of their waste at specific disposal sites and such collection, treatment and disposal is required to be monitored by the concerned State Pollution Control Board (SPCB) or Pollution Control Committee (PCC) in Union Territory. The following problems are generally encountered in cities and towns while dealing with industrial solid waste (Anandakumar, 2005).

- There are no specific disposal sites where industries can dispose their waste.
- Mostly, industries generating solid waste in city and town limits are of small scale nature and even do not seek consents of SPCBs/PCCs.
- Industries are located in non-conforming areas and as a result they cause water and air pollution problems besides disposing solid waste.
- Industrial estates located in city limits do not have adequate facilities so that industries can organize their collection, treatment and disposal of liquid and solid waste.
- There is no regular interaction between urban local bodies and SPCBs/PCCs to deal such issues relating to treatment and disposal of waste and issuance of licenses in non-conforming areas.

The major generators of industrial solid wastes are the thermal power plants producing coal ash, the integrated Iron and Steel mills producing blast furnace slag and steel melting slag, non-ferrous industries like aluminum, zinc and copper producing red mud and tailings, sugar industries generating press mud, pulp and paper industries producing lime and fertilizer and allied industries producing gypsum.

Table 1b: Source and quantum of generation of some major industrial waste

S.No.	Name	Quantity (million tonnes per annum)	Source / origin
1.	Steel and Blast furnace	35.0	Conversion of pig iron to steel and manufacture of iron
2.	Brine mud	0.02	Caustic soda industry
3.	Copper slag	0.0164	By product from smelting of copper
4.	Fly ash	70.0	Coal based thermal power plants
5.	Kiln dust	1.6	Cement plants
6.	Lime sludge	3.0	Sugar, paper, fertilizer tanneries, soda ash, calcium carbide industries
7.	Mica scraper waste	0.005	Mica mining areas
8.	Phosphogypsum	4.5	Phosphoric acid plant, ammonium phosphate
9.	Red mud/ Bauxite	3.0	Mining and extraction of alumina from Bauxite
10.	Coal washery industry	3.0	Coal mines
11.	Iron tailing	11.25	Iron ore
12.	Lime stone wastes	50.0	Lime stone quarry
13.	Pressmud (solid distillery waste)	5.0	Sugar mill and distilleries

(Source: National Waste Management Council- Ministry of Environment & Forests- 1990/1999)

2.2.3.1 Integrated Iron and Steel Plant Slag

The Blast Furnace (BF) and Steel Melting Shop (SMS) slags in integrated iron and steel plants are at present dumped in the surrounding areas of the steel plants making hillocks encroaching on the agricultural land. Although, the BF slag has potential for conversion into granulated slag, which is a useful raw material in cement manufacturing, it is yet to be practised in a big way. Even the use of slag as road subgrade or land-filling is also very limited (Emery and Matchett, 1979; World Bank, 1996).

2.2.3.2 Phosphogypsum

Phosphogypsum is the waste generated from the phosphoric acid, ammonium phospate and hydrofluoric acid plants. This is very useful as a building material. At present very little attention has been paid to its utilisation in making cement, gypsum board, partition panel, ceiling tiles, artificial marble, fiber boards etc. (Degirmenci, 2008; Sen and Mishra, 2010).

2.2.3.3 Red Mud

Red mud as solid waste is generated in non-ferrous metal extraction industries like aluminum and copper. The red mud at present is disposed in tailing ponds for settling, which more often than not finds its course into the rivers, especially during monsoon. However, red mud has recently been successfully tried and a plant has been set up in the country for making corrugated sheets. Demand for such sheet should be popularised and encouraged for use. This may replace asbestos which is imported and also banned in developed countries for its hazardous effect. Attempts are also made to manufacture polymer and natural fibres composite panel doors from red mud (Parekh and Goldberer, 1976; Prasad and Sharma, 1986).

2.2.3.4 Lime Mud

Lime sludge, also known as lime mud, is generated in pulp & paper mills which are not recovered for reclamation of calcium oxide for use except in the large mills. The lime mud disposal by dumping into low-lying areas or into water courses directly or as run-off during monsoon is not only creating serious pollution problem but also wasting the valuable non- renewable resources. The reasons for not reclaiming the calcium oxide in the sludge after recalcination is that it contains high amount of silica. Although a few technologies have been developed to desilicate black liquor before burning, none of the mills in the country are adopting desilication technology (Sthiannopkao and Sreesai, 2009).

2.2.3.5 Waste Sludge and Residues

Treatment of industrial wastes/effluents results in generation of waste sludge/residues which, if not properly disposed, may cause ground and surface water pollution (Hasselgren, 1999).

2.2.3.6 Coal Ash

In general, a 1,000 MW station using coal of 3,500 kilo calories per kg and ash content in the range of 40-50 per cent would need about 500 hectares for disposal of fly ash for about 30 years' operation. It is, therefore, necessary that fly ash should be utilized wherever possible to minimize environmental degradation. The thermal power plant should take into account the capital and operation/maintenance cost of fly ash disposal system as well as the associated environmental protection cost, vis-a-vis dry system of collection and its utilization by the thermal power plant or other industry, in evaluating the feasibility of such system. The research and development carried out in India for utilization of fly ash for making building materials has proved that fly ash can be successfully utilized for production of bricks, cement and other building materials. Indigenous technology for construction of building materials utilizing fly ash is available and is being practiced in a few industries (El-Mogazi *et al.,* 1988). However, large scale utilization is yet to take off. Even if the full potential of fly ash utilization through manufacture of fly ash bricks and blocks is explored, the quantity of fly ash produced by the thermal power plants is so huge that major portion of it will still remain unutilized. Hence, there is a need to evolve strategies and plans for safe and environmentally sound method of disposal.

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 (Jala and Goyal, 2006). 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 (Hart *et al.,* 2003).

2.2.3.6a Disposal of fly ash

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).

2.2.3.6b Use of fly ash in Agroforestry

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). The high concentration of elements (K, Na, Zn, Ca, Mg and Fe) in fly-ash increases the yield of many agricultural crops (Basu *et. al.,* 2009). Fly ash addition generally has shown positive impact on plant biomass production and nutrient uptake (Ciravolo and

Adriano, 1979; Elfving *et al*., 1981). Fly ash has been found to increase yield of alfalfa *(Medicago sativa)*, barley *(Hordeum vulgare),* bermudagrass *(Cynodon dactylon)* and white clover *(Trifolium repens)* and improves physical and chemical characteristics of the soil (Martens, 1971; Page *et al.,* 1979; Hill and Lamp, 1980; Elseewi *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 and brick kiln dust amendment in soil improved plant growth and yield of eggplant. Highest increase was observed at 20% level in fly ash and 30% level in brick kiln dust. The study showed that fly ash and brick kiln dust were beneficial to the plant at lower levels i.e. 20% and 30% respectively (Rizvi and Khan, 2009). Fly ash can be used as a potential nutrient supplement for degraded soils thereby solving the solid waste disposal problem to some extent. However, the bioaccumulation of toxic heavy metals and their critical levels for human health in plant parts and soil should be investigated (Kishor *et. al.,* 2010).

Exploitation of metal uptake by plant biomass as a method of soil decontamination is limited by plant productivity and the concentration of metals achieved (Baker *et al*., 1991). 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). Compared to traditional soil conditioning materials as asbestos, fly ash seems to be more advantageous as it is an environmental safe material, contains plant nutrients and can be used in biological cultivation (EU dir 889/08). Additionally, it is a low cost material and thanks to its granular composition, is readily applicable (Chassapis *et al.,* 2010).

2.2.3.6c Use of fly ash and microbial consortium with organic manure

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 (Gaind 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).

Tiwari *et al.,* (1988*)* found that *Azotobacter* and phosphobacteria in the rhizospheric soils were more when plants were inoculated with consortium of these organisms in comparison of individual inoculation. Addition of 2% farmyard manure to the richer soil enhanced this effect. Plant growth was more when seedlings were inoculated with both *Azotobacter* and the phosphobacteria (Tiwari *et al.,* 1988). These microorganisms are successful in getting established in the soil ecosystem due to their high adaptability in a wide variety of environments, their faster growth rate, and their biochemical versatility to metabolize a variety of natural and xenobiotic compounds. The establishment of large numbers of metabolically active populations of beneficial soil microbes is critical for the success of several environmental remediation and agricultural practices (Metting, 1992). Microbial metal mobilization from particulate fly ash by *Acidithiobacilli* resulted in cadmium, copper and zinc mobilization of > 80%, whereas, lead, chromium and nickel were mobilized by 2, 11 and 32% respectively. In addition, the potential of HCN-forming bacteria (*Chromobacterium violaceum, Pseudomonas fluorescens*) was investigated to mobilize metals when grown in the presence of solid materials (e.g., Cu containing ores, electronic scrap etc.) (Brandl and Faramarzi, 2006). To study establishment of inoculum, it is necessary to have a sensitive and reliable method for specifically detecting and quantifying the inoculated strain in the field. In recent years, new techniques based on molecular markers or polymerase chain reaction (PCR) have been used to specifically monitor the bacteria in the field. Use of marker genes such as *gusA, lacZ, celB, xylA, luxAB* has become an important tool in studies on microbial ecology. The *lacZ* marker has been used to study nodule infection by *Rhizobium* (Boivin *et al*., 1990) and root colonization by *Azospirillum* (Katupitiya *et al.,* 1995a, 1995b). *E.coli lacZ* has proved to be a highly versatile reporter gene in *Sacchromyces cerevisiae* and has been used to study many aspects of signal-transduction pathways and other cellular processes (Guarente and Ptashne, 1981; Rose *et al.,* 1981; Guarente, 1983; Burns *et al.,* 1994; Uhl and Johnson, 2001).

2.2.3.7 Distillery waste

India is a major producer of sugar in the world, and sugar industry offers employment potential and contributes substantially to economic development. There are about 579 sugar mills and 285 distilleries in India. Apart from the sugar and alcohol, these factories generate many by-products and waste materials. For example, more than 5 million tonnes of solid waste (pressmud) is being produced from sugar industries. The disposal of wastes from industrial sources is becoming a serious problem throughout the world. The distillery waste is loaded with organic and inorganic salts, resulting in high EC (30-45 dS/m). Being plant originated, the waste also contains considerable amounts of plant nutrients and organic matter. High nitrogen, phosphorus and potassium content is there. Calcium, magnesium, sulphate and chloride are also present in appreciable amounts. Thus, it can effectively be used as a source of plant nutrients and as soil amendment. Recently, the presence of appreciable amounts of plant growth promoters *viz.*, gibberellic acid and indole acetic acid have also been detected which further enhances the nutrient value of waste (Rajukkannu and Manickam, 1997; Valliappan, 1998; Murugaragavan, 2002). The high concentration of calcium in waste may have the potential in reclaiming the sodic soils similar to that of gypsum effect. The unpleasant odour due to the presence of skatole, indole and other sulphur compounds, which are not effectively decomposed by yeast or methanogenic bacteria during distillation, is also an issue of public concern.

The viability of recycling the solid wastes generated by the winery and distillery industry by means of co-composting with animal manures, as well as to evaluate the quality of the composts obtained. Two piles, using exhausted grape marc and cattle manure or poultry manure, respectively (at ratios, on a fresh weight basis, of 70:30), were composted by the Rutgers static pile composting system. Throughout the composting process, a number of parameters were monitored, such as pH, electrical conductivity, organic matter, water-soluble carbon, water-soluble polyphenols, different forms of nitrogen (organic nitrogen, ammonium and nitrate) and humification indices (humification ratio, humification index, percentage of humic acid-like C, polymerisation ratio and cation exchange capacity), as well as the germination index. Organic matter losses followed first-order kinetics equation in both piles, the highest organic matter mineralisation rate being observed with exhausted grape marc and cow manure. On the other hand, the mixture with the lowest

C/N ratio, using exhausted grape marc and poultry manure, showed the highest initial ammonium contents, probably due to the higher and more labile N content of poultry manure. The increase in the cation exchange capacity revealed the organic matter humification during composting. In contrast, other humification parameters, such as the humification ratio and the humification index, did not show the expected evolution and, thus, could not be used to assess compost maturity. Composting produced a degradation of the phytotoxic compounds, such as polyphenols, to give composts without a phytotoxic character. Therefore, composting can be considered as an efficient treatment to recycle this type of wastes, due to composts presented a stable and humified organic matter and without phytotoxic effects, which makes them suitable for their agronomic use (Bustamante *et al.,* 2008). The beneficial effect of distillery waste on crop production is well documented (Joshi *et al.,* 1996; Ramana *et al.,* 2002).

2.3 Remediation of heavy metals from soil

A large portion of biosphere is contaminated by heavy metals as a result of human activities. Conventional solutions such as disposal of contaminated soil in landfills, which relies heavily on 'dig and dump' or encapsulation, neither of which takes into consideration the issue of decontamination of the soil, account for a large proportion of the remediation operations at present (Pulford and Watson, 2003). Remediation techniques like immobilization or extraction by physico-chemical techniques are expensive and are often appropriate only for small areas. On the other hand for the decontamination of polluted sites phytoremediation seems attractive as it offers site restoration, partial decontamination, maintenance of biological activity and biorecovery of metals (Baker *et al*., 1991, 1994). It has attracted attention for its low cost of implementation and environmental benefits.

2.3.1 Phytoremediation

Phytoremediation is defined as the use of green plants as well as associated rhizospheric microbes to remove pollutants from the environment or to render them harmless (Raskin *et al*., 1994; Salt *et al*., 1998). It is an innovative biological technique that can be applied for the cleanup of both organic and inorganic pollutants present in the soil. A number of

articles have been written on the important aspects of phytoremediation, a novel plant based technology (Salt *et al*., 1995, 1998; Chaney *et al*., 1997; Chaudhry *et al.,* 1998; Meagher, 2000; Lasat, 2002; Pulford and Watson, 2003; Prasad and Freitas, 2003; Alkorta *et al.,*, 2004).

Phytoremediation of metals occurs due to following activities:

- Phytoextraction: Pollutant accumulating plants remove metals from the soil and concentrate them in the harvestable part of plants (Kumar *et al*., 1995a).
- Rhizofiltration: Removal of contaminants from aqueous waste streams by absorption onto plant roots (Dushenkov *et al.,* 1995).
- Phytostabilization: Immobilization or prevention of migration of contaminants in the environment by plant exudates, leading to the reduction in the mobility and bioavailability of the contaminants (Vangronsveld *et al*., 1995).
- Phytomining: Plant ability to extract large amounts of metals from soils can be exploited to recover metals of economic value from ore deposits and other soils (Glass, 2000).

It has been estimated that the market for phytoremediation of metals from soils in the USA alone was nearly $1-2 million in 1997, with a potential to increase to $15-25 million by 2000 and $70-100 million by 2005 (Glass, 2000). Governments worldwide are establishing research and decontamination programs to use this potential. Environment Canada has developed a database (PHYTOREM) of 775 plants with capabilities to accumulate or hyper accumulate one or several of 19 key metallic elements (McIntyre, 2003). Phytoremediation is easier to manage because it is an autotrophic system of large biomass that requires little nutrient input (Evans and Furlong, 2003). Moreover, plants offer protection against water and wind erosion and in preventing spreading of contaminants (Pulford and Watson, 2003).

The success of phytoremediation as an environmental cleanup technology depends upon number of factors including the extent of soil contamination, metal availability for uptake into roots and plant ability to intercept, absorb and accumulate metals in shoots (Ernst,

1996). This review aims to give a broad overview of the various phytoremediation technologies and their potential role in clean up of pollutants especially heavy metals.

2.3.1.1 Phytoextraction

The concept of using plants to clean up contaminated environment is very old and cannot be traced to any particular source (Blaylock and Huang, 2000). About 300 years ago, plants were proposed for use in the treatment of wastewater (Hartman, 1975). At the end of the 19th century, *Thlaspi caerulescens* and *Viola calaminria* were the first plant species documented to accumulate high levels of metals in leaves (Bauman, 1885). Plants able to accumulate upto 1% Ni in shoots were identified (Minguzzi and Vergnano, 1948). The idea of using plants to extract metals from contaminated soil was reintroduced and developed by Utsunamyia (1980) and Chaney (1983). At present, there are two basic strategies of metal phytoextraction; natural phytoextraction and induced or chemically assisted phytoextraction (Salt *et al.,* 1998).

Phytoextraction is based on the use of pollutant accumulating plants for the removal of metals and organics from soil by concentrating them in the harvestable parts (Brooks *et al.,* 1977; Salt *et al.,* 1998; Reeves and Baker, 2000; Vassilev *et al*., 2004). Metal phytoextraction is not as extreme as conventional metal removal methods but still involves considerable alterations in the environment, which includes elimination of the existing vegetation cover and application of fertilizers and various soil amendments to increase metal availability to plants (Ebbs *et al*., 1998; Huang *et al*., 1998; Tichy *et al*., 1997; Pawlowska *et al.,* 2000). Plants for phytoextraction, i.e., metal removal from soil, should have the following characteristics: (i) tolerant to high levels of the metal, (ii) accumulate reasonably high levels of the metal, (iii) rapid growth rate, (iv) produce reasonably high biomass in the field and (v) profuse root system (Garbisu *et al.,* 2002).

The roots of the established hyperaccumulators absorb metal elements from the soil and translocate them to the above ground shoots, where they get accumulated in high concentration (Prasad and Freitas, 2003). It is also based on high biomass producing plants used together with chemical agents enhancing both metal solubility and uptake by plants (Huang, 1997; Blaylock *et al.,* 1997). After sufficient plant growth and metal

accumulation, the above ground portions of the plants are harvested and removed, which results in the permanent removal of the metals from site. After removal of heavy metals from the soil, the disposal of the contaminated material is an environmental concern. Some researchers suggested incineration (Kumar *et al.,* 1995a), while others suggested about the extraction of valuable metals from the metal rich ash (Comis, 1996; Cunningham and Ow, 1996).

In general, the reports assessing metal phytoextraction potential are based on pot experiments, when compared to field experiments higher metal extracting values have been observed, which might be due to higher solubility of metals and the effect of amendments aiming at mobilizing the metals etc. (Vassilev *et al*., 2004). The selection of heavy metal tolerant species is a reliable tool to achieve success in phytoremediation. 163 plant taxa belonging to 45 families have been found to be metal tolerant and are capable of growing on elevated conc. of toxic metals (Prasad and Freitas, 2003). The use of metal tolerant species and their metal indication and accumulation is a function of immense use for biogeochemical prospecting (Brooks, 1983; Badri and Springuel, 1994; Mcinnes *et al.,* 1996). Chemically assisted phytoextraction is based on the use of non-accumulator plants with metal accumulation levels far below those of hyperaccumulators but with high biomass potential (Vassilev *et al*., 2004). This is aimed to overcome the main limitations of natural phytoextraction, i.e., a limited number of suitable hyperaccumulators for some important metal pollutants such as Pb (Huang *et al*., 1997; Lasat, 2000), as well as their low biomass production.

The first field trial on natural phytoextraction was conducted in 1991-92 in sewage sludge treated plot at Woburn, England (Mcgrath *et al*., 1993). The maximum Zn uptake was found in *Thlaspi caerulescens* (*T. caerulescens*) accumulating 2000-8000 mg Zn/kg. *T.caerulescens* was shown to accumulate 1000-4000 mg/kg Cd (Brooks *et al.,* 1998). *Sedum alfredii* Hance has been identified as a new Zn and Cd hyperaccumulating plant species (Yang *et al.,* 2004). Zn conc. in its shoot can reach over 20 g/kg when grown at 80 mg Zn/l in nutrient solution without showing any toxic symptoms (Yang *et al*., 2002). Cd conc. in leaves and stem of *S. alfredii* increased with increasing Cd supply levels, and reached a maximum upto 9000 – 65000 mg/kg dry weight (Yang *et al*., 2004). Seedlings of *Sesbania drummondii* can hyperaccumulate Pb in a controlled hydroponic environment

(Barlow *et al.,*, 2000). High Cu conc. has been found in *Betula* roots (Kozlov *et al*., 1995; Maurice and Lagerkvist, 2000) as well as in *Salix* roots (Punshon and Dickinson, 1997). The Pb, Zn and Cd phytoextraction potential of 14 different plants was assessed in a chelate induced phytoextraction experiment. The phytoextraction potential of water hyacinth (*Eichchornia crassipes*) was assessed for the removal of selenium and copper individually and from binary solutions. Plant growth, estimated on day 16 of metal treatment, decreased at all concentrations of selenium (2–12 ppm), whereas it increased at lower concentrations of copper (4–12 ppm) and decreased at higher exposure levels. The results revealed that a water hyacinth-based system could successfully remove selenium and copper from water/wastewater (Pal and Rai, 2010).

EDTA (ethylenediamine-tetracetic acid) and EDDS (ethylenediamine disuccinic acid) were used as chelates. The addition of these chelates increased the proportion of phytoavailable Pb, Zn and Cd in the soil and also their uptake by tested plants upto 48 times by *Sinapsis alba*, 4.6 times by *Raphanus sativus oleiformis* and 3.3 times by *Amaranthus sp*. respectively. *Cannabis sativa* hyperaccumulated 105 times Pb, 2.3 times Zn and 31.7 times Cd higher than control (Kos *et al.,*, 2003). Blaylock *et al*. (1997) and Huang *et al*. (1997) found that application of EDTA at 2 g/kg soil resulted in a conc. of more than 1.5 % Pb in the shoots of *Brassica juncea* and 1 % in maize and pea plants. It was also shown that other chelators such as EGTA (ethylene–bis [oxy ethyletrinitrilo] tetracetic acid) had high affinity to Cd, while DTPA (diethylene-triamine-pentacetic acid) showed high affinity to Zn (Blaylock *et al*., 1997).

Restrictions apply, however, to both use of complexing agents and artificial soil acidification. It was found that EDTA and EDTA-heavy metal complexes are toxic for some plants and high doses of EDTA inhibited the development of arbuscular mycorrhiza (Geebelen *et al.,* 2002; Dirilgen 1998; Creman *et al*., 2001). *In-situ* application of chelating agents can cause groundwater pollution by uncontrolled metal dissolution and leaching (Creman *et al*., 2001; Sun *et al.,*, 2001). An experiment was carried out tp see the effects of EDTA and citric acid on accumulation potential of marigold (Tagetes erecta) to Zn, Cu, Pb, and Cd and also to evaluate the impacts of these chelators (EDTA and citric acid) in combination with all the four heavy metals on the growth of marigold. The plants were grown in pots and treated with Zn (7.3 mg l^{-1}), Cu (7.5 mg l^{-1}), Pb (3.7

mg l^{-1}) and Cd (0.2 mg l^{-1}) alone and in combination with different doses of EDTA i.e., 10, 20 and 30 mg l^{-1}. All the three doses of EDTA i.e., 10, 20 and 30 mg l^{-1} significantly increased the accumulation of Zn, Cu, Pb and Cd by roots, stems and leaves as compared to control treatments (Sinhal *et. al.,* 2010).

The ideal plant species for metal phytoextraction has to be highly productive in biomass and to uptake and translocate a significant part of metals to its shoots (Vassilev *et al.,* 2004). Some tree sps. mainly *Salix* sps. and *Populus* sps. exhibit these traits and are already used in phytoremediation programmes and for Cd phytoextraction from lightly polluted agricultural soils (Landberg and Greger, 1994). In fact, *Salix* sps. are not metal hyperaccumulators, but it was shown that among different clones there are high accumulators of Cd and Zn. About 150 clones of different *Salix* sps. have been screened for uptake, transport of metals to shoots and tolerance to Cd, Zn and Cu (Landberg and Greger, 1996; Landberg and Greger, 1994). Some reports by Grant and Bailey (1997), Griga *et al.,* (2003), Yankov *et al.,* (2000) and Yankov and Tashin (2001) showed that crops for fibre or oil production could be used for profitable crop production accompanied by phytoextraction of metal from polluted soils.

The main advantage of this technology is its lower cost as compared to other known remediation technologies (Glass, 2000; EPA, 2000). The possible metal recycling should provide further economic advantage as the ash of some hyperaccumulators consists of significant amount of metals (20-40 % Zn for *T. caerulescens*) and there is no need to pay for safe disposal (Chaney *et al*., 1997), it can work without further disturbing the site, which is of great importance for its public acceptance (Vassilev *et al*., 2004). Besides all its advantages, it has certain limitations also, the major limitation is that it can only be used for low to moderately contaminated soils and it is applicable only to surface soils and is a time consuming process (Robinson *et al.,* 1998; Blaylock and Huang, 2000). It is still at developmental stage, small companies and universities are driving much of its innovation and research, whereas, environmental engineering firms are involved in application projects.

In our own lab, i.e., at Thapar Institute of Engineering & Technology campus, metal accumulation in root, stem and leaves of three and a half year old *Eucalyptus tereticornis*

planted on a marginal land amended with unweathered ESP fly ash @ 0-24% (v/v) was studied. Uptake of Pb, Fe, Ni, Cr and Cu was highest by roots followed by leaves and stem. Accumulation of Pb, Fe, Ni, Cr and Cu was 18, 2500, 36, 39 and 44 µg/g respectively, by the roots. The accumulation of Mn and Zn was more in leaves. (Suryan *et al*., 2004).

2.3.1.2 Rhizofiltartion

Rhizofiltation refers to the use of plant roots to sorb, concentrate and precipitate metal contaminants from surface or groundwater (Dushenkov *et al.,* 1995). It is effective in cases where wetlands can be created and all of the contaminated water is allowed to come into contact with roots. Contaminants should be those that sorb strongly to roots such as hydroponic organics, Pb, Cr(III) etc. Rhizofiltration is primarily used to remediate extracted groundwater, surface water and wastewater with low concentration of contaminant and it can be very cost effective (Salt *et al.,* 1995). It can be used for Pb, Cd, Cu, Ni, Zn and Cr, which are primarily retained within the roots (USEPA, 2000). An ideal plant for rhizofiltation should have rapidly growing roots with the ability to remove toxic metals from solution over extended periods of time. Dushenkov *et al.,* (1995) demonstrated that many 'large root' species have the ability to absorb and precipitate heavy metals from solution, such as sunflower (*Helianthus* sp.), rye (*Elymus* sp.), corn (*Zea mays*), Indian mustard (*Brassica juncea*). The mechanisms of toxic metal removal by plant roots are not necessarily similar for different metals.

In rhizofiltation plants used have the ability to remove upto 60% of their dry weights as toxic metals (Salt *et al.,* 1995). The process involves raising plants hydroponically and transplanting them into metal polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov *et al*., 1995; Salt *et al*., 1995; Flathman and Lanza, 1998 and Zhu *et al.,* 1999). Root exudates and changes in the pH of rhizosperic soil may also cause metals to precipitate onto root surfaces.

Plants for rhizofiltation should be able to accumulate and tolerate significant amounts of the target metals alongwith easy handling, low maintenance cost and a minimum of secondary waste requiring disposal (Dushenkov and Kapulnik, 2000). Several aquatic

species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes*; Kay *et al*., 1984; Zhu *et al.,* 1999), pennywort (*Hydrocotyle umbellate* L.; Dierberg *et al*., 1987) and duckweed (*Lemna minor* L.; Mo *et al.,* 1989). As a result of their small, slow growing roots these plants are not much efficient at metal removal and have limited potential for rhizofiltation (Dushenkov *et al*., 1995). Sunflower (*Helianthus sp*.), Indian mustard (*Brassica juncea*), Tobacco (*Nicotiana tabacum*), Rye (*Elymus sp*.), Spinach (*Spinacea Oleracea*) and Corn (*Zea mays*) have been studied for their ability to remove Pb from water, with sunflower having the greatest ability. In a study, after only one hour of treatment Sunflower reduced Pb conc. significantly (Raskin and Ensley, 2000). Indian mustard had bioaccumulation coefficient of 563 for Pb and has proven to be effective in removing a wide conc. range of Pb (4 mg/l – 500 mg/l) (Raskin and Ensley, 2000; USEPA, 2000). The hairy root cultures of *Brassica napus* were used to study the removal of 2,4-dichlorophenol (2,4-DCP), a common contaminant in industrial effluents that is highly toxic for human and aquatic life. High removal efficiencies (93-95%) were observed in a broad pH range (pH 3-9), reaching 98-99% in the range pH 4-8 (Agostini *et al*., 2003). The hairy root systems of *Brassica napus* and *Chenopodium amaranticolor* were used for removal of uranium from the solution of concentration up to 5,000 microM. The results indicated that the hairy roots could remove uranium from the aqueous solution within a short period of incubation. *Brassica juncea* could take up 20-23% of uranium from the solution containing up to 5,000 µM, when calculated on g/g dry weight basis. *Chenopodium amaranticolor* showed a slow and steady trend in taking up uranium, with 13% uptake from the solution of 5,000 µM concentration (Eapen *et al*., 2003). More than 80% of the initial uranium from an artificially uranium contaminated solution and three genuine groundwater samples was removed within 24 h by using sunflower and the residual uranium concentration of the treated water was lower than 30 µg/L (USEPA drinking water limit). For bean, the uranium removal efficiency of the rhizofiltration was roughly 60–80%. The maximum uranium removal via rhizofiltration for the two plant cultivars occurred at pH 3–5 of solution and their uranium removal efficiencies exceeded 90%. The lab-scale continuous rhizofiltration clean-up system delivered over 99% uranium removal efficiency (Lee and Yang, 2010). A higher amount of toxic metals was accumulated in the roots of *Hydrocotyle umbellata* L. than in the

shoots. The bioconcentration factor of Cr was higher than that of Zn and Cu at the same exposure time, indicating a higher accumulation potential of Cr by *H. umbellata.* The order of uptake efficiency was Cr > Zn > Na > Cu and the maximum metal uptake was observed after 90 days of exposure of *H. umbellate*. This plant not only tolerated up to 60% concentration of tannery sludge but also reduced chromium content of sludge to a considerable extent (Khilji and Bareen, 2008).

The advantages associated with the rhizofiltation are the ability to use both terrestrial and aquatic plants for either *in situ* or *ex situ* applications, applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling and likelihood of public acceptance (Dushenkov *et al.,* 1995; Kumar *et al.,* 1995b and Raskin and Ensley, 2000). The disadvantages include the constant need to maintain pH, plants may first need to be grown in a nursery and then transplantation, maintenance of successful hydroponic systems in the field will require expertise of qualified personnel, periodic harvesting and plant disposal and a good understanding of the chemical speciation (USEPA, 2000).

2.3.1.3 Phytostabilization

Phytostabilization is defined as immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone of plants. It is also known as phytorestoration. It is a plant based remediation technology that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Cunningham *et al.,* 1995; Salt *et al.,* 1995; Flathman and Lanza, 1998; Berti and Cunningham, 2000 and Schnoor, 2000). Pytostabilization of organic pollutants that are foreign to living organisms is based on sequestration processes, such as humification (McCutcheon and Schnoor, 2003).

Table 1c: Plants with the potential for phytoremediation of various heavy metals and organic compounds

Metal or Organic compound	Plant species	Method/Activity	Reference
Cd	*Brassica juncea*	Phytoextraction Rhizofiltration	Kumar *et al*., 1995a Dushenkov *et al*., 1995
	Sedum alfredii	Phytoextraction	Yang *et al*., 2004
	Thlaspi caerulescens	-do-	Brown *et al*., 1995
	Cannabis sativa	-do-	Citterio *et al*., 2003
	Tagetes erecta	-do-	Sinhal *et al.,* 2010
Cr	*Brassica juncea*	Phytoextraction Rhizofiltration	Kumar *et al*., 1995a Dushenkov *et al*., 1995
	Cannabis sativa	Phytoextraction	Citterio *et al*., 2003
Cu	*Festuca rubra*	Phytostabilization	Smith and Bradshaw, 1992
	Agrotis tenuis	-do-	-do-
	Brassica juncea	Phytoextraction Rhizofiltration	Ebbs *et al.,* 1997 Dushenkov *et al*., 1995
	Tagetes erecta	Phytoextraction	Sinhal *et al.,* 2010
Co	*Berkheya coddii*	Phytoextraction	Keeling *et al*., 2003
Pb	*Sesbania drummondi*	-do-	Barlow *et al*.,, 2000
	Festuca rubra	Phytostabilization	Smith and Bradshaw, 1992
	Agrotis tenuis	-do-	-do-
	Brassica juncea	Rhizofiltration	Dushenkov *et al*., 1995
	Tagetes erecta	Phytoextraction	Sinhal *et al.,* 2010
Ni	*Brassica juncea*	Phytoextraction Rhizofiltration	Ebbs *et al*., 1997 Dushenkov *et al*., 1995
	Thlaspi caerulescens	Phytoextraction	Baker *et al.,* 1991
	Berkheya coddii	-do-	Keeling *et al*., 2003
	Alyssum murale	Phytoextraction Phytomining	Whiting *et al.,* 2003 Broadhurst *et al*., 2004
	Cannabis sativa	Phytoextraction	Citterio *et al*., 2003
Zn	*Sedum alfredii*	-do-	Yang *et al*., 2004
	Thlaspi caerulescens	-do-	Baker and Walker, 1990
	Festuca rubra	Phytostabilization	Smith and Bradshaw, 1992
	Agrotis tenuis	-do-	-do-
	Brassica juncea	Rhizofiltration	Dushenkov *et al*., 1995
	Alyssum murale	Phytoextraction	Whiting *et al*.,, 2003
	Tagetes erecta	-do-	Sinhal *et al.,* 2010
Se	*Typha latifolia L.*	Phytovolatilization	Pilon-Smits *et al.,* 1999
	Eichchornia crassipes	Phytoextraction	Pal and Rai, 2010
U	*Brassica napus*	Rhizofiltration	Eapen *et al*., 2003
	Chenopodium amaranticolor	-do-	-do-

Phyostabilization involves root zone microbial and chemical processes. It can change metal solubility and mobility or impact the dissociation of organic compounds. The plant affected soil environment can convert metals from a soluble to an insoluble oxidation state (Salt *et al.,* 1995). Phytostabilization can occur through sorption, precipitation, complexation or metal valence reduction (EPA, 1997). In a vegetative cap for phytostabilization, a combination of trees and grasses may be used. Fast transpiring trees such as poplar maintain an upward flow to prevent downward leaching, while grasses prevent wind erosion and lateral runoff with their dense root systems (Bennett *et al*., 2003; McCutcheon and Schnoor, 2003).

Sometimes there is no immediate effort to clean metal polluted sites, either because the responsible companies no longer exist or because the sites are of no high priority on a remediation agenda (Berti and Cunningham, 2000). Metal tolerant plants are required for heavy metal contaminated soils. Plants chosen for phytostabilization should be poor translocaters of metal contaminants to above ground plant tissues that could be consumed by humans and animals. The plants selected should be easy to establish and care for, grow quickly, have dense canopies and root systems and be tolerant of metal contaminants and other site conditions that may limit plant growth. *Brassica juncea* has been shown to reduce leaching of metals from soil by over 98% (Raskin *et al.,* 1994). Arsenic might be taken up by plants because it is similar to the plant nutrient phosphate, although poplar leaves in a field study did not accumulate amounts of As. Poplars were grown in soil containing an average of 1250 mg/kg As (Pierzynski *et al.,* 2002). Mine wastes containing Cu were stabilized by grasses (Salt *et al.,* 1995). The research of Smith and Bradshaw (1992) led to the development of two cultivars of *Agrotis tenuis* and one *Festuca rubra*, which are now commercially available for phytostabilization of Pb, Zn and Cu, contaminated soils. Soil amendments can also be used to stabilize metals in soils. Amendments should be selected that will maximize the growth of vegetation, which then also helps to phytostabilize the soil (Berti and Cunningham, 1997). Rye corn is a potentially useful plant for rapid phytostabilization of gold mine tailings, with only minor phosphate amendment required (Mains *et. al.,* 2006).

It has advantages over other soil remediation practices in that it has a lower cost and is less disruptive than other more vigorous soil remedial technologies (EPA, 2000), easy to

implement (Schnoor, 2000). Revegetation offers aesthetic value and enhances ecosystem restoration. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). The main disadvantage is that the contaminants remain in place. The vegetation and soil may require long term maintenance to prevent release of the contaminants and future leaching (EPA, 2000). Highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is not possible (Berti and Cunningham, 2000).

2.3.1.4 Phytomining

Phytomining is defined as the production of crop of metal by growing high biomass plants that accumulate high metal conc. Some of these plants are natural metal hyperaccumulators, while in others the property can be induced (Brooks, 1998). Plant ability to extract large amount of metals from soils can be exploited to recover metals of economic value from ore deposits and other soils (Glass, 2000). Studies have shown that using certain plants to extract metals from soil is commercially feasible. It may be a green alternative to destructive, opencast mining practices. It could be used to mine metals that are uneconomic by conventional methods (Brooks, 1998).

Research conducted at the University of Texas in El Paso, USA, has shown that gold accumulated by alfaalfa plants and stored in leaf and stem biomass can be present as discrete nano-particles of pure metal (Gardea-Torresdey *et al*., 2002). This discovery was made after alfaalfa sprouts germinated on gold chloride enriched agar (320 mg/kg Au) were analysed using X-ray absorption spectroscopy (XAS) and transmission electron microscopy (TEM). The gold recovery rate in plants has been observed for many tested artificial and real substrates (Anderson *et al*., 1998). *Chilopsis Linearis* exposed to 160 mg L^{-1} had a growth reduction in the first growth stage, but those plants had the capacity to successfully recover from the toxic effects of the gold. In addition, the results showed that the gold uptake by desert willow had a concentration-dependent trend and that the amount of gold absorbed by the plant increased as the age of the plant increased. The amount of gold found in the leaves suggests that desert willow could potentially be a gold hyperaccumulator. In addition, the XAS data showed that desert willow produced gold nanoparticles within the tissues (Rodriguez *et. al.,* 2007). Broadhurst *et al.,* (2004) have

developed commercially viable phytomining technologies employing *Alyssum* Ni-hyperaccumulator species, where the majority of Ni is stored either in the leaf epidermal cell vacuoles, or in the basal portions of the numerous stellate trichomes. The metal concentration in the trichome basal compartment was the highest ever reported for healthy vascular plant tissue, approximately 15-20% dry weight (Broadhurst *et al.*, 2004).

Keeling *et al.* (2003) investigated the potential of South African high biomass Ni hyper accumulator *Berkheya coddii* to phytoextract Co and Ni from artificial metalliferous media. Plant accumulations of both metals from single element substrates indicate that the bioaccumulation coefficient increases as total metal conc. increase. An important step in the phytomining operations is the recovery of metals from harvested plant material. In this work, a laboratory scale horizontal tube furnace was used to generate Ni enriched bio-ore from the dried biomass of Ni hyperaccumulator plants. Prior to furnace treatment, hairy roots of *Alyssum bertolonii* were exposed to Ni in liquid medium to give biomass Ni conc. of 1.9% to 7.7% dry weight; whole plants of *Berkheya coddii* biomass was about 15 times greater than in *A. bertolonii*. After furnace treatment at 1200°C under air, Ni bearing residues with crystalline morphology and containing upto 82% Ni were generated from *A. bertolonii* (Boominathan *et al.*, 2004).

Plants and associated microorganisms can remediate heavy metal contaminated soil via phytoextraction, rhizofiltration, phytostabilization and phytomining. Phytoremediation works effectively for a wide range of inorganic pollutants, the underlying biological processes are still largely unknown in most of the cases (Pilon-Smits, 2005). However, biology alone cannot make phytoremediation to work, the complexity of phytoremediation needs co-ordination with many other disciplines, such as developments in the field of environmental and agricultural engineering will have a major impact on the efficiency of plant cultivation, amendment application and final disposal of metal-enriched plant biomass (Salt *et al.*, 1998). In recent years, phytoremediation has emerged as a promising ecoremediation technology, particularly for soil and water cleanup of large volumes of contaminated sites. Identification and selection of more efficient plant varieties for phytoremediation, optimized doses of soil amendments and agronomic practices can increase the efficiency of the phytoremediation. Remediation system that is

applicable to specific contaminated soils becomes significant, provided both fundamental and applied research at the primary level is pursued.

Phytoremediation in appropriate situations is a low-cost technique especially relevant for moderately polluted areas and is environment friendly and is gradually approaching commercialization. Its major disadvantage is in requiring longer remediation period. Despite the remaining gaps in our knowledge regarding the complexity of phytoremediation, there is a need for more field studies to demonstrate the effectiveness of phytoremediation technology for its public acceptance and policy makers, which makes it a novel approach.

Chapter 3
Materials and Methods

3.1 Analysis of soil and industrial solid waste

Chemicals: Chemicals used for the physico-chemical and biological analysis were procured from

1. HiMedia, Mumbai
2. Sdfine Chemicals Ltd., Mumbai
3. CDH Laboratory Reagent, New Delhi
4. Ranbaxy, Mohali
5. SISCO (SRL), Mumbai
6. Merck, Mumbai
7. Sigma-Aldrich, USA
8. Bangalore Genei, Bangalore

Instruments: Instruments used for the analysis are as follows:

1. Atomic Absorption Spectrophotometer (GBC 932AA, Australia)
2. Orbital Shaker (Scigenics Biotech)
3. pH meter (Thermo Orion)
4. EC meter (Thermo Orion)
5. Gel Electrophoresis (Tarsons)
6. Vaccum Pump (Millipore)
7. Hot Plate (Tarsons)
8. Vortex Mixer, Spinix (Tarsosns)
9. Shaker Incubator (Labcon)
10. B.O.D. Incubator (Metrex Scientific Instruments)
11. Muffel Furnace (SICO)
12. UV-Vis Spectrophotometer (Hitachi)
13. Weighing Balance (Sartorius)
14. Electroporator (Biorad)

15. Hot Air Oven (NSW, Narang Scientific Works)
16. Microwave Oven (LG)
17. Refrigerator (LG)
18. -20°C Deep Freezers (Vestfrost)
19. Autoclave (Equitron)
20. Laminar Air Flow Bench (Thermadyne)

3.1.1 Collection of soil and industrial solid waste (FA & DW) and Farm yard manure (FYM)

Two nursery experiments were carried out (each for 180 days) at Thapar University, Patiala (Punjab). The soil for the experiments was collected from agricultural field, Thapar University Campus Patiala (Punjab), fly ash was brought from GGS Superthermal Plant, Ropar (Punjab); Distillery waste from Patiala Distilleries, Vill. Main, Patiala (Punjab) and farmyard manure was collected from local market, Patiala (Punjab). The physico-chemical analysis of the soil and all the amending materials, viz., fly ash (FA), distillery waste (DW) and farmyard manure (FYM) was carried out following standard protocols as follows. Prior to analysis collected soil and all the soil amending materials were air dried and passed through 2mm size sieve for physical analysis and ground and passed through 0.2mm size sieve for chemical analysis.

3.1.2 Chemical analysis

3.1.2.1 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 20ml 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, prepared by using standard standard buffer tablets from HiMedia.

3.1.2.2 Electrical conductivity

Electrical conductivity was measured in µS 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 µS cm^{-1}).

3.1.2.3 Organic carbon

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

Reagents

1. 1 N $K_2Cr_2O_7$: 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%).
6. 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 $K_2Cr_2O_7$. 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

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

3.1.2.4 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 $NaHCO_3$ 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 H_2SO_4, mixed thoroughly and volume made upto 2 l 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 H_2SO_4 diluted to 1 l.
5. Stock Standard P solution (50 ppm P): 0.2917 g KH_2PO_4 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 1 l.

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 upto 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 sample (mg kg^{-1}) = P in extract (mg l^{-1}) x 20 (the standard sample to solution ratio)

3.1.2.5 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 upto 2 l.
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 2 l 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 1 l.
5. Stock Standard P solution (50 ppm P): Dissolved 0.2917g of KH_2PO_4 in water to a final volume of 1 l.
6. Working Standard P solution (1 ppm): Diluted 20 ml of (50 ppm P) solution to 1 l.
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 (1:1) and filtered through Whatman No. 42 filter paper and the filtrate was 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. A 2 ml aliquot was taken and analysis carried out as in step 3 and 4.

Calculation

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

3.1.2.6 Available Nitrogen

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

Reagents

1. 0.32% Potassium per manganate: 3.2 g of $KMnO_4$ dissolved in water and final volume made up to 1 l.
2. 2.5% NaOH: 25 g of sodium hydroxide pellets dissolved in water and volume made up to 1 l.
3. 2% boric acid: 20 g of boric acid powder dissolved in warm water by stirring and diluted to 1 l.

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 pthalate: Dissolve 20.422 g of the salt in water and dilute to 1 l.
6. 0.1N NaOH: 4g of NaOH dissolved in water and diluted to 1l, standardized against 0.1 N potassium hydrogen pthalate 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 0.1N 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 burrette until a pink colour started appearing. A blank was run without soil.

Calculation

Available N in sample in ppm = (X) x 0.00028 x 100/5 x 10,000

where, X stands for the titre value of 0.02 N H_2SO_4 consumed

3.1.2.7 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.
7. 50% NaOH.
8. 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 condensor 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

$$\text{Total N \%} = \frac{(T-B) \times 0.0014 \times 100}{\text{Weight of sample}}$$

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

3.1.2.8 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 1 l.
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 rpm. 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 addition of 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. 10 ml of 0.15% calcium chloride, 1.0 g barium chloride, 1 ml gum acacia were added and volume was made up with distilled water. The absorbance was read at 420 nm.
5. 1 g soil was digested with HNO_3 and $HClO_4$ in the ratio 3:1 on hot plate at 100ºC 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.

Calculation

S in sample (mg kg^{-1}) = S in extract (mg l^{-1}) x 6.25 (the standard sample to solution ratio)

3.1.2.9 Total Fe, Mn, Ni, Cr, Pb, Zn, Ca, Mg, Na, Cd, Co

Estimation of total metals was done as per the method given by Page et al. (1982).

Reagents

1. Concentrated perchloric acid ($HClO_4$) and nitric acid (HNO_3).
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. HNO_3 and $HClO_4$ 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 root, stem and leaves of *Populus deltoides* and *Toona ciliata* using an atomic absorption spectrophotometer (GBC 932AA). The various metals along with their sensitivity limits are as follows.

Element	Sensitivity ($\mu g\ ml^{-1}$)
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
Cd	0.009
Co	0.04

Calcultion

$$\text{Heavy metals in sample (mg kg}^{-1}\text{)} = \frac{\text{heavy metal in extract (mg l}^{-1}\text{) x dilution factor}}{\text{Wt. of sample taken}}$$

3.1.2.10 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 1 l after adjusting the pH to 7.0.
3. Whatman No.42 filter paper.

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).

Calculation

$$\text{K in sample (mg kg}^{-1}\text{)} = \frac{\text{K in extract (mg l}^{-1}\text{) x dilution factor}}{\text{Wt. of sample taken}}$$

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

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

Reagents

1. Deionized water.
2. 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 $CaCl_2.2H_2O$ added to 5l of deionized water followed by DTPA-TEA mixture and pH adjusted to 7.3±0.05 using 1.0 N HCl.
3. Whatman No.42 filter paper.

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 25ºC 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).

Calculation

$$\text{Elements in sample (mg kg}^{-1}\text{)} = \frac{\text{elements in extract (mg l}^{-1}\text{) x dilution factor}}{\text{Wt. of sample taken}}$$

3.1.3 Physical analysis

3.1.3.1 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 was fitted with a brass lid.

Procedure

1. A filter paper disc 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 80º 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
Moisture absorbed by filter paper	W4
Moisture held by soil alone	W3-W2-W4
Water holding capacity of the soil (%)	W3-W2-W4/W2-W1x100

3.1.3.2 Bulk density

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

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

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

3.1.3.3 Particle size distribution

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

Procedure

1. 100 g of soil sample was weighed and placed in a 500 ml beaker followed by addition of 200 ml distilled H_2O and 100 ml sodium oxalate solution.
2. The contents were stirred well in a mechanical stirrer and given 4-5 washings with distilled H_2O, 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 ºF- 68) x0.2

Time	Temperature	Hydrometer reading	Correction	Correct reading	% suspension
40 secs	78º F	33.5	2.2	35.7	35.7
2 hrs	77º F	15.0	2.0	17.0	17.0

Sand per cent = 64.0

Clay per cent =17.0

Silt per cent =18.7

Reading at 40 secs = 35.7

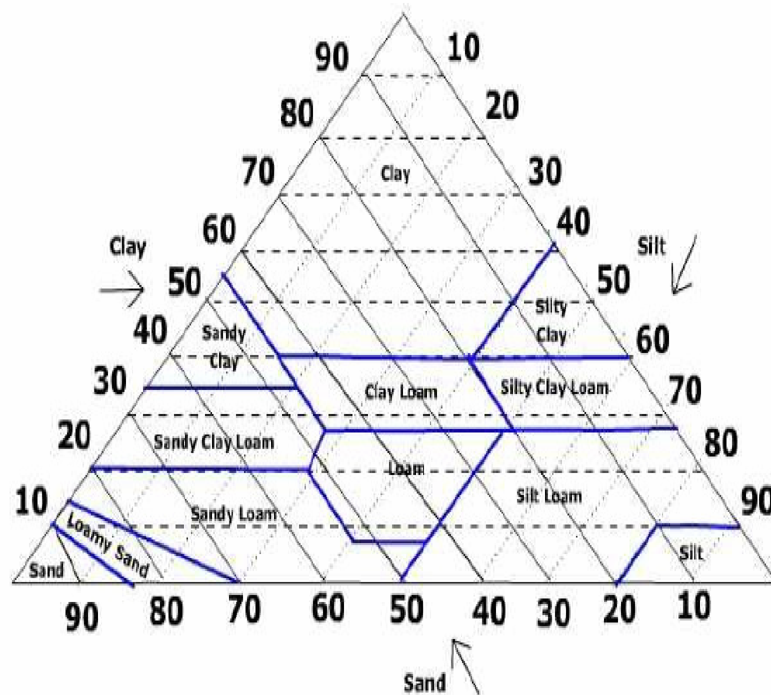
Reading at 2 hours = 17.0

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.

3.1.3.4 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.

3.2. Microbiological Analysis

Bacterial cultures and Growth conditions

The pure cultures of *Pseudomonas striata* and *Azotobacter* CBD15 were procured from Division of Microbiology, IARI, New Delhi and were maintained on Pikovskaya's media (PKV) and Jensen's media plates respectively at 30 ± 2°C. A loopful of full grown cultures from the selective media plates were inoculated in PKV broth and Jensen's broth for their mass cultivation in flasks, the flasks were incubated at 30 ± 2°C and 120 rpm for 48 hours.

Both the bacterial strains were transformed with plasmid pMMB277 (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) carrying dual marker *lacZ* and chloramphenicol resistant gene isolated from *E. coli* 2842 (procured from IMTECH, Chandigarh) and inoculated in soil with and without soil amending agents to study its proliferation and to study survival multiplication or colonization of inoculum of labeled strain, the bacterial count and expression was determined.

3.2.1 Microbial biomass

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

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.1filter 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. Repeated washing was 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 vaccum dessicator.
7. The dessicator was connected to a vaccum pump and switched on until the chloroform boiled for about five minutes.
8. The outlet was closed and dessicator was incubated in the dark for 24 hours.
9. After 24 hours the vaccum 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 K_2SO_4.
12. The samples were kept on a shaker for 30 minutes and the suspension was filtered through Whatman No.1filter paper.
13. 10 ml of filtrate was transferred to a 500 ml conical flask and 2 ml of 0.2 N $K_2Cr_2O_7$ was added.
14. 10 ml concentrated H_2SO_4 and 5 ml of orthophosphoric acid was added to each flask.
15. The flasks were kept on hot plate at 100ºC 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 was 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):

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

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

VS (ml) = wet soil weight (g) – oven-dry soil weight (g)+ extractant volume (ml)

4. Determination of extractable carbon (EC in mg ml^{-1})

4.1. Standardisation of FAS solution:

Normality of FAS (x N) =

$$\frac{\text{Vol. of } K_2Cr_2O_7 \text{ (1 ml) x Strength of } K_2Cr_2O_7 \text{ (0.2 N)}}{\text{Average titre value for the blank (ml)}}$$

4.2. Volume of $K_2Cr_2O_7$ solution consumed by FAS in any sample (Y ml):

$$\frac{\text{Normality of FAS (}x\text{N) x Titre value (ml)}}{\text{Normality of } K_2Cr_2O_7 \text{ (0.2 N)}}$$

4.3. Volume of $K_2Cr_2O_7$ consumed for oxidizing easily mineralisable C in 10 ml of extractant = 2-Y ml.

4.4 Extractable C (EC) in µg ml^{-1} :

(2-Y) ml of 0.2 N $K_2Cr_2O_7$ oxidises 600 x (2-Y) µg of C

Amount of extractable C (EC):

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

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

EC_F or EC_{UF} (µg g^{-1} soil) = EC (µg ml^{-1}) x VS (Ml) /MS (g)

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

B-C (µg g^{-1} soil) = $(EC_F - EC_{UF})/ K_{EC}$

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

3.2.2 Enumeration of Bacteria

3.2.2.1 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
sterile water blanks	10 and 9 ml

Procedure

1. 1g of soil was added to 10 ml sterile water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml sterile water making a dilution corresponding to 10^{-1}.
3. Further dilutions were prepared in a similar way up to 10^{-6}.
4. 100µl 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.

Calculation

$$\text{CFU/gm of sample} = \frac{\text{No. of colonies/plate x 10 x dilution factor}}{\text{Wt of sample taken}}$$

3.2.2.2 Enumeration of free-living nitrogen fixing bacteria

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

Jensen's Medium (g l^{-1})

Sucrose	20.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.5g
NaCl	0.2g
$CaCO_3$	2.0g
$FeSO_4.7H_2O$	0.1g

pH	7.5
Agar	15.0 g
sterile water blanks	10 and 9 ml

Procedure

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

Calculation

$$\text{CFU/gm of sample} = \frac{\text{No. of colonies/plate x 10 x dilution factor}}{\text{Wt of sample taken}}$$

3.2.2.3 Enumeration 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
$(NH_4)_2SO_4$	0.5g
NaCl	0.2g
$MgSO_4.7H_2O$	0.1g
KCl	0.2g
Yeast extract	0.5g
$MnSO_4$	0.025g
$FeSO_4.7H_2O$	0.020g
Agar	15g

pH	7±0.2
sterile water blanks	10 and 9 ml

Procedure

1. 1g of soil was added to 10 ml sterile water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml sterile water making a dilution corresponding to 10^{-1}.
3. Further dilutions were prepared in a similar way upto 10^{-6}.
4. 100µl 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 30ºC for 24 hours and observations recorded.
6. The single colonies were streaked repeatedly on 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 24 hour incubation at 30ºC.

Calculation

$$\text{CFU/gm of soil} = \frac{\text{No. of colonies/plate x 10 x dilution factor}}{\text{Wt. of soil (gm)}}$$

3.2.2.4 Enumeration of E.coli 2842 *(lacZ$^+$), Pseudomons striata (lacZ$^+$) transformant and Azotobacter CBD15 (kan^R)*

Bacterial counts in soil with and without various soil amendments were carried out as per the standard plate count agar method for *E.coli* 2842 *(lacZ$^+$), Pseudomons striata (lacZ$^+$)* transformant and *Azotobacter* CBD15 *(kan^R)* on nutrient agar, Pikovskaya's (PKV) plates containing 10µg ml^{-1} chloramphenicol, IPTG and X-gal and Jensen's medium plates containing 50µg ml^{-1} kanamycin (Cappuccino, 1987).

Requirements

1. IPTG + X-gal + chloramphenicol containing nutrient agar and PKV media plates
2. IPTG + X-gal + kanamycin containing nutrient agar and Jensen's media plates
3. Standard solutions of IPTG, X-gal, Chloramphenicol and Kanamycin

Preparation of stock solutions

1. IPTG *(Isopropyl-β-D-thiogalactopyranoside)* :

   100 mg ml^{-1} of sterile water and filter sterilized by 0.22µ disposable filter.
2. 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.
3. Chloramphenicol Stock solution (10 mg ml^{-1}) and working solution (10µg ml^{-1}).
4. Kanamycin Stock solution (10 mg ml^{-1}) and working solution (50µg ml^{-1}).

Procedure

1. 1g of control soil and from various treatments was added to 10 ml sterile water blank individually and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml sterile water making a dilution corresponding to 10^{-1}.
3. Further dilutions were prepared in a similar way upto 10^{-6}.
4. 100µl inoculum was taken from dilution 10^{-3} and 10^{-4} and added to nutrient agar, Pikovskaya's (PKV) plates containing 10µg ml^{-1} chloramphenicol, IPTG and X-gal and Jensen's medium plates containing 50µg ml^{-1} Kanamycin and the inoculum was spreaded with the help of a spreader.
5. The plates containing inoculum were incubated at 30ºC for 24 hours and observations were recorded.

Calculation

$$\text{CFU/gm of sample} = \frac{\text{No. of colonies/plate x 10 x dilution factor}}{\text{Wt of sample taken}}$$

3.2.3 Growth curve

The growth curve of bacterial strains *Pseudomonas striata* and *Azotobacter* CBD15 were studied by plotting the absorbance of culture grown in their respective media at hourly interval (Cappuccino, 1987).

Requirement

1. Overnight grown bacterial cultures
2. Test tubes containing nutrient broth
3. Mechanical shaker
4. Cuvettes
5. Autopippetes
6. Spectrophotometer

Procedure

1. The overnight grown bacterial culture (in triplicate) 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 isolate was measured as a function of time by plotting the absorbance against each hour.

3.2.4 Antibiotic profiling of bacterial cultures

Antibiotic profiling of bacterial strains *Pseudomonas striata* and *Azotobacter* CBD15 using standard antibiotics (Cappuccino, 1987) was done as follows:

Requirements

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 µg ml^{-1}

Procedure

1. 50 ml media was dispensed in flasks (250 ml) and broth in test tubes (10 ml each).
2. Working solutions of antibiotics were made and filter-sterilized in the laminar air flow cabin.
3. Five plates each of control, Str 50, Kan 50, Nal 50 and Chl 10 were prepared.
4. The cultures of *Pseudomonas striata* and *Azotobacter* CBD15 were inoculated in 5ml broth and were kept for overnight growth at 30°C.
5. The absorbance of the cultures was checked at 600nm 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.
7. Observations were recorded.

3.2.5 Plasmid isolation

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

Requirements

1. Sol. I : 50 mM glucose + 10mM EDTA + 25 mM Tris HCl (pH 8)
2. Sol. II : 0.2 N NaOH + 1% w/v SDS (pH 12)
3. Sol. III : 60 ml 5 M Potassium acetate + 11.5 ml glacial acetic acid + 28.5 ml D/W (pH 5.8) -store at 4^0C.
4. TE buffer: 10 mM Tris HCl + 1 mM EDTA -store at -20°C
5. Micro-centrifuge
6. Auto-pipettes

Procedure

1. Suspended 1.5 ml of actively growing culture in an eppendorf.
2. Spinned down at 10,000 rpm for 5 min.

3. Discarded the supernatant completely and dried out the pellet by inclining it.
4. Added Sol. I (200 µl), left for 1.5 min.
5. Prepared lysozyme (10mg/ml) freshly.
6. Added 30 µl of freshly prepared lysozyme in each eppendorf and mixed it thoroughly.
7. Added 400 µl of freshly prepared Sol. II. Mixed it well and incubated in ice bath. Left for 5 min.
8. Added 300 µl of Sol. III and kept in ice bath for 20-60 min.
9. Centrifuged at 10,000 rpm for 10 min. Collected supernatant.
10. DNA was precipitated by adding equal volume of isopropanol for 1hr.
11. Isolated pellet by centrifugating at 12,000 rpm for 20 min, dissolved in 30 µl of TE buffer, stored at – 4°C.

Solvent extraction

1. RNAse was activated for 8-10 minutes at 100°C and slowly cooled to room temperature after which 1-2 µl 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 µl: 250µl) 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 µl 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 airdried.
6. The pellet was redissolved in 50µl of TE (pH 8.0) and stored at -20°C followed by gel electrophoresis using 0.7% agarose.

3.2.6 Gel Electrophoresis

Requirements

1. TBE buffer (5x) : 5.4 gm Tris-base + 2.74 gm Boric acid + 2 ml of 0.5M EDTA + distilled water to make final volume 100 ml.
2. Loading buffer (5x): 30% Sucrose + 0.1% bromophenol blue + 20mM EDTA.
3. Ethidium bromide stock: 5mg/ml.
4. Gel electrophoresis assembly
5. UV-illuminator.

Procedure:

1. Set the Gel electrophoresis assembly.
2. Prepared 0.7% agarose gel in 0.5x TBE buffer.
3. Added ethidium bromide in the agarose gel (running conc. 1 μg/ml).
4. Poured the gel in the assembly and let it get set.
5. Removed the comb, and loaded the samples in the well along with loading buffer in the required conc. (to make running conc. 1x).
6. Connected the wires to the voltage supplier and let the gel torun and saw with the help of UV-illuminator.

3.2.7 Transformation of Bacterial strains

Transformation of *P. striata* and *Azotobacter* CBD15 with plasmid pMMB277 of *E.coli 2842* was carried out by calcium chloride treatment method (Mendel and Higa, 1970).

Requirements

1. 100 mM $CaCl_2$
2. Nutrient broth
3. Auto-pipettes

Procedure

1. 200 μl of overnight grown culture was inoculated in 25 ml broth and was grown at 37°C for 2 ½ hr in shaker incubator.

2. Transferred freshly grown culture in sterile centrifugation tubes and centrifuged at 7,000 rpm for 10 min. at 4°C to get pellet.
3. Discarded the supernatant and suspended the pellet in ice chilled 100 mM (5 ml) $CaCl_2$ solution. Kept it in ice for 10 min.
4. Again centrifuged it at 7,000 rpm for 10 min. to get pellet at 4°C.
5. Discarded the supernatant and resuspended the pellet in 1 ml of ice chilled $CaCl_2$ solution, kept it in ice bath for 2-½ hr to make competent cells.
6. Transferred 100 µl of competent cells in an eppendorf; added 10 µl of purified plasmid.
7. Mixed completely and kept for 30 min. for incubation in ice.
8. Gave heat shock at 42°C for 2 min. and then cooled at room temp.
9. Added 1 ml of Nutrient broth in each eppendorf. Gave it incubation at 37°C for 1 hr.
10. Centrifuged at 6,000 rpm for 6 min. and discarded 600 µl of supernatant.
11. Plated 100 µl in each plate (Selection plating).

3.3. Nursery Trials

Nursery experiments were carried out at Science & Technology Entrepreneur's Park, Thapar University, Patiala (Punjab) to see the effect of microbes and soil amendments of industrial solid waste on the growth and biomass production of *Populus deltoides* and *Toona ciliata* and to study the microbial interaction, also to check the extent of heavy metal uptake by these tree species.

The soil was mixed with different amending materials to make twelve treatments as shown below for *Populus deltoides* and *Toona ciliata.* In one experiment shoot cuttings of *Populus deltoides* M. were grown singly in all the twelve treatments in 16 replications / treatment and in another seedling of *Toona ciliata* R. were transplanted (one seedling / pot) from seed raised bed in all the different treatments from T1 to T12. Irrigation from surface was used to maintain soil moisture at approximately water holding capacity.

T1- Soil (Control)	T2- Soil + MA
T3- Soil + FA	T4- Soil + FA + MA
T5- Soil + FA + DW	T6- Soil + FA + DW + MA
T7- Soil + FA + FYM	T8- Soil + FA + FYM + MA
T9- Soil + DW	T10- Soil + DW + MA
T11- Soil + FYM	T12- Soil + FYM + MA

(MA: Microbial amendment (*P. striata* and *Azotobacter sp.*) @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

- *Populus deltoides* M.

Kingdom	Plantae - Plants
Plants Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Salicales
Family	Salicaceae – Willow family
Genus	*Populus* L. – cottonwood
Species	*Populus deltoides* Bartram ex Marsh. – eastern cottonwood
Subspecies	*Populus deltoides* Bartram ex Marsh. ssp. *monilifera*

- *Toona ciliata* R.

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Sapindales
Family	Meliaceae – Mahogany family
Genus	*Toona* (Endl.) Roem. – redcedar
Species	*Toona ciliata* Roem. – Australian redcedar

3.3.1 Biometric data collection

Plant height and collar diameter of the stem of both the tree species were recorded on day 180. Height was measured by elongating the plant and measuring to the top of the upper leaf with a ruler and collar diameter of the stem above the soil surface was measured with a digital vernier caliper. After the measurement the plants were harvested, roots and shoots were separated at the crown and then leaves were separated from the stem. The soil adhering to roots was removed by washing under a gentle stream of water under the tap. Roots, stem and leaves were dried in a hot air oven at 80°C for 48 hours, after that the dry weights of the plant parts were measured gravimetrically.

3.3.2 Heavy metal uptake by root, stem and leaves of P. deltoides and T. ciliata

Dried leaves stem and roots were crushed in a pestle and mortar and were powdered in a mixer grinder after which they were sieved through 0.2 mm sieve to obtain a fine powder form. For heavy metal analysis of root, stem and leaves, 1 g oven dried (70ºC), powderized and sieved sample (0.02 mm mesh size sieve) of each was digested by wet digestion method with concentrated HNO_3 and $HClO_4$ in the ratio 3:1 (Page, 1982). The samples were digested on a hot plate at a temperature corresponding to 100ºC for 3-4 hrs. Heating was done till it dried up completely and whitish brown dry mass was obtained. It was then cooled and the precipitate/digest mixture was extracted in acid water mixture (concentrated HCl: MilliQ water in the ratio 1:1), filtered through whatman filter paper No. 42 and the volume was made up to 50 ml. The filtrate was analyzed for metal content using Atomic Absorption Spectrophotometer (GBC 932 AA, Australia). The instrument was calibrated using standard solution of Cu.

3.4. Statistical analysis

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

3.4.1. 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.

3.4.2. Variance

The variance is measured as the square of the units in which the variable X is measure3.4. For example, if X is the height in centimeters (cm), the variance will be measured in cm^2 (square centimeters). The formula for variance is:

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

= Sum of the squares of the deviations of individual values from the

mean x ¸ sample size

where n is the number of observations; x is the arithmetic mean of the observations of X_{2s} are the individual observations – x_1, x_2…………x_i…………x_n.

3.4.3 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,

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

3.4.4 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 form the same of population. It is used to compare means with one another.

$$\text{Formula for } S^2 \qquad = \text{variance} = \sum (X_i - \overline{X})^2 / (n-1)$$

$$= (\sum X_i^2 - n\overline{X}^2 / (n-1) = (SS\text{-}CF) \div (n-1)$$

Standard deviation = square root of variance = √S2

Standard error = √ (variance/sample size) = √(S2/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.

3.4.5 Two way Analysis of Variance

Two way ANOVA, also called two-factor ANOVA, determines how a response is affected by two factors. Two-way ANOVA simultaneously asks three questions:

1. Does the first factor systematically affect the results?
2. Does the second factor systematically affect the results?
3. Does the two factors interact?

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 skew ness 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 bock 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 $T_{.j} = \sum_{i=1}^{k} x_{ij}$

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

Blocks	Sample values Treatments 1	2	3	…	k	Sample size	Total	mean
i.	x_{11}	x_{12}	x_{13}	…	x_{1k}	k	$T_{.1}$	$\overline{x_{1\cdot}}$
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	$T_{.3}$	$\overline{x_{3\cdot}}$
.	.	.	.	…		.	.	.
.	.	..	.	…		.	.	.
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	$\overline{x}_{.1}$	$\overline{x}_{.2}$	$\overline{x}_{.3}$	…	$\overline{x}_{.k}$			$\overline{x}..$

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

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

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

The mean of the jth treatment $= \dfrac{\text{total of the } j\text{th block}}{\text{sample size}} = \dfrac{T_{\cdot j}}{k} = \dfrac{\sum_j x_{ij}}{k}$

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 ($SS_{treatments}$)
+ sum of squares
of residual ($SS_{residual}$)

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

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

$\sum_{i=1}^{n} \sum_{j=1}^{k} (\overline{x_{i.}} - \overline{x_{..}})^2$ = block SS ;

$\sum_{i=1}^{n} \sum_{j=1}^{k} ((x_{ij} - \overline{x_{i.}} - \overline{x_{.j}} - \overline{x_{..}})^2$ = residual SS.

The computation formula when simplified are :

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

$$(SS_{treatments}) = \sum_{i=1}^{n} \sum_{j=1}^{k} (\overline{x_{.j}} - \overline{x_{.i}})^2 = \sum_{i=1}^{n} \sum_{j=1}^{k} x^2_{.j} - C = \sum_{j=1}^{k} \frac{T_{.j}^2}{n} - C$$

$$(SS_{blocks}) = \sum_{i=1}^{n} \sum_{j=1}^{k} (\overline{x_{i.}} - \overline{x_{..}})^2 = \sum_{i=1}^{n} \sum_{j=1}^{k} x^2_{i} - C = \sum_{i=1}^{k} \frac{T_{i.}^2}{k} - C$$

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

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

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

Total = N-1 = kn – 1

Blocks = 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)			

F_1 = Variance ratio for blocks with df of (n-1) Vs (n-1) (k-1)
F_2 = 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).

Chapter 4
Results

4.1 To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Populus deltoides* and *Toona ciliata*

4.1.1 Physico-chemical characterization of soil, fly ash, distillery waste and farmyard manure

The texture analysis revealed that the experimental soil had sandy loam texture having 64.0% sand, 17.0% silt and 18.4% clay respectively, and fly ash had loamy sand texture (Table 2a). Distillery waste showed highest water holding capacity (117.52%) followed by farmyard manure (74.38%), fly ash (65.50%) and soil showed the least water holding capacity of 34.68%, whereas, the same experimental soil showed the maximum bulk density of 1.19 g $cm^{-3,}$ followed by fly ash and distillery waste (0.78 cm^{-3}) and farmyard manure (0.41 cm^{-3}) (Table 2a).

Table 2a: Physical characterization of soil, fly ash, distillery waste and farmyard manure

Physical Properties	**Soil**	**Fly ash**	**Distillery waste**	**Farmyard manure**
Water holding capacity (%)	34.68	65.50	117.52	74.38
Bulk density (g cm^{-3})	1.19	0.78	0.78	0.41
Particle size distribution				
	Sand	Silt	Clay	Texture
Soil	64.0	17.0	18.4	Sandy loam
Fly ash	87.5	5.5	7.0	Loamy sand

The selected physical and chemical characteristics of sandy loam soil, fly ash, distillery waste and farmyard manure showed that pH of soil, distillery waste and farm yard manure are alkaline ranging from 8.68 to 8.89, whereas, fly ash had an acidic pH of 6.09.

Electrical conductivity was maximum in case of distillery waste, i.e., 16 mS/cm followed by farmyard manure (2.40 mS/cm), fly ash (0.16 mS/cm) and soil (0.10 mS/cm).

4.1.1.1 Major Elements

Total organic carbon was found to be maximum in farmyard manure, i.e., 8.72%, whereas, distillery waste had maximum amount of total and available nitrogen (2.180 and 0.48%), total and available phosphorus (2577.79 and 594.46 mg/kg) and total and available potassium (12862.0 and 7691.00 mg/kg) followed by farmyard manure and fly ash respectively (Table 2b).

4.1.1.2 Secondary Elements

Total and available S was maximum in distillery waste, i.e., 21152.55 and 7619.36 mg/kg respectively, followed by soil and farmyard manure and had its minimum concentration in fly ash. Ca in both total and available forms had its highest concentration in distillery waste (33725.33 and 4943.0 mg/kg respectively). Total and available forms of Mg were also found to be present in maximum concentration in distillery waste followed by farmyard manure. The same trend had been observed for Na. Total and available Na had its maximum concentration (1123.0 and 819.33 mg/kg) in distillery waste (Table 2b).

4.1.1.3 Micronutrients

Total Cu was ranging from 1.53 mg/kg in fly ash to 90.32 mg/kg in distillery waste; total Zn was found to be ranging from 38.0 mg/kg in soil to 534.33 mg/kg in distillery waste; total Mn was ranging from 21.05 mg/kg in fly ash to 125.38 mg/kg in soil, whereas, total Fe was ranging from 2227.33 mg/kg to 11894.33 mg/kg in soil. Both total Cu and Zn had their maximum concentration in distillery waste, whereas, soil possessed maximum concentration of total Mn and Fe. Available form of Cu, Zn and Fe was below detection limit in soil (Table 2b).

4.1.1.4 Heavy Metals

The results showed that the concentration of lead in both total and available forms was maximum in distillery waste (54.78 and 15.08 mg/kg) followed by farmyard manure and fly ash. The concentration of chromium was maximum in that of soil (6.92 mg/kg)

followed by fly ash, farmyard manure and distillery waste, also, chromium was below detectable limit in available form in all the test components. Cadmium had its highest concentration in total form in soil (2.65 mg/kg), followed by distillery waste (2.52 mg/kg), fly ash (1.12 mg/kg) and farmyard manure (0.80 mg/kg), whereas, Cd was maximally available in distillery waste followed by farmyard manure, soil and fly ash. Both Ni and Co in total and available forms had their highest concentration in distillery waste (15.67 and 10.08 mg/kg) and (8.15 and 4.78 mg/kg) respectively (Table 2b).

4.1.2 Bacterial enumeration of soil, fly ash, distillery waste and farmyard manure

Microbiological characterization revealed that farmyard manure had maximum bacterial count (3.2×10^8 cfu gm^{-1}) followed by soil (3.1×10^6 cfu gm^{-1}), distillery waste (2.6×10^4 cfu gm^{-1}) and fly ash which did not possess any kind of bacterial microflora. Upon enumeration in Jensen's media and Pikovskaya's media for the count of nitrogen fixing and phosphorus solubilizing bacteria, it was found that the same trend followed as on nutrient agar, i.e., farmyard manure had maximum population followed by soil, distillery waste and fly ash. Microbial biomass showed the following trend: farmyard manure (276 $\mu g\ g^{-1}$) > distillery waste (208 $\mu g\ g^{-1}$) > soil (172 $\mu g\ g^{-1}$) > fly ash (nil) (Table 2c).

4.1.3 Nursery Trial

The soil was mixed with different amending materials to make twelve treatments as shown below for *Populus deltoides* and *Toona ciliata*

T1- Soil (Control)	T2- Soil + MA
T3- Soil + FA	T4- Soil + FA + MA
T5- Soil + FA + DW	T6- Soil + FA + DW + MA
T7- Soil + FA + FYM	T8- Soil + FA + FYM + MA
T9- Soil + DW	T10- Soil + DW + MA
T11- Soil + FYM	T12- Soil + FYM + MA

(MA: Microbial amendment (*P. striata* and *Azotobacter sp.*) @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Characterization of nursery soil in treatments (T1 to T12) before and after nursery trial of *P. deltoides* and *T. ciliata was carried out.*

Table 2b: Chemical characterization of soil, fly ash, distillery waste and farmyard manure

Chemical Properties		Soil	Fly ash	Distillery waste	Farmyard manure
pH		8.75 ± 0.24	6.09 ± 0.07	8.68 ± 0.03	8.89 ± 0.01
EC (mS/cm)		0.10 ± 0.00	0.16 ± 0.00	16.00 ± 0.09	2.40 ± 0.00
TOC (%)		0.10 ± 0.01	0.05 ± 0.01	8.17 ± 0.09	8.72 ± 0.12
Major Elements (mg/kg)					
Phosphorus	T	111.77 ± 5.36	238.73 ± 4.50	2577.79 ± 27.49	594.46 ± 20.89
	Ava	1.95 ± 0.04	17.36 ± 0.81	594.46 ± 20.89	276.00 ± 1.44
Nitrogen (%)	T	0.026 ± 0.00	0.014 ± 0.00	2.180 ± 0.017	2.043 ± 0.01
	Ava	0.006 ± 0.00	0.005 ± 0.00	0.484 ± 0.00	0.454 ± 0.00
Potassium	T	1490.3 ± 139.49	209.0 ± 12.29	12862.0±256.02	5577.0 ±103.95
	Ava	10.67 ± 5.67	70.67 ± 8.84	7691.0 ± 27.30	4131.0 ± 70.77
Secondary Elements (mg/kg)					
Sulphur	T	4435.48±352.57	162.41 ± 20.19	21152.55±330.53	3052.20±121.29
	Ava	48.39 ± 3.70	288.71 ± 3.52	7619.36 ± 12.04	1186.29 ± 62.97
Calcium	T	2755.67 ± 36.45	1136.33 ± 8.69	33725.33 ± 13.33	6360.67±178.69
	Ava	1883 ± 40.28	801.33±110.70	4943.0 ± 23.71	2541.67±178.48
Magnesium	T	4689.0 ± 76.07	520.67 ± 30.33	8182.33 ± 188.98	5668.33 ± 55.18
	Ava	285.67 ± 12.20	107.67 ± 12.00	1490.33 ± 14.40	682.33 ± 9.06
Sodium	T	332.33 ± 4.84	435.0 ± 31.14	1123.0 ± 19.50	826.0 ± 5.51
	Ava	265.67 ± 4.81	335.0 ± 23.71	819.33 ± 8.88	625.0 ± 7.21
Micronutrients (mg/kg)					
Copper	T	6.02 ± 0.31	1.53 ± 0.07	90.32 ± 0.70	21.62 ± 0.41
	Ava	BDL	0.22 ± 0.04	15.88 ± 0.16	3.17 ± 0.02
Zinc	T	38.0 ± 3.51	59.67 ± 0.88	534.33 ± 7.97	139.33 ± 8.11
	Ava	BDL	41.33 ± 3.38	183.0 ± 8.72	30.33 ± 1.20
Manganese	T	125.38 ± 4.69	21.05 ± 0.81	114.33 ± 4.89	80.17 ± 0.21
	Ava	1.90 ± 0.06	1.60 ± 0.10	8.18 ± 0.32	7.60 ± 0.20
Iron	T	11894.33±53.76	2227.33±86.04	8195.67 ± 51.75	4712.67 ± 78.50
	Ava	BDL	BDL	384.0 ± 15.13	BDL
Heavy Metals (mg/kg)					
Lead	T	13.15 ± 0.84	15.67 ± 0.30	54.78 ± 1.09	51.68 ± .72
	Ava	10.27 ± .26	5.28 ± 0.19	15.08 ± 0.99	13.57 ± .94
Chromium	T	2.92 ± 0.28	5.42 ± 0.06	1.32 ± 0.02	4.17 ± 0.20
	Ava	BDL	BDL	BDL	BDL
Cadmium	T	2.65 ± 0.06	1.12 ± 0.10	2.52 ± 0.15	0.80 ± 0.10
	Ava	0.12 ± 0.02	0.05 ± 0.03	0.47 ± 0.12	0.30 ± 0.03
Nickel	T	14.97 ± 0.25	8.10 ± 0.23	15.67 ± 0.66	9.80 ± 0.52
	Ava	2.57 ± 0.19	3.82 ± 0.31	8.15 ± 0.88	5.20 ± 0.23
Cobalt	T	5.23 ± 0.08	5.98 ± 0.24	10.08 ± 0.33	7.02 ± 0.21
	Ava	2.17 ± 0.06	3.60 ± 0.19	4.78 ± 0.04	3.12 ± 0.14

Values are Mean ± S.E. (n=3); T: total; Ava: Available; BDL: Below Detection Limit

Table 2c: Microbiological characterization of soil, fly ash, distillery waste and farmyard manure

Microbiological Properties	Soil	Fly ash	Distillery waste	Farmyard manure
Microbial enumeration (Nutrient agar) Cfu gm^{-1}	3.1×10^6	Nil	2.6×10^4	3.2×10^8
Microbial enumeration (Jensen's medium) Cfu gm^{-1}	4.2×10^4	Nil	3.4×10^2	5.0×10^5
Microbial enumeration (Pikovskaya's medium) Cfu gm^{-1}	2.0×10^3	Nil	2.6×10^2	2.8×10^4
Microbial biomass (µg g^{-1})	172	Nil	208	276

4.1.3.1 Percentage Survival

The percentage survival of *P. deltoides* was found to be around 75% and for *T. ciliata* it was around 25% in control soil. For both the tree species survival was maximum in treatments T8 (soil amended with fly ash and farm yard manure, co-inoculated with microbial consortium in T8), i.e., for *P. deltoides* it was 93.75% and for *T. ciliata* it was 83.33% respectively (Table 2d and 2e). Both the plants showed least survival in treatments amended with distillery waste T5, T6, T9 and T10.

In case of *P. deltoides* survival was maximum in treatment T8 followed by T7, treatments T4, T7, T11 and T12 had the same effect on the survival of the plant, which showed that farmyard manure and fly ash led to better survival of the plant. The addition of microbial consortium also had a marked effect on the survival of plants by improving survival rate by 6% (Table 2d).

In case of *T. ciliata* survival was maximum in treatments T7, T8 and T12 followed by treatment T4, which showed that farmyard manure and fly ash had marked positive effects on the survival of the plant (Table 2e).

Table 2d: Percentage survival of *P. deltoides* in nursery trial after a period of 180 days in 16 initial replicates

Treatments	Final survival (no. of plants)	Percentage survival
T1 (CS)	12	75.0
T2 (S+MA)	13	81.3
T3 (S+FA)	13	81.3
T4 (S+FA+MA)	14	87.5
T5 (S+FA+DW)	09	56.3
T6 (S+FA+DW+MA)	10	62.5
T7 (S+FA+FYM)	14	87.5
T8 (S+FA+FYM+MA)	15	93.8
T9 (S+DW)	08	50.0
T10(S+DW+MA)	11	68.8
T11(S+FYM)	14	87.5
T12(S+FYM+MA)	14	87.5

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 2e: Percentage survival of *T. ciliata* in nursery trial after a period of 180 days in 12 initial replicates

Treatments	Final survival (no. of plants)	Percentage survival
T1 (CS)	3	25.0
T2 (S+MA)	4	33.3
T3 (S+FA)	6	50.0
T4 (S+FA+MA)	9	75.0
T5 (S+FA+DW)	6	50.0
T6 (S+FA+DW+MA)	5	41.7
T7 (S+FA+FYM)	10	83.3
T8 (S+FA+FYM+MA)	10	83.3
T9 (S+DW)	6	50.0
T10(S+DW+MA)	8	66.7
T11(S+FYM)	8	66.7
T12(S+FYM+MA)	10	83.3

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.1.3.2 Biometric Parameters

4.1.3.2a Plant height

At day 180, the plant height of *P. deltoides* was maximum in treatment T8 (138.67 cm) amended with fly ash, farmyard manure and microbial consortium. It was significantly 55.05% more as compared to control (Table 2f). The treatments having distillery waste as an amendment significantly decreased the height of *P. deltoides*. Treatments T4, T7, T11 and T12 significantly increased the height of plant as compared to the control (T1) treatment, yet it was lesser than the height of plant in treatment T8 (Fig. 1a).

For *Toona ciliata* the plant height was maximum in treatment T8 (45.0 cm), whereas, treatments T5 and T9 significantly decreased the plant height which were comparable to the T1 (control) treatment (Table 2g and Fig. 1b). All the other treatments increased the height of plant comparable to each other but were not statistically significant. Treatment T8 was the only treatment that showed significant impact on the height of plant of both the agroforestry tree species.

Fig. 1a: Height of *Populus deltoides* in various soil amendments over a period of 180 days

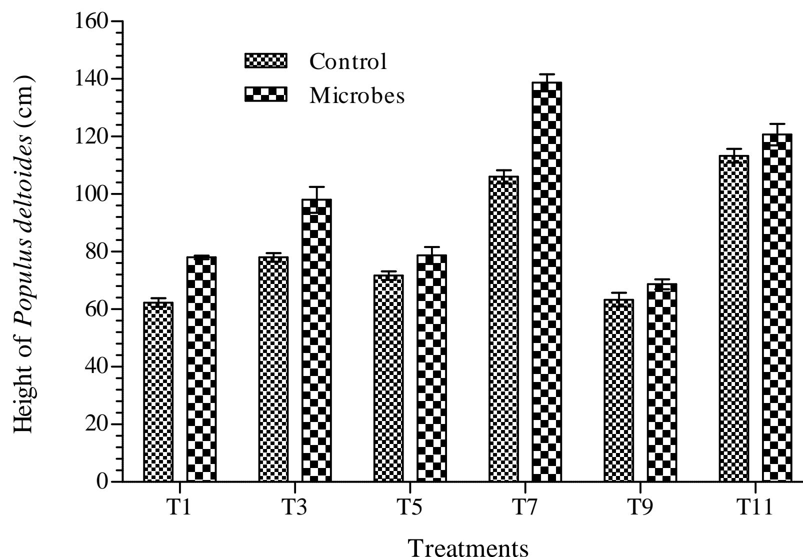
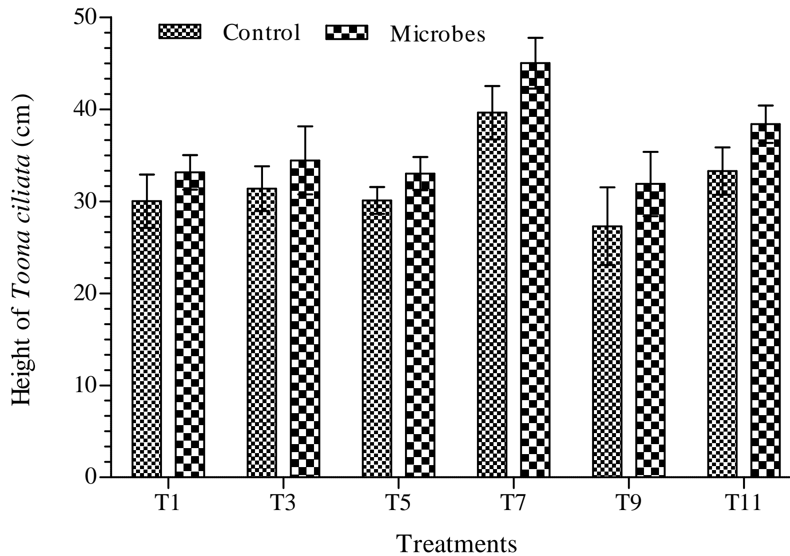


Fig. 1b: Height of *Toona ciliata* in various soil amendments over a period of 180 days



4.1.3.2b Collar diameter

At day 180, *P. deltoides* in treatment T8 had the maximum collar diameter (1.13 cm) followed by treatment T12 (0.97 cm), leading to significant increase (46.90%) in the collar diameter of the plant followed by treatments T7 and T11 which were not statistically significant but were comparable to each other. All the rest of the treatments had decreased the collar diameter of the tree species comparable to (T1) control (Table 2f and Fig. 1c).

The collar diameter of *T. ciliata* was maximum in treatment T8 (45.0 cm), followed by treatments T7 (39.62 cm), T4 (34.44 cm) and T2 (33.12 cm), respectively, but this increase in collar diameter was not statistically significant (20.63%) (Table 2g), the increase in these four treatments was comparable to each other. Collar diameter of *T. ciliata* decreased significantly in treatments T10 and T9, respectively, having distillery waste as one of the soil ameliorant (Fig. 1d).

Fig. 1c: Collar diameter of *Populus deltoides* in various soil amendments over a period of 180 days

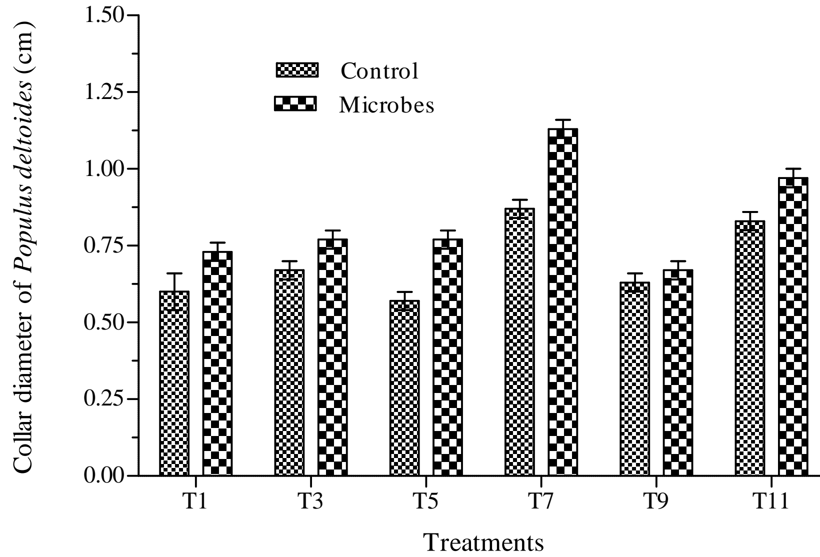
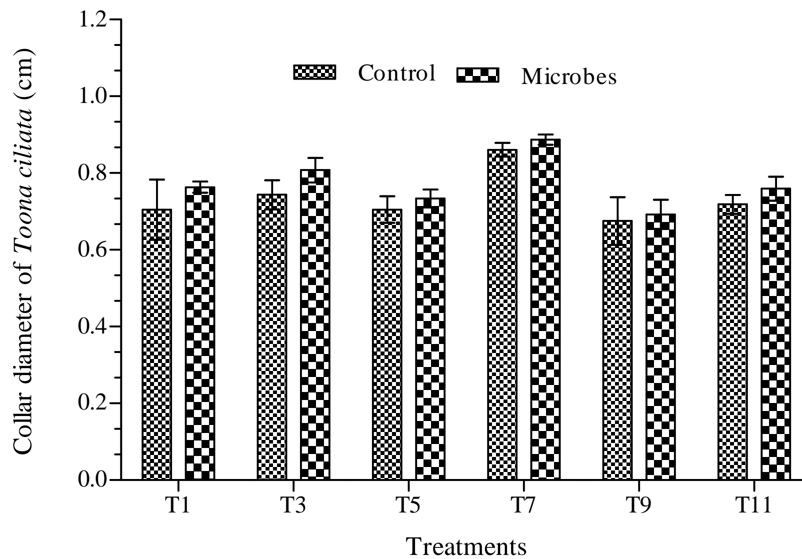


Fig. 1d: Collar diameter of *Toona ciliata* in various soil amendments over a period of 180 days



4.1.3.2c Total dry biomass

After harvesting at day 180, total dry biomass of *P. deltoides* was maximum in treatment T8 (62.49 g) and the increase was (72.21%) as comparable to control (T1), which was statistically significant (Table 2f), whereas, treatment T9 significantly decreased the total dry biomass of the plant, comparable to T1 (control soil). Treatments T2–T6, T10 and T12 increased the dry biomass as compared to T1 and T9, but were statistically insignificant (Fig. 1e).

The total dry biomass of *T. ciliata* was highest in treatment T8 (8.46 g) followed by T3 (7.51 g) and T7 (7.49 g), respectively, but the increase was not statistically significant (Table 2g), although it was significantly more than T1, T5, T9 and T10 treatments (Fig. 1f). The treatments T2, T4, T6 and T12 having microbial consortium as one of the soil ameliorant increased the production of total dry biomass, but the increase was not statistically significant.

Fig. 1e: Total dry biomass of *Populus deltoides* in various soil amendments over a period of 180 days

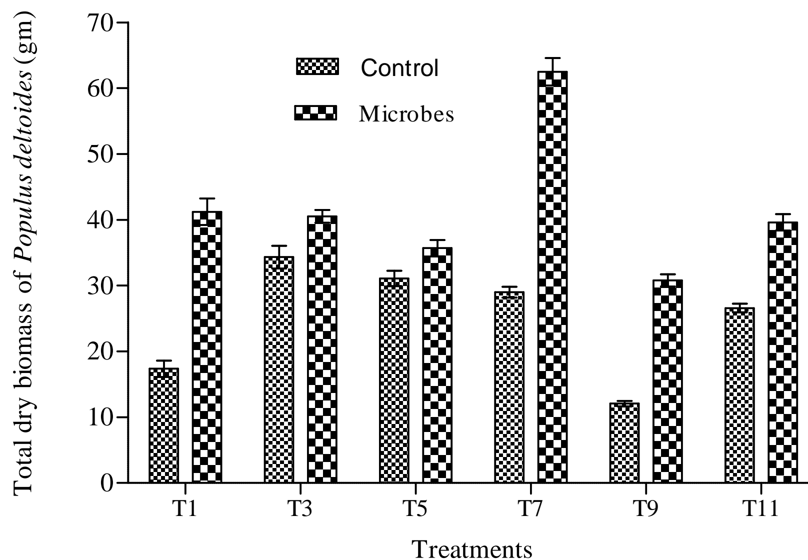


Fig. 1f: Total dry biomass of *Toona ciliata* in various soil amendments over a period of 180 days

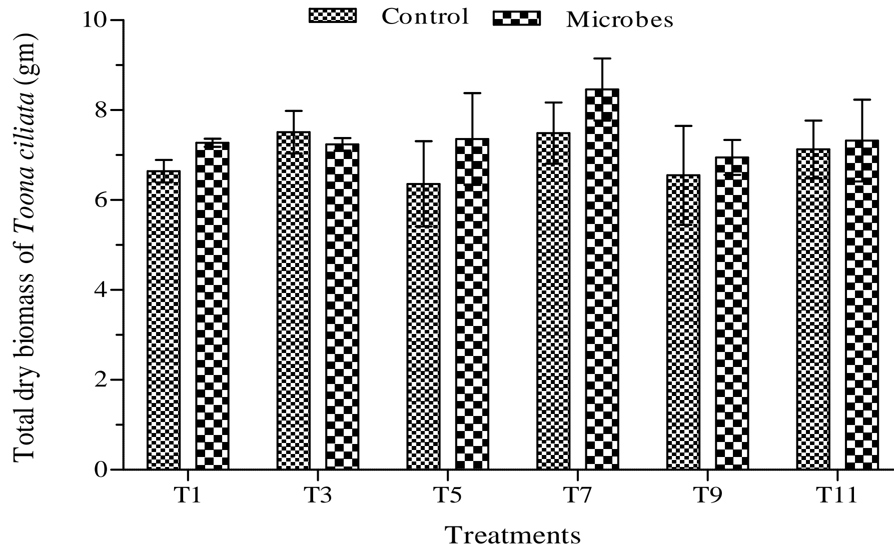


Table 2f: Plant height (cm), collar diameter (cm) and total dry biomass (gm) of *Populus deltoides* grown in twelve different treatments over a period of 180 days

Treatments		Plant height (cm)	Collar diameter (cm)	Total dry biomass (gm)
T1	CS	62.33 b	0.60 b	17.36 de
T2	S + MA	78.00 b	0.73 b	41.21 abcde
T3	S + FA	78.00 b	0.67 b	34.33 abcde
T4	S + FA + MA	98.00 ab	0.77 b	40.51 abcde
T5	S + FA + DW	71.67 b	0.57 b	31.07 abcde
T6	S + FA + DW + MA	78.67 b	0.77 b	35.69 abcde
T7	S + FA + FYM	106.00 ab	0.87 ab	28.98 bcde
T8	S + FA + FYM + MA	138.67 a	1.13 a	62.49 a
T9	S + DW	63.33 b	0.63 b	12.06 e
T10	S + DW + MA	68.67 b	0.67 b	30.81 abcde
T11	S + FYM	113.33 ab	0.83 ab	26.59 cde
T12	S + FYM + MA	120.67 ab	0.97 a	39.61 abcde

Mean values followed by the same letter within a column are not statistically different
(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 2g: Plant height (cm), collar diameter (cm) and total dry biomass (gm) of *Toona ciliata* grown in twelve different treatments over a period of 180 days

Treatments		Plant height (cm)		Collar diameter (cm)		Total dry biomass (gm)	
T1	CS	30.00	b	0.70	ab	6.64	b
T2	S + MA	33.13	ab	0.76	a	7.27	ab
T3	S + FA	31.38	ab	0.74	ab	7.51	a
T4	S + FA + MA	34.44	ab	0.81	a	7.24	ab
T5	S + FA + DW	30.08	b	0.71	ab	6.36	b
T6	S + FA + DW + MA	33.00	ab	0.73	ab	7.36	ab
T7	S + FA + FYM	39.63	ab	0.86	a	7.49	a
T8	S + FA + FYM + MA	45.00	a	0.89	a	8.46	a
T9	S + DW	27.29	b	0.68	b	6.55	b
T10	S + DW + MA	31.88	ab	0.69	b	6.95	b
T11	S + FYM	33.28	ab	0.72	ab	7.13	ab
T12	S + FYM + MA	38.38	ab	0.76	ab	7.32	ab

Mean values followed by the same letter within a column are not statistically different
(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

The comparative differences of plant height, collar diameter and total dry biomass between control soil, different soil amendments un-inoculated and inoculated with microbial consortium over a period of 180 days showed that the microbial consortium had a significant impact on the plant height (Fig 1a), collar diameter (1c) and total dry biomass (1e) of *P. deltoides*. As compared to the plants in un-inoculated controls the plants in microbial consortium inoculated treatments had comparatively higher values of all the biometric parameters. The combined effect of fly ash, farmyard manure and microbial consortium had more pronounced impact on the growth of the plant.

The inoculation of microbial consortium to the nursery seedlings of *Toona ciliata* did not show significant impact on collar diameter and total dry biomass of the plant (Fig. 1d and 1f), whereas, showed significant impact on the plant height (Fig 1d). The impact on height was more pronounced in treatment T8 (33.33% increase), all others were comparable to each other, whereas, in case of collar diameter and total dry biomass all the inoculated and un-inoculated treatments had shown almost comparable impact.

4.2 To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves.

To study the effect of interaction between beneficial soil microbes and solid waste on plant growth parameters in nursery trials revealed that the amendment with fly ash and farmyard manure had a significant impact on total dry biomass, collar diameter and height of the plant with a P value < 0.0001, and F values were 77.17, 38.29 and 172.2 respectively in *P. deltoides*. The addition of microbial consortium to different treatments also showed a significant impact on total dry biomass and collar diameter (P value $<$ 0.0001), whereas, the impact on height was non-significant with a P value of 1.0 (Table 3a). The interaction between fly ash and microbial consortium; farmyard manure and microbial consortium and between fly ash, farmyard manure and microbial consortium was also significant and latter had significant impact on all the biometric parameters with P value < 0.0001 for total dry biomass and stem height and P value $= 0.0496$ for collar diameter. The percentage of variance for interaction between fly ash, farmyard manure and microbial consortium for total dry biomass was 16.58%, for collar diameter it was 4.61% and for stem height it was 4.70% (Table 3a). The interaction between distillery waste and microbial consortium was non significant.

Two-way ANOVA for *T. ciliata* showed that the addition of fly ash and farmyard manure had significant impact only on collar diameter and stem height with a P value of <0.0001 and 0.0032 respectively, it did not have a significant impact on total dry biomass of the plant. The addition of distillery waste had non significant impact on the biometric parameters. The F value for total dry biomass, collar diameter and stem height was 0.6073, 6.772 and 3.952 respectively. The addition of microbial consortium had significant impact only on the stem height of the plant and showed non significant impact on the rest of the biometric parameters, viz. total dry biomass and collar diameter (Table 3b). The interaction between fly ash and microbial consortium; farmyard manure and microbial consortium was non significant and the interaction between fly ash, farmyard manure and microbial consortium was also non significant and the percentage of variance of interaction was 1.17% for total dry biomass, 0.63% for collar diameter and 0.30% for stem height (Table 3b).

Table 3a: Analysis of variance of biometric parameters of *Populus deltoides*

Biometric Parameters	Soil Amendment								
	Fly ash and Farmyard manure			Microbial consortium			Interaction		
	P	F	% var	P	F	% var	P	F	% var
Total dry biomass (gm)	<0.0001 s	77.17	35.82	<0.0001 s	488.7	45.37	<0.0001 s	35.71	16.58
Collar diameter (cm)	<0.0001 s	38.29	67.26	<0.0001 s	56.07	19.70	0.0496 s	2.627	4.61
Stem height (cm)	<0.0001 s	172.2	85.19	1.000 ns	78.19	7.74	<0.0001 s	9.501	4.70

P- P value; F- F value; % var- % variance; s- significant; ns- non significant

Table 3b: Analysis of variance of biometric parameters of *Toona ciliata*

Biometric Parameters	Soil Amendment								
	Fly ash and Farmyard manure			Microbial consortium			Interaction		
	P	F	% var	P	F	% var	P	F	% var
Total dry biomass (gm)	0.6945 ns	0.6073	4.00	0.3041 ns	1.072	1.41	0.9698 ns	0.1786	1.17
Collar diameter (cm)	<0.0001 s	6.772	31.01	0.0602 ns	3.647	3.34	0.9829 ns	0.1381	0.63
Stem height (cm)	0.0032 s	3.952	20.24	0.358 s	4.574	4.68	0.9977 ns	0.0585	0.30

P- P value; F- F value; % var- % variance; s- significant; ns- non significant

4.2.1 Physico-chemical characterization of soil in various treatments on day zero of nursery trial

The addition of microbial consortium on day zero did not have any effect on the physico-chemical characteristics of the various treatments, except for the microbiological characterization of various treatments.

The results showed that pH is highly alkaline in all the treatments ranging from 8.43 (T3) – 8.75 (T1). Electrical conductivity was highest in the treatments amended with distillery waste, i.e., treatments T9, T10 (2.62 mS/cm) followed by treatments T5, T6 (2.60 mS/cm). It was least in treatments T1 and T2 (control soil). Water holding capacity was maximum in treatments T9 and T10 (45.71%) followed by T5 and T6 (45.11%), T7 and T8 (44.55%) and it was minimum for control soil (34.68%). The bulk density was maximum for treatments T9 and T10 (1.41 gm/cm^3) and was least for treatments T7 and T8 (1.05 gm/cm^3). The texture analysis revealed that treatments T1 and T2 had sandy loam texture, whereas, treatments T3, T4, T7 and T8 (amended with fly ash) had loamy sand texture, and treatments T5, T6, T9, T10, T11 and T12 had clay loam texture (Table 3c).

Table 3c: Physico-chemical characterization of soil in different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	**pH**	**EC (mS/cm)**	**WHC (%)**	**Bulk Density (gm/cm^3)**	**Texture**
T1 (CS)	8.75 ± 0.24	0.10 ± 0.01	34.7	1.19	Sandy loam
T2 (S+MA)	8.75 ± 0.24	0.10 ± 0.01	34.7	1.19	Sandy loam
T3 (S+FA)	8.66 ± 0.16	0.11 ± 0.01	37.4	1.15	Loamy sand
T4 (S+FA+MA)	8.66 ± 0.16	0.11 ± 0.01	37.4	1.15	Loamy sand
T5 (S+FA+DW)	8.43 ± 0.07	2.60 ± 0.01	45.1	1.12	Clay loam
T6 (S+FA+DW+MA)	8.43 ± 0.07	2.60 ± 0.01	45.1	1.12	Clay loam
T7 (S+FA+FYM)	8.55 ± 0.06	0.32 ± 0.01	44.6	1.05	Loamy sand
T8 (S+FA+FYM+MA)	8.55 ± 0.06	0.32 ± 0.01	44.6	1.05	Loamy sand
T9 (S+DW)	8.50 ± 0.05	2.62 ± 0.02	45.7	1.41	Clay loam
T10(S+DW+MA)	8.50 ± 0.05	2.62 ± 0.02	45.7	1.41	Clay loam
T11(S+FYM)	8.60 ± 0.04	0.33 ± 0.01	44.0	1.06	Clay loam
T12(S+FYM+MA)	8.60 ± 0.04	0.33 ± 0.01	44.0	1.06	Clay loam

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.1.1 Total Organic Carbon

Total organic carbon in all the treatments was ranging from 0.10% (T1) to 0.89% (T9 and T10), the maximum being in treatment amended with distillery waste, followed by treatments T5 and T6 (0.82%) and T3, T4, T11 and T12 (0.63%) amended with farmyard manure (Table 3d), the treatments having fly ash as an ameliorant had less organic carbon content.

4.2.1.2 Major Elements

Total and available nitrogen was also present in the maximum amount in treatment T9 and T10 (0.222 % and 0.049% respectively), followed by T5 and T6 (0.203% and 0.045%) , treatments T7, T8, T11 and T12 had same total and available nitrogen content, i.e., 0.156% and 0.034% respectively.

Total phosphorus in all the treatments was ranging from 111.77 mg/kg (T1) to 806.21 mg/kg (T5), treatments amended with distillery waste had high total P content, treatment T9 had 612.48 mg/kg total P, treatment T7 had 412.79 mg/kg, T11 had 220.26 mg/kg and T3 had 164.22 mg/kg of total P. Same was the trend with available P in all the treatments, i.e., control soil had least available P (1.95 mg/kg), followed by treatment T3 (2.88 mg/kg), treatment T11 (65.42 mg/kg), treatment T7 amended with fly ash and farmyard manure (69.47 mg/kg) and treatment T9 (202.45 mg/kg). It showed that both total and available phosphorus content was maximum in the treatments amended with distillery waste (Table 3d).

Total K was also found to be in highest concentration in the treatments amended with distillery waste, it was maximum in T9 – 3760.67 mg/kg, followed by T5 – 3300.0 mg/kg, T3 – 2396.0 mg/kg, T7 – 2367.33 mg/kg, T11 – 1890.0 mg/kg and least concentration was found in control soil (T1) – 1490.33 mg/kg. Available K followed slightly different pattern and was present in maximum concentration in treatment T5 – 3095.33 mg/kg, followed by T9 – 1899.67 mg/kg, T11 – 144.0, T7 – 74.33 mg/kg, T3 - 12.67 mg/kg (Table 3d) and least in control soil (T1) – 10.67 mg/kg. This indicates that most of the K that was present in soils amended with distillery waste was in the available form, whereas, in rest of the treatments it was present in the bound form.

4.2.1.3 Secondary Elements

The concentration of secondary elements, i.e., total and S, Ca, Mg and Na showed almost the same pattern of being having their maximum concentrations in the treatments amended with distillery waste and had their least concentrations in control soil (un-inoculated and inoculated with microbial consortium). Total S was ranging from 3435.48 mg/kg (T1) – 73.64.47 mg/kg, and available s was ranging from 48.39 mg/kg (T1) – 1716.13 mg/kg (T5). Total Ca showed its maximum concentration in treatment T9 (16134.33 mg/kg) and minimum concentration in treatment T1 (2755.67 mg/kg), whereas, available Ca was ranging from 1883.0 (T1) – 4112.0 (T5) (Table 3e). Total Mg was ranging from 3689.0 mg/kg in treatment T1 – 6824.0 mg/kg in treatment T5 and available Mg had its highest concentration in treatment T5 (980.0 mg/kg) and lowest concentration in treatment T1 (285.67 mg/kg). In case of total Na, its concentration was ranging from 332.33 mg/kg (T1) – 535.67 mg/kg (T9), and available Na had its maximum concentration in T9 (437.67 mg/kg) and minimum concentration in T3 (250.0 mg/kg) (Table 3e).

4.2.1.4 Micronutrients

Copper a micronutrient was ranging from 4.47 mg/kg (T3) – 14.07 mg/kg (T5), the availability of Cu was below detection limit in control soil (T1) and was maximum in T5 (2.20 mg/kg) (Table 3f). Total Zn was ranging from 35.0 mg/kg (T3) – 92.67 mg/kg (T5), whereas, available Zn was also below detection limit in T1 and was present in its maximum concentration in treatment T7 (43.33 mg/kg). Total Mn was present in its maximum concentration in treatment T1 (125.38 mg/kg), while the minimum concentration was 91.67 mg/kg (T5). The availability of Mn was minimum, i.e., 1.42 mg/kg in treatment T3 as compared to its maximum availability (8.92 mg/kg) in treatment T5. Total Fe was ranging from 10046.0 mg/kg (T3) – 11894.33 mg/kg (T1), whereas, available Fe was below detection limit in all the treatments except T5 (50.33 mg/kg) (Table 3i d).

4.2.1.5 Heavy Metals

Pb was ranging from 13.15 mg/kg (T1) - 35.32 mg/kg (T11), Cr had its maximum concentration in treatment T11 (8.70 mg/kg) and minimum concentration in treatment T3 (4.83 mg/kg), Cd was present in maximum amount 3.83 mg/kg in treatment T3 and minimum amount was found as 1.82 mg/kg in treatment T7. Ni was ranging from 12.58 mg/kg (T3) – 17.03 mg/kg (T11), whereas, Co had its maximum concentration (6.82 mg/kg) in treatment T9 and had its minimum concentration (4.27 mg/kg) in T5.

Similarly availability of Pb was ranging from 4.20 mg/kg (T5) – 18.45 mg/kg (T3), available Cr was below detection limit in all the 12 treatments. Available Cd was ranging from 0.03 mg/kg (T3 and T7) – 0.35 mg/kg (T5), available Ni had its maximum concentration 5.18 mg/kg in treatment T9 and minimum concentration 2.53 mg/kg in treatment T7, whereas, available Co was ranging from 0.77 mg/kg (T3) – 3.08 mg/kg (T7) (Table 3g).

Table 3d: Organic carbon and major nutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	**TOC (%)**	**Major Elements**					
		N (%)		**P (mg/kg)**		**K (mg/kg)**	
		Total	Available	Total	Available	Total	Available
T1 (CS)	0.10 ± 0.01	0.026 ± 0.001	0.006 ± 0.001	111.77 ± 5.36	1.95 ± 0.04	1490.33 ± 139.49	10.67 ± 5.67
T2 (S+MA)	0.10 ± 0.01	0.026 ± 0.001	0.006 ± 0.001	111.77 ± 5.36	1.95 ± 0.04	1490.33 ± 139.49	10.67 ± 5.67
T3 (S+FA)	0.14 ± 0.01	0.034 ± 0.001	0.008 ± 0.001	164.22 ± 7.82	2.88 ± 0.44	2396.0 ± 127.23	12.67 ± 2.40
T4 (S+FA+MA)	0.14 ± 0.01	0.034 ± 0.001	0.008 ± 0.001	164.22 ± 7.82	2.88 ± 0.44	2396.0 ± 127.23	12.67 ± 2.40
T5 (S+FA+DW)	0.82 ± 0.01	0.203 ± 0.001	0.045 ± 0.001	806.21 ± 19.16	203.88 ± 2.14	3300.0 ± 18.33	3095.33 ± 89.85
T6 (S+FA+DW+MA)	0.82 ± 0.01	0.203 ± 0.001	0.045 ± 0.001	806.21 ± 19.16	203.88 ± 2.14	3300.0 ± 18.33	3095.33 ± 89.85
T7 (S+FA+FYM)	0.63 ± 0.02	0.156 ± 0.003	0.034 ± 0.001	412.79 ± 13.07	69.47 ± 1.87	2367.33 ± 21.14	74.33 ± 13.93
T8 (S+FA+FYM+MA)	0.63 ± 0.02	0.156 ± 0.003	0.034 ± 0.001	412.79 ± 13.07	69.47 ± 1.87	2367.33 ± 21.14	74.33 ± 13.93
T9 (S+DW)	0.89 ± 0.03	0.222 ± 0.003	0.049 ± 0.001	612.48 ± 24.67	202.45 ± 2.71	3760.67 ± 112.88	1899.67 ± 46.82
T10(S+DW+MA)	0.89 ± 0.03	0.222 ± 0.003	0.049 ± 0.001	612.48 ± 24.67	202.45 ± 2.71	3760.67 ± 112.88	1899.67 ± 46.82
T11(S+FYM)	0.63 ± 0.004	0.156 ± 0.001	0.034 ± 0.001	220.26 ± 1.58	65.72 ± 0.70	1890.0 ± 31.38	144.0 ± 5.69
T12(S+FYM+MA)	0.63 ± 0.004	0.156 ± 0.001	0.034 ± 0.001	220.26 ± 1.58	65.72 ± 0.70	1890.0 ± 31.38	144.0 ± 5.69

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3e: Chemical analysis of secondary nutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	**Secondary Elements**							
	S (mg/kg)		**Ca (mg/kg)**		**Mg (mg/kg)**		**Na (mg/kg)**	
	Total	Available	Total	Available	Total	Available	Total	Available
T1 (CS)	3435.48 ± 352.57	48.39 ± 3.70	2755.67 ± 36.45	1883.0 ± 40.28	3689.0 ± 76.07	285.67 ± 12.20	332.33 ± 4.84	265.67 ± 4.81
T2 (S+MA)	3435.48 ± 352.57	48.39 ± 3.70	2755.67 ± 36.45	1883.0 ± 40.28	3689.0 ± 76.07	285.67 ± 12.20	332.33 ± 4.84	265.67 ± 4.81
T3 (S+FA)	3690.64 ± 82.50	57.26 ± 7.169	2833.33 ± 18.55	1014.0 ± 91.65	4309.67 ± 84.69	447.67 ± 59.88	336.0 ± 2.65	250.0 ± 7.09
T4 (S+FA+MA)	3690.64 ± 82.50	57.26 ± 7.169	2833.33 ± 18.55	1014.0 ± 91.65	4309.67 ± 84.69	447.67 ± 59.88	336.0 ± 2.65	250.0 ± 7.09
T5 (S+FA+DW)	6093.19 ± 213.70	1716.13 ± 32.38	15674.67 ± 201.53	4112.0 ± 103.93	6824.0 ± 33.86	980.0 ± 12.49	475.33 ± 7.22	360.33 ± 7.06
T6 (S+FA+DW+MA)	6093.19 ± 213.70	1716.13 ± 32.38	15674.67 ± 201.53	4112.0 ± 103.93	6824.0 ± 33.86	980.0 ± 12.49	475.33 ± 7.22	360.33 ± 7.06
T7 (S+FA+FYM)	5471.55 ± 84.75	223.39 ± 4.49	4530.67 ± 152.98	3500.0 ± 122.08	3830.0 ± 48.0	722.0 ± 24.17	534.33 ± 7.51	432.0 ± 6.66
T8 (S+FA+FYM+MA)	5471.55 ± 84.75	223.39 ± 4.49	4530.67 ± 152.98	3500.0 ± 122.08	3830.0 ± 48.0	722.0 ± 24.17	534.33 ± 7.51	432.0 ± 6.66
T9 (S+DW)	7364.47 ± 5.60	1668.55 ± 21.83	16134.33 ± 153.17	4045.33 ± 99.56	5113.67 ± 99.99	499.0 ± 11.93	535.67 ± 11.84	437.67 ± 6.44
T10(S+DW+MA)	7364.47 ± 5.60	1668.55 ± 21.83	16134.33 ± 153.17	4045.33 ± 99.56	5113.67 ± 99.99	499.0 ± 11.93	535.67 ± 11.84	437.67 ± 6.44
T11(S+FYM)	5667.56 ± 363.13	100.00 ± 0.81	2639.67 ± 2.186	2169.33 ± 117.95	4235.33 ± 166.12	627.67 ± 6.17	414.0 ± 6.11	320.33 ± 7.69
T12(S+FYM+MA)	5667.56 ± 363.13	100.00 ± 0.81	2639.67 ± 2.186	2169.33 ± 117.95	4235.33 ± 166.12	627.67 ± 6.17	414.0 ± 6.11	320.33 ± 7.69

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3f: Chemical analysis of micronutrients in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	**Micronutrients**							
	Cu (mg/kg)		**Zn (mg/kg)**		**Mn (mg/kg)**		**Fe (mg/kg)**	
	Total	Available	Total	Available	Total	Available	Total	Available
T1 (CS)	6.02 ± 0.31	BDL	38.0 ± 3.51	BDL	125.38 ± 4.69	1.90 ± 0.06	11894.33 ± 53.76	BDL
T2 (S+MA)	6.02 ± 0.31	BDL	38.0 ± 3.51	BDL	125.38 ± 4.69	1.90 ± 0.06	11894.33 ± 53.76	BDL
T3 (S+FA)	4.47 ± 0.09	0.20 ± 0.05	35.0 ± 2.65	BDL	107.92 ± 0.40	1.42 ± 0.24	10046.0 ± 96.42	BDL
T4 (S+FA+MA)	4.47 ± 0.09	0.20 ± 0.05	35.0 ± 2.65	BDL	107.92 ± 0.40	1.42 ± 0.24	10046.0 ± 96.42	BDL
T5 (S+FA+DW)	14.07 ± 0.49	2.80 ± 0.20	92.67 ± 7.22	23.33 ± 1.86	91.67 ± 1.36	8.92 ± 0.49	10561.33 ± 11.47	50.33 ± 4.48
T6 (S+FA+DW+MA)	14.07 ± 0.49	2.80 ± 0.20	92.67 ± 7.22	23.33 ± 1.86	91.67 ± 1.36	8.92 ± 0.49	10561.33 ± 11.47	50.33 ± 4.48
T7 (S+FA+FYM)	7.40 ± 0.25	0.52 ± 0.19	56.33 ± 3.76	43.33 ± 1.67	106.88 ± 1.07	1.88 ± 0.07	10756.67 ± 107.28	BDL
T8 (S+FA+FYM+MA)	7.40 ± 0.25	0.52 ± 0.19	56.33 ± 3.76	43.33 ± 1.67	106.88 ± 1.07	1.88 ± 0.07	10756.67 ± 107.28	BDL
T9 (S+DW)	13.40 ± 0.84	1.83 ± 0.15	85.67 ± 2.33	19.0 ± 1.53	109.35 ± 4.00	6.68 ± 0.11	10870.67 ± 116.10	BDL
T10(S+DW+MA)	13.40 ± 0.84	1.83 ± 0.15	85.67 ± 2.33	19.0 ± 1.53	109.35 ± 4.00	6.68 ± 0.11	10870.67 ± 116.10	BDL
T11(S+FYM)	8.00 ± 0.59	0.13 ± 0.02	79.33 ± 0.33	33.33 ± 3.18	122.02 ± 5.14	2.28 ± 0.17	11755.0 ± 136.95	BDL
T12(S+FYM+MA)	8.00 ± 0.59	0.13 ± 0.02	79.33 ± 0.33	33.33 ± 3.18	122.02 ± 5.14	2.28 ± 0.17	11755.0 ± 136.95	BDL

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3g(I): Chemical analysis of heavy metals in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	**Heavy Metals**					
	Pb (mg/kg)		**Cr (mg/kg)**		**Cd (mg/kg)**	
	Total	Available	Total	Available	Total	Available
T1 (CS)	13.15 ± 0.84	10.27 ± 0.26	6.92 ± 0.28	BDL	2.65 ± 0.06	0.12 ± 0.02
T2 (S+MA)	13.15 ± 0.84	10.27 ± 0.26	6.92 ± 0.28	BDL	2.65 ± 0.06	0.12 ± 0.02
T3 (S+FA)	23.08 ± 0.29	18.45 ± 0.49	4.83 ± 0.31	BDL	3.83 ± 0.07	0.03 ± 0.02
T4 (S+FA+MA)	23.08 ± 0.29	18.45 ± 0.49	4.83 ± 0.31	BDL	3.83 ± 0.07	0.03 ± 0.02
T5 (S+FA+DW)	32.92 ± 0.97	4.20 ± 0.65	6.07 ± 0.67	BDL	2.58 ± 0.08	0.35 ± 0.03
T6 (S+FA+DW+MA)	32.92 ± 0.97	4.20 ± 0.65	6.07 ± 0.67	BDL	2.58 ± 0.08	0.35 ± 0.03
T7 (S+FA+FYM)	14.05 ± 0.30	8.32 ± 1.36	5.30 ± 0.09	BDL	1.82 ± 0.07	0.03 ± 0.03
T8 (S+FA+FYM+MA)	14.05 ± 0.30	8.32 ± 1.36	5.30 ± 0.09	BDL	1.82 ± 0.07	0.03 ± 0.03
T9 (S+DW)	32.27 ± 0.60	14.87 ± 0.71	5.18 ± 0.22	BDL	2.08 ± 0.19	0.25 ± 0.03
T10(S+DW+MA)	32.27 ± 0.60	14.87 ± 0.71	5.18 ± 0.22	BDL	2.08 ± 0.19	0.25 ± 0.03
T11(S+FYM)	35.32 ± 0.39	15.45 ± 0.32	8.70 ± 0.05	BDL	2.03 ± 0.09	0.32 ± 0.02
T12(S+FYM+MA)	35.32 ± 0.39	15.45 ± 0.32	8.70 ± 0.05	BDL	2.03 ± 0.09	0.32 ± 0.02

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

contd..

Table 3g (II): Chemical analysis of heavy metals in soil of different treatments on day zero of nursery trial of *P. deltoides* and *T. ciliata*

Treatments	Heavy Metals			
	Ni (mg/kg)		Co (mg/kg)	
	Total	Available	Total	Available
T1 (CS)	14.97 ± 0.25	2.57 ± 0.19	5.23 ± 0.08	2.17 ± 0.06
T2 (S+MA)	14.97 ± 0.25	2.57 ± 0.19	5.23 ± 0.08	2.17 ± 0.06
T3 (S+FA)	12.58 ± 0.67	4.40 ± 0.14	4.75 ± 0.10	0.77 ± 0.12
T4 (S+FA+MA)	12.58 ± 0.67	4.40 ± 0.14	4.75 ± 0.10	0.77 ± 0.12
T5 (S+FA+DW)	14.17 ± 0.42	3.20 ± 0.50	4.27 ± 0.20	2.73 ± 0.20
T6 (S+FA+DW+MA)	14.17 ± 0.42	3.20 ± 0.50	4.27 ± 0.20	2.73 ± 0.20
T7 (S+FA+FYM)	15.92 ± 0.51	2.53 ± 0.27	5.57 ± 0.34	3.08 ± 0.10
T8 (S+FA+FYM+MA)	15.92 ± 0.51	2.53 ± 0.27	5.57 ± 0.34	3.08 ± 0.10
T9 (S+DW)	15.45 ± 0.24	5.18 ± 0.25	6.82 ± 0.14	2.45 ± 0.13
T10(S+DW+MA)	15.45 ± 0.24	5.18 ± 0.25	6.82 ± 0.14	2.45 ± 0.13
T11(S+FYM)	17.03 ± 0.43	4.07 ± 0.37	5.37 ± 0.32	2.55 ± 0.06
T12(S+FYM+MA)	17.03 ± 0.43	4.07 ± 0.37	5.37 ± 0.32	2.55 ± 0.06

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.2 Physico-chemical analysis of soil in various treatments on completion of nursery trial of P. deltoides (on day 180)

Analysis of soil after six months of nursery trial showed a marked variation in pH in different treatments (T1 – T12). pH was ranging from neutral to alkaline, i.e., 7.06 (T6) – 9.09 (T4) (Table 3h). Electrical conductivity was least in control soil (T1), i.e., 363.00 μS/cm and was maximum in treatment T10, i.e., 1079.33 μS/cm. Water holding capacity was minimum in treatment (T9) – 34.96 % and was maximum in treatment (T3) – 45.56% (Table 3h). It showed that distillery waste had a marked effect on pH and electrical conductivity of the soil, while addition of fly ash @10% (v/v) improved the water holding capacity of the soil. Bulk density was ranging from 1.03 gm/cm^3(T9) - 1.20 gm/cm^3 (T3).

Organic carbon was least in case of control soil (0.04 %), whereas, was maximum in treatment T6 and T10 (0.54 %) (Table 3i), due to the amendment with distillery waste as distillery waste is rich in organic carbon. Available N was ranging from 0.0058% in control soil to 0.1083% in treatment T7 (amended with FA and FYM). Available phosphorus had its minimum value in control soil (T1) – 40.43 mg/kg, whereas, available P was maximum in treatment T8 – 135.28mg/kg; available S was ranging from 30.65 mg/kg (T2) - 183.87 mg/kg (T10) (Table 3i).

Table 3h: pH, EC, WHC (%) and bulk density of soil in different treatments after six months of nursery trial of *P. deltoides*

Treatments	**Parameters Analysed (Soil)**			
	pH	**EC (μS/cm)**	**WHC (%)**	**Bulk density (gm/cm^3)**
T1 (CS)	8.37 ± 0.07	363.00 ± 1.15	37.8	1.10
T2 (S+MA)	8.88 ± 0.02	486.33 ± 7.22	34.9	1.04
T3 (S+FA)	8.73 ± 0.04	564.00 ± 14.01	45.6	1.26
T4 (S+FA+MA)	9.09 ± 0.02	596.67 ± 0.88	42.4	1.15
T5 (S+FA+DW)	7.82 ± 0.02	593.67 ± 1.45	38.1	1.18
T6 (S+FA+DW+MA)	7.06 ± 0.03	847.33 ± 7.22	37.3	1.12
T7 (S+FA+FYM)	9.02 ± 0.02	809.00 ± 5.57	39.1	1.20
T8 (S+FA+FYM+MA)	8.69 ± 0.02	674.00 ± 6.56	38.5	1.17
T9 (S+DW)	7.23 ± 0.03	1073.67 ± 12.78	35.0	1.03
T10(S+DW+MA)	7.96 ± 0.01	1079.33 ± 9.40	36.6	1.10
T11(S+FYM)	8.63 ± 0.01	728.33 ± 16.02	36.3	1.09
T12(S+FYM+MA)	8.67 ± 0.01	711.00 ± 8.39	38.9	1.18

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3i: Organic carbon, available phosphorus and available sulphur in different treatments after six months of nursery trial of *P. deltoides*

Treatments	Parameters Analysed (Soil)			
	Org. C (%)	**Ava N (%)**	**Ava P (mg/kg)**	**Ava S (mg/kg)**
T1 (CS)	0.04 ± 0.01	0.006 ± 0.00	40.43 ± 0.46	52.42 ± 12.04
T2 (S+MA)	0.06 ± 0.01	0.006 ± 0.00	54.06 ± 3.18	30.65 ± 5.82
T3 (S+FA)	0.04 ± 0.01	0.006 ± 0.00	46.33 ± 0.34	100.81 ± 1.61
T4 (S+FA+MA)	0.05 ± 0.00	0.007 ± 0.00	43.08 ± 1.85	137.10 ± 19.57
T5 (S+FA+DW)	0.53 ± 0.01	0.008 ± 0.00	42.79 ± 0.53	118.55 ± 38.58
T6 (S+FA+DW+MA)	0.54 ± 0.01	0.006 ± 0.00	48.91 ± 0.75	179.03 ± 8.72
T7 (S+FA+FYM)	0.36 ± 0.01	0.108 ± 0.00	130.20 ± 1.90	123.39 ± 20.29
T8 (S+FA+FYM+MA)	0.26 ± 0.01	0.008 ± 0.00	135.28 ± 1.25	104.84 ± 7.69
T9 (S+DW)	0.38 ± 0.01	0.007 ± 0.00	46.55 ± 1.86	114.52 ± 18.60
T10(S+DW+MA)	0.54 ± 0.01	0.011 ± 0.00	67.51 ± 1.55	183.87 ± 36.63
T11(S+FYM)	0.14 ± 0.00	0.008 ± 0.00	113.29 ± 0.62	66.13 ± 0.81
T12(S+FYM+MA)	0.21 ± 0.00	0.010 ± 0.00	121.26 ± 0.31	91.94 ± 18.90

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.3 Analysis of major, secondary and micronutrients in soil and uptake by root, stem and leaves of P. deltoides

Nutrition status

In soil: Major element total phosphorus was minimum in treatment T1 (97.46 mg/kg) and was maximum in treatment T6 – 617.25 mg/kg and total K was minimum in treatment T2 – 65.00 mg/kg and was maximum in treatment T5 – 942.50 mg/kg (Table 3j).

Secondary elements were as follows: Ca was ranging from 30.00 mg/kg (T1) – 1203.33 mg/kg (T7); Mg was ranging from 730.00 mg/kg (T2) – 3870.00 mg/kg (T12) (Table 3j); Na was having minimum value in treatment T9 – 15.83 mg/kg, whereas, maximum in treatment T5 – 48.83 mg/kg (Table 3j).

Micronutrients were as follows: Cu was ranging from 4.83 mg/kg (T4) – 9.52 mg/kg (T6); Zn was ranging from 1.77 mg/kg (T2) – 22.48 mg/kg (T12); Mn was having minimum value in treatment T2 – 40.72 mg/kg and was having maximum value in treatment T12 – 235.15 mg/kg; Fe was ranging from 1186.67 mg/kg (T2) – 5493.33 mg/kg (T12) (Table 3k).

In plants: Total P was minimum in roots of *Populus deltoides* (233.97 mg/kg) in treatment T1 and was maximum in leaves, i.e., 6280.10 mg/kg in treatment T9; K was ranging from 1335.00 mg/kg in roots (T2) – 17590.00 mg/kg in leaves (T12); Ca was minimum in root (1545.00 mg/kg) in treatment T6 and was maximum in leaves (6796.67 mg/kg) in treatment T4 and Mg was ranging from 1431.67 mg/kg in root (T4) – 8403.33 mg/kg in leaves (T1) (Table 3j). Na was ranging from 128.33 mg/kg in Stem (T5) – 3195.00 mg/kg in root (T11). It showed that P, K, Ca and Mg were having their maximum concentration in leaves of *P. deltoides* and Na had its maximum concentration in roots of the plant.

Cu was ranging from 2.52 mg/kg in stem (T4) – 28.47 mg/kg in leaves (T1); Zn was present in minimum concentration 8.65 mg/kg in root (T2), whereas, it was maximum in leaves (29.32 mg/kg) in treatment T9; Mn was ranging from 6.13 mg/kg in Stem (T4) – 195.90 mg/kg in leaves (T5) and Fe was minimum in stem (87.50 mg/kg) in T4 and was maximum in root (1432.50 mg/kg) in treatment T11 (Table 3k). It showed that Fe was having their maximum concentration in root, whereas, Mn, Cu and Zn were maximally present in leaves of *P. deltoides.*

Table 3j: Major and secondary elements (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial

Treatments		P	K	Ca	Mg	Na
T1 (CS)	Soil	97.46 ± 2.73	595.00 ± 51.50	30.00 ± 2.50	1737.50 ± 262.40	30.83 ± 3.63
	Root	233.97 ± 11.88	1546.67 ± 55.85	4729.17 ± 70.16	1440.00 ± 90.01	1668.33 ± 80.50
	Stem	390.14 ± 6.31	3277.50 ± 182.51	1925.00 ± 68.76	1908.33 ± 34.65	298.33 ± 21.62
	Leaves	979.67 ± 15.53	11706.67 ± 420.09	7595.00 ± 161.30	8403.33 ± 216.92	616.67 ± 18.39
T2 (S+MA)	Soil	112.36 ± 3.63	65.00 ± 13.77	117.50 ± 29.19	730.00 ± 81.86	32.50 ± 6.61
	Root	295.96 ± 6.77	1335.00 ± 115.50	4325.83 ± 34.83	1812.50 ± 83.12	1446.67 ± 24.25
	Stem	456.31 ± 13.32	2657.50 ± 39.87	1689.17 ± 131.87	1623.33 ± 35.28	209.17 ± 13.10
	Leaves	1098.30 ± 41.56	11442.50 ± 466.23	4306.67 ± 116.71	7497.50 ± 183.43	750.83 ± 33.11
T3 (S+FA)	Soil	127.86 ± 2.73	542.50 ± 43.37	41.67 ± 10.44	1580.83 ± 110.42	25.00 ± 2.89
	Root	340.67 ± 7.80	1725.00 ± 200.42	2971.67 ± 127.03	2288.33 ± 57.03	1324.17 ± 157.33
	Stem	545.72 ± 6.22	2733.33 ± 182.99	2103.33 ± 85.69	2095.00 ± 98.79	278.33 ± 38.63
	Leaves	2149.20 ± 67.64	10683.33 ± 247.20	6502.50 ± 71.46	8290.83 ± 247.02	513.33 ± 39.06
T4 (S+FA+ MA)	Soil	135.01 ± 3.10	204.17 ± 50.42	377.50 ± 11.27	1485.83 ± 164.77	33.33 ± 3.00
	Root	353.18 ± 6.45	1883.33 ± 106.03	2522.50 ± 32.24	1431.67 ± 74.67	1127.50 ± 60.68
	Stem	589.83 ± 9.02	3041.67 ± 173.82	2071.67 ± 52.62	1873.33 ± 27.02	187.50 ± 30.55
	Leaves	2544.41 ± 30.93	12369.17 ± 267.96	6796.67 ± 31.37	8089.17 ± 287.58	340.83 ± 28.22
T5 (S+FA+ DW)	Soil	579.10 ± 14.83	942.50 ± 41.16	34.17 ± 5.46	2060.00 ± 225.73	48.33 ± 10.14
	Root	886.23 ± 12.87	3306.67 ± 198.88	2005.83 ± 40.04	1755.00 ± 68.94	1191.67 ± 32.03
	Stem	1109.03 ± 17.19	5320.00 ± 174.69	1960.83 ± 178.43	2116.67 ± 20.88	128.33 ± 22.65
	Leaves	2310.58 ± 26.42	12007.50 ± 502.17	3944.17 ± 50.26	5527.50 ± 22.55	97.50 ± 1090
T6 (S+FA+ DW+MA)	Soil	617.25 ± 6.22	925.83 ± 15.90	42.50 ± 7.22	2263.33 ± 231.67	20.00 ± 7.64
	Root	926.13 ± 4.85	2060.83 ± 62.07	1545.00 ± 31.76	1792.50 ± 41.61	1448.33 ± 38.44
	Stem	1025.14 ± 6.28	4958.33 ± 330.18	2340.83 ± 240.38	2425.00 ± 104.76	256.67 ± 82.67
	Leaves	2340.18 ± 17.86	14369.17 ± 645.67	4406.67 ± 126.12	6020.00 ± 60.02	303.33 ± 39.19
T7 (S+FA+ FYM)	Soil	319.21 ± 5.16	730.83 ± 89.05	1203.33 ± 50.20	3294.17 ± 44.75	40.83 ± 3.63
	Root	884.90 ± 11.33	2455.83 ± 88.28	3608.33 ± 72.98	2086.67 ± 42.14	3077.50 ± 164.35
	Stem	1367.73 ± 13.48	5235.83 ± 150.92	2070.83 ± 223.34	2779.17 ± 45.79	870.00 ± 50.52
	Leaves	5288.81 ± 146.68	16653.33 ± 348.93	3470.00 ± 103.51	7790.83 ± 212.32	1596.67 ± 77.05
T8 (S+FA+ FYM+MA)	Soil	354.97 ± 4.13	529.17 ± 79.51	239.17 ± 53.86	3029.17 ± 93.51	18.33 ± 3.00
	Root	943.31 ± 7.80	3820.00 ± 103.21	3501.67 ± 137.25	2083.33 ± 43.43	1420.00 ± 58.47
	Stem	1486.95 ± 11.50	4017.50 ± 186.53	1960.00 ± 123.05	2289.17 ± 12.28	223.33 ± 31.70
	Leaves	5803.23 ± 109.06	15209.17 ± 237.34	5087.50 ± 74.54	8022.50 ± 69.93	515.00 ± 58.97
T9 (S+DW)	Soil	428.89 ± 13.48	510.00 ± 47.72	67.50 ± 17.56	2816.67 ± 154.41	15.83 ± 4.64
	Root	1236.00 ± 13.07	1531.67 ± 28.00	1821.67 ± 53.08	2350.83 ± 72.06	1299.17 ± 34.65
	Stem	1690.21 ± 44.10	4834.17 ± 170.61	2296.67 ± 177.67	2415.83 ± 30.49	358.33 ± 35.95
	Leaves	6280.10 ± 17.77	16214.17 ± 310.23	4835.83 ± 128.49	5582.50 ± 173.55	359.17 ± 59.44
T10(S+ DW+MA)	Soil	499.82 ± 2.06	522.50 ± 45.21	251.67 ± 47.44	2823.33 ± 142.59	25.83 ± 7.27
	Root	1045.56 ± 4.85	2775.83 ± 185.39	1969.17 ± 43.21	2609.17 ± 73.62	1716.67 ± 65.42
	Stem	1879.46 ± 14.12	5026.67 ± 171.37	2513.33 ± 114.91	3025.00 ± 94.38	504.17 ± 48.51
	Leaves	5789.24 ± 16.89	13945.00 ± 329.37	4802.50 ± 121.98	6571.67 ± 176.35	679.17 ± 36.10
T11(S+ FYM)	Soil	146.34 ± 2.60	615.00 ± 18.43	222.50 ± 24.11	3090.00 ± 137.99	35.00 ± 9.01
	Root	258.40 ± 12.17	2592.50 ± 168.68	5102.50 ± 78.76	3030.83 ± 65.36	3195.00 ± 128.32
	Stem	651.23 ± 6.87	4429.17 ± 163.82	2724.17 ± 180.80	2461.67 ± 67.92	320.00 ± 51.66
	Leaves	866.42 ± 14.19	16585.83 ± 77.01	5119.17 ± 112.27	7649.17 ± 34.92	1325.00 ± 60.02
T12(S+ FYM+MA)	Soil	171.38 ± 3.63	884.17 ± 53.33	620.00 ± 23.63	3870.00 ± 207.04	24.17 ± 5.83
	Root	408.02 ± 14.50	2780.00 ± 75.79	2551.67 ± 41.47	2262.50 ± 59.11	1304.17 ± 79.67
	Stem	764.48 ± 11.17	5769.17 ± 414.60	1916.67 ± 45.88	2611.67 ± 53.65	240.00 ± 31.66
	Leaves	979.67 ± 15.53	17590.00 ± 299.02	4984.17 ± 44.26	7735.00 ± 101.81	666.67 ± 71.22

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3k: Micro nutrients (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial

Treatments		**Cu**	**Zn**	**Mn**	**Fe**
T1 (CS)	Soil	5.22 ± 0.24	12.43 ± 0.02	137.65 ± 0.26	3150.83 ± 81.86
	Root	18.12 ± 0.77	10.05 ± 0.68	62.53 ± 0.98	778.33 ± 24.42
	Stem	10.03 ± 0.38	16.87 ± 0.36	24.10 ± 0.10	203.33 ± 2.07
	Leaves	28.47 ± 0.56	23.05 ± 0.19	90.90 ± 0.35	147.50 ± 5.20
T2 (S+MA)	Soil	5.55 ± 0.26	1.77 ± 0.63	40.72 ± 1.96	1186.67 ± 173.21
	Root	19.08 ± 0.25	8.65 ± 0.40	74.62 ± 0.46	579.17 ± 31.17
	Stem	4.18 ± 0.21	13.73 ± 0.43	16.15 ± 0.19	126.67 ± 0.83
	Leaves	11.05 ± 0.16	23.63 ± 0.96	79.18 ± 0.80	219.17 ± 8.21
T3 (S+FA)	Soil	5.60 ± 0.28	13.77 ± 0.68	118.85 ± 3.21	3060.83 ± 173.70
	Root	19.45 ± 0.58	9.68 ± 0.46	78.78 ± 0.32	791.67 ± 3.00
	Stem	7.73 ± 0.12	13.58 ± 0.89	12.97 ± 0.15	109.17 ± 5.07
	Leaves	12.20 ± 0.35	21.85 ± 0.35	74.95 ± 0.25	111.67 ± 3.33
T4 (S+FA+ MA)	Soil	4.83 ± 0.29	4.45 ± 0.26	80.47 ± 1.10	1938.33 ± 29.49
	Root	9.13 ± 0.33	11.53 ± 0.37	68.53 ± 0.26	790.83 ± 20.28
	Stem	2.52 ± 0.16	15.18 ± 0.53	6.13 ± 0.27	87.50 ± 7.64
	Leaves	24.47 ± 0.37	20.92 ± 0.89	69.33 ± 0.36	160.00 ± 4.33
T5 (S+FA+ DW)	Soil	5.58 ± 0.37	9.33 ± 0.90	161.05 ± 0.69	4622.50 ± 56.86
	Root	19.73 ± 0.62	15.90 ± 0.22	195.90 ± 1.73	878.33 ± 14.24
	Stem	3.98 ± 0.12	16.77 ± 0.61	34.77 ± 0.15	109.17 ± 9.28
	Leaves	27.32 ± 0.58	26.13 ± 0.95	145.63 ± 0.48	511.67 ± 3.63
T6 (S+FA+ DW+MA)	Soil	9.52 ± 0.24	15.03 ± 0.28	172.38 ± 1.70	4732.50 ± 60.02
	Root	8.30 ± 0.65	10.60 ± 0.79	104.17 ± 0.64	415.00 ± 3.82
	Stem	5.25 ± 0.10	17.43 ± 0.58	35.12 ± 0.54	96.67 ± 5.46
	Leaves	18.07 ± 0.70	27.88 ± 0.99	194.23 ± 0.81	231.67 ± 12.11
T7 (S+FA+ FYM)	Soil	5.92 ± 0.44	15.20 ± 0.68	204.63 ± 0.79	4365.83 ± 183.32
	Root	11.82 ± 0.33	10.02 ± 0.44	106.42 ± 0.27	927.50 ± 31.25
	Stem	7.23 ± 0.15	13.03 ± 0.70	16.48 ± 0.16	124.17 ± 7.12
	Leaves	17.05 ± 0.06	20.28 ± 0.46	81.48 ± 0.31	144.17 ± 6.01
T8 (S+FA+ FYM+MA)	Soil	5.83 ± 0.11	10.57 ± 0.12	181.70 ± 2.25	4175.00 ± 135.03
	Root	16.52 ± 0.15	16.45 ± 0.10	102.47 ± 1.11	995.00 ± 31.66
	Stem	4.60 ± 0.23	11.22 ± 0.38	16.88 ± 0.25	127.50 ± 7.22
	Leaves	15.78 ± 0.38	24.05 ± 0.63	135.12 ± 0.24	180.00 ± 8.78
T9 (S+DW)	Soil	6.53 ± 0.14	11.45 ± 0.43	210.90 ± 2.53	4552.50 ± 130.97
	Root	16.90 ± 0.51	15.17 ± 0.50	82.93 ± 0.20	946.67 ± 13.64
	Stem	9.13 ± 0.31	15.22 ± 0.77	19.62 ± 0.42	112.50 ± 8.78
	Leaves	16.68 ± 0.33	29.32 ± 0.71	120.28 ± 0.60	240.83 ± 3.63
T10(S+ DW+MA)	Soil	6.07 ± 0.36	16.68 ± 0.48	207.68 ± 1.51	4565.00 ± 153.08
	Root	10.82 ± 0.41	15.12 ± 0.54	116.08 ± 0.32	684.17 ± 20.28
	Stem	9.55 ± 0.30	19.42 ± 0.49	36.68 ± 0.19	162.50 ± 15.88
	Leaves	20.23 ± 0.21	28.50 ± 0.36	110.32 ± 0.25	158.33 ± 5.46
T11(S+ FYM)	Soil	6.10 ± 0.23	17.20 ± 0.66	201.27 ± 2.51	4365.00 ± 99.51
	Root	23.57 ± 0.66	17.70 ± 0.58	136.30 ± 0.99	1432.50 ± 19.84
	Stem	6.72 ± 0.43	15.38 ± 0.67	26.62 ± 0.13	205.00 ± 3.82
	Leaves	18.87 ± 0.11	21.07 ± 0.27	108.90 ± 0.44	195.83 ± 1.67
T12(S+ FYM+MA)	Soil	8.92 ± 0.69	22.48 ± 0.51	235.15 ± 1.12	5493.33 ± 131.99
	Root	12.40 ± 0.28	12.05 ± 0.53	79.95 ± 1.02	675.83 ± 5.07
	Stem	7.20 ± 0.08	9.00 ± 0.35	19.35 ± 0.10	172.50 ± 10.41
	Leaves	17.62 ± 0.27	22.83 ± 0.03	93.42 ± 0.18	205.83 ± 4.64

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.4 Analysis of heavy metals in soil and uptake by root, stem and leaves of P. deltoides

Heavy metals

In soil: Heavy metals in soil after six months of nursery trial were as follows: Pb was ranging from 0.95 mg/kg (T4) – 10.17 mg/kg (T12); Cr was below detection limit in treatments T5 – T6, whereas, it was maximum in treatment T11 – 8.37 mg/kg; Cd was ranging from 1.05 mg/kg in treatment T7 – 3.32 mg/kg in T3. Ni was ranging from 2.33 mg/kg (T2) – 12.50 mg/kg (T12) and Co was having minimum value in treatment T1 (0.38 mg/kg) – 2.40 mg/kg as maximum value in treatment T12 (Table 3l).

In plants: Pb was ranging from 0.13 mg/kg in roots in treatments T3 to 10.92 mg/kg in stem in treatment T9; Cr was below detection limit in stem of all the treatments and was having its maximum value 96.62 mg/kg in leaves (T6); Cd was ranging from 0.27 mg/kg in root (T1) – 3.40 mg/kg in stem (T10); Ni was ranging from 1.73 mg/kg in leaves (T7) – 9.15 mg/kg in root (T9) and Co was below detection limit in the stem of all the treatments, whereas, it was having its maximum concentration in leaves of the plant (4.25 mg/kg) T5 (Table 3l).

4.2.5 Reduction in concentration of heavy metals in soil after completion of nursery trial of P. deltoides

The results showed that the percentage reduction in concentration of Pb was maximum in treatment T4 (95.88%), followed by T3 (93.07%) and was least in control soil (T1), i.e., 65.78% (Table 3m). Concentration of Cr was completely reduced in soil in treatments T5 and T6 (100%), and was least reduced in T11 – 3.79% only. Treatment T4 showed the maximum reduction potential for the reduction in concentration of Cd (72.06%), as compared to treatment T12 which had the least potential for the reduction in concentration of Cd (5.42%). Concentration of Ni was maximally reduced in treatment T2 (84.44%) and was minimally reduced in T12 (26.60%). The percentage reduction in concentration of Co was ranging from 92.73% (T1) – 43.79% (T6) (Table 3m).

Table 3l: Heavy metals (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Populus deltoides* after six months of nursery trial

Treatments		**Pb**	**Cr**	**Cd**	**Ni**	**Co**
T1 (CS)	Soil	4.50 ± 0.09	3.47 ± 0.25	1.07 ± 0.04	6.90 ± 0.38	0.38 ± 0.03
	Root	1.58 ± 0.13	18.92 ± 0.72	0.27 ± 0.04	2.05 ± 0.15	0.72 ± 0.10
	Stem	3.10 ± 0.08	BDL	1.65 ± 0.14	6.65 ± 0.33	BDL
	Leaves	5.65 ± 0.52	57.62 ± 0.51	1.00 ± 0.03	2.27 ± 0.30	1.67 ± 0.14
T2 (S+MA)	Soil	2.45 ± 0.13	4.23 ± 0.35	2.32 ± 0.07	2.33 ± 0.07	0.75 ± 0.06
	Root	2.43 ± 0.22	21.28 ± 0.20	0.68 ± 0.04	5.02 ± 0.19	1.10 ± 0.16
	Stem	8.25 ± 0.20	BDL	1.60 ± 0.13	7.13 ± 0.32	BDL
	Leaves	8.37 ± 0.31	59.82 ± 0.11	1.35 ± 0.06	3.57 ± 0.12	2.20 ± 0.09
T3 (S+FA)	Soil	1.60 ± 0.18	2.20 ± 0.35	3.32 ± 0.11	7.75 ± 0.33	0.68 ± 0.15
	Root	2.13 ± 0.04	26.27 ± 0.71	0.38 ± 0.06	3.08 ± 0.25	1.48 ± 0.15
	Stem	4.52 ± 0.25	BDL	2.12 ± 0.07	4.82 ± 0.22	BDL
	Leaves	8.52 ± 0.22	62.12 ± 0.58	1.62 ± 0.03	4.28 ± 0.21	1.82 ± 0.22
T4 (S+FA+ MA)	Soil	0.95 ± 0.19	2.98 ± 0.09	1.07 ± 0.09	5.67 ± 0.32	1.98 ± 0.20
	Root	3.52 ± 0.06	22.15 ± 0.32	0.40 ± 0.03	5.60 ± 0.29	2.60 ± 0.21
	Stem	6.37 ± 0.48	BDL	1.97 ± 0.09	4.67 ± 0.22	BDL
	Leaves	7.27 ± 0.46	59.70 ± 0.33	1.42 ± 0.06	2.00 ± 0.06	2.53 ± 0.11
T5 (S+FA+ DW)	Soil	2.30 ± 0.39	BDL	1.68 ± 0.14	7.43 ± 0.27	0.72 ± 0.20
	Root	4.05 ± 0.10	25.08 ± 0.16	1.02 ± 0.04	4.23 ± 0.26	1.45 ± 0.10
	Stem	6.02 ± 0.37	BDL	1.73 ± 0.09	5.02 ± 0.27	BDL
	Leaves	6.45 ± 0.28	72.15 ± 0.50	1.18 ± 0.10	4.28 ± 0.12	4.25 ± 0.18
T6 (S+FA+ DW+MA)	Soil	3.83 ± 0.53	BDL	2.27 ± 0.09	8.82 ± 0.15	2.40 ± 0.18
	Root	4.85 ± 0.19	17.87 ± 0.24	0.90 ± 0.08	3.15 ± 0.16	2.38 ± 0.09
	Stem	7.55 ± 0.15	BDL	2.37 ± 0.12	6.10 ± 0.25	BDL
	Leaves	10.45 ± 0.25	96.62 ± 1.06	1.77 ± 0.04	3.92 ± 0.22	3.85 ± 0.16
T7 (S+FA+ FYM)	Soil	3.10 ± 0.52	2.55 ± 0.10	1.05 ± 0.14	8.30 ± 0.35	1.48 ± 0.32
	Root	5.02 ± 0.51	20.15 ± 0.30	0.78 ± 0.09	4.22 ± 0.17	2.30 ± 0.21
	Stem	10.25 ± 0.30	BDL	2.78 ± 0.15	4.75 ± 0.13	BDL
	Leaves	6.35 ± 0.64	18.73 ± 0.43	1.77 ± 0.06	1.73 ± 0.04	2.90 ± 0.20
T8 (S+FA+ FYM+MA)	Soil	3.62 ± 0.50	4.27 ± 0.12	1.08 ± 0.06	8.45 ± 0.09	1.12 ± 0.12
	Root	5.92 ± 0.32	35.32 ± 0.32	1.10 ± 0.08	6.43 ± 0.15	0.88 ± 0.03
	Stem	9.08 ± 0.31	BDL	2.65 ± 0.08	7.28 ± 0.48	BDL
	Leaves	5.33 ± 0.58	21.03 ± 0.11	1.40 ± 0.05	2.63 ± 0.29	2.62 ± 0.16
T9 (S+DW)	Soil	5.73 ± 0.78	2.70 ± 0.20	1.57 ± 0.09	10.27 ± 0.50	1.38 ± 0.15
	Root	5.23 ± 0.37	18.47 ± 0.32	1.23 ± 0.09	9.15 ± 0.36	1.70 ± 0.10
	Stem	10.92 ± 0.56	BDL	3.00 ± 0.06	7.12 ± 0.31	BDL
	Leaves	6.00 ± 0.49	18.80 ± 0.29	1.80 ± 0.05	4.53 ± 0.17	3.72 ± 0.20
T10(S+ DW+MA)	Soil	5.27 ± 0.12	3.50 ± 0.48	1.20 ± 0.10	9.53 ± 0.24	0.62 ± 0.14
	Root	4.03 ± 0.22	18.23 ± 0.78	0.82 ± 0.04	4.87 ± 0.24	3.30 ± 0.10
	Stem	8.67 ± 0.48	BDL	3.40 ± 0.10	6.10 ± 0.67	BDL
	Leaves	7.23 ± 0.34	18.40 ± 0.53	2.02 ± 0.02	3.13 ± 0.20	3.25 ± 0.22
T11(S+ FYM)	Soil	8.63 ± 0.27	8.37 ± 0.16	1.20 ± 0.13	10.43 ± 0.32	0.67 ± 0.12
	Root	5.73 ± 0.49	35.25 ± 0.38	1.20 ± 0.08	9.03 ± 0.31	2.10 ± 0.12
	Stem	9.50 ± 0.49	BDL	2.82 ± 0.09	5.90 ± 0.10	BDL
	Leaves	8.68 ± 0.56	22.12 ± 0.07	1.60 ± 0.08	2.35 ± 0.13	2.95 ± 0.20
T12(S+ FYM+MA)	Soil	10.17 ± 0.54	7.27 ± 0.46	1.92 ± 0.04	12.50 ± 0.44	0.97 ± 0.04
	Root	6.43 ± 0.30	19.18 ± 0.57	1.52 ± 0.06	4.27 ± 0.10	2.25 ± 0.20
	Stem	9.40 ± 0.45	BDL	3.07 ± 0.12	5.97 ± 0.26	BDL
	Leaves	5.13 ± 0.26	21.38 ± 0.04	1.48 ± 0.07	3.02 ± 0.28	3.45 ± 0.10

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3m: Heavy metal (mg/kg) analysis in soil before plantation and after harvesting of *Populus deltoides* and percentage reduction (R) in concentration of heavy metals in soil

Treatments	Time period	Heavy metals (mg / kg) analysed in Soil									
		Pb		Cr		Cd		Ni		Co	
		In soil	R	In soil	R	In soil	R	In soil	R	In soil	R
T1 (CS)	Day 0	13.15 ± 0.84	65.78 e	6.92 ± 0.28	49.46 c	2.65 ± 0.06	59.62 b	14.97 ± 0.25	53.91 b	5.23 ± 0.08	92.73 a
	Day 180	4.50 ± 0.09		3.47 ± 0.25		1.07 ± 0.04		6.90 ± 0.38		0.38 ± 0.03	
T2 (S+MA)	Day 0	13.15 ± 0.84	81.37 c	6.92 ± 0.28	38.87 d	2.65 ± 0.06	12.45 f	14.97 ± 0.25	84.44 a	5.23 ± 0.08	85.66 b
	Day 180	2.45 ± 0.13		4.23 ± 0.35		2.32 ± 0.07		2.33 ± 0.07		0.75 ± 0.06	
T3 (S+FA)	Day 0	23.08 ± 0.29	93.07 a	4.83 ± 0.31	54.45 b	3.83 ± 0.07	13.32 f	12.58 ± 0.67	38.39 e	4.75 ± 0.10	85.68 b
	Day 180	1.60 ± 0.18		2.20 ± 0.35		3.32 ± 0.11		7.75 ± 0.33		0.68 ± 0.15	
T4 (S+FA+MA)	Day 0	23.08 ± 0.29	95.88 a	4.83 ± 0.31	38.30 d	3.83 ± 0.07	72.06 a	12.58 ± 0.67	54.93 b	4.75 ± 0.10	58.32 d
	Day 180	0.95 ± 0.19		2.98 ± 0.09		1.07 ± 0.09		5.67 ± 0.32		1.98 ± 0.20	
T5 (S+FA+DW)	Day 0	32.92 ± 0.97	93.01 a	6.07 ± 0.67	100.00 a	2.58 ± 0.08	34.88 d	14.17 ± 0.42	47.57 c	4.27 ± 0.20	83.14 b
	Day 180	2.30 ± 0.39		BDL		1.68 ± 0.14		7.43 ± 0.27		0.72 ± 0.20	
T6 (S+FA+DW+MA)	Day 0	32.92 ± 0.97	88.37 b	6.07 ± 0.67	100.00 a	2.58 ± 0.08	12.02 f	14.17 ± 0.42	37.76 e	4.27 ± 0.20	43.79 e
	Day 180	3.83 ± 0.53		BDL		2.27 ± 0.09		8.82 ± 0.15		2.40 ± 0.18	
T7 (S+FA+FYM)	Day 0	14.05 ± 0.30	77.94 c	5.30 ± 0.09	51.89 b	1.82 ± 0.07	42.31 c	15.92 ± 0.51	47.86 c	5.57 ± 0.34	73.43 c
	Day 180	3.10 ± 0.52		2.55 ± 0.10		1.05 ± 0.14		8.30 ± 0.35		1.48 ± 0.32	
T8 (S+FA+FYM+MA)	Day 0	14.05 ± 0.30	74.23 d	5.30 ± 0.09	19.43 f	1.82 ± 0.07	40.66 c	15.92 ± 0.51	46.92 d	5.57 ± 0.34	79.89 b
	Day 180	3.62 ± 0.50		4.27 ± 0.12		1.08 ± 0.06		8.45 ± 0.09		1.12 ± 0.12	
T9 (S+DW)	Day 0	32.27 ± 0.60	82.24 b	5.18 ± 0.22	47.88 c	2.08 ± 0.19	24.52 e	15.45 ± 0.24	33.53 f	6.82 ± 0.14	80.94 b
	Day 180	5.73 ± 0.78		2.70 ± 0.20		1.57 ± 0.09		10.27 ± 0.50		1.30 ± 0.15	
T10(S+DW+MA)	Day 0	32.27 ± 0.60	83.67 b	5.18 ± 0.22	32.43 e	2.08 ± 0.19	42.31 c	15.45 ± 0.24	38.32 e	6.82 ± 0.14	90.91 a
	Day 180	5.27 ± 0.12		3.50 ± 0.48		1.20 ± 0.10		9.53 ± 0.24		0.62 ± 0.14	
T11(S+FYM)	Day 0	35.32 ± 0.39	75.56 c	8.70 ± 0.05	03.79 g	2.03 ± 0.09	40.89 c	17.03 ± 0.43	38.76 e	5.37 ± 0.32	87.52 a
	Day 180	8.63 ± 0.27		8.37 ± 0.16		1.20 ± 0.13		10.43 ± 0.32		0.67 ± 0.12	
T12(S+FYM+MA)	Day 0	35.32 ± 0.39	71.21 d	8.70 ± 0.05	16.44 f	2.03 ± 0.09	05.42 g	17.03 ± 0.43	26.60 g	5.37 ± 0.32	81.94 b
	Day 180	10.17 ± 0.54		7.27 ± 0.46		1.92 ± 0.04		12.50 ± 0.44		0.97 ± 0.04	

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v) Values followed by the same letter within a column are not statistically different

4.2.6 Physico-chemical analysis of soil in various treatments on completion of nursery trial of T. ciliata (on day 180)

Analysis of soil after six months of nursery trial showed marked variation in pH in different treatments (T1 – T12). All treatments had alkaline pH ranging from 7.22 – 8.45 (Table 3n) except treatment T4 which had a pH of 6.94. Electrical conductivity was least in treatment T3 (amended with fly ash), i.e., 423.00 µS/cm and was maximum in treatment T10, i.e., 845.00 µS/cm. Water holding capacity was minimum in treatment (T9) – 36.02 % and was maximum in treatment (T3) – 40.12% (Table 3n). It showed that distillery waste had a marked effect on pH and electrical conductivity of the soil, while addition of fly ash @10% (v/v) improved the water holding capacity of the soil. Bulk density was ranging from 1.42 gm/cm^3 (T3) maximum – 1.12 gm/cm^3(T9) minimum.

Organic carbon was least in case of treatments T3 and T4 amended with fly ash (0.21%), whereas, was maximum in treatment T11 (1.14%) (Table 3o), due the amendment with farmyard manure as farmyard manure is rich in organic carbon. Available phosphorus and sulphur both were having minimum values in control soil (T1) – 19.05 and 164.52 mg/kg respectively, whereas, available P was maximum in treatment T12 – 75.84 mg/kg and available S in treatment (T8) – 966.94 mg/kg (Table 3o).

Table 3n: pH, EC, WHC (%) and bulk density of soil in different treatments after six months of nursery trial of *Toona ciliata*

Treatments	**Parameters Analysed (Soil)**			
	pH	**EC (µS/cm)**	**WHC (%)**	**Bulk density (gm/cm^3)**
T1 (CS)	8.22 ± 0.09	786.67 ± 1.68	38.3	1.23
T2 (S+MA)	8.45 ± 0.04	602.33 ± 3.02	35.4	1.15
T3 (S+FA)	8.45 ± 0.03	423.00 ± 3.06	40.1	1.42
T4 (S+FA+MA)	8.45 ± 0.01	477.00 ± 0.57	39.3	1.26
T5 (S+FA+DW)	7.30 ± 0.03	699.00 ± 2.85	39.2	1.3
T6 (S+FA+DW+MA)	7.41 ± 0.03	818.33 ± 2.36	38.8	1.24
T7 (S+FA+FYM)	8.44 ± 0.03	391.67 ± 1.17	47.0	1.35
T8 (S+FA+FYM+MA)	8.12 ± 0.02	409.00 ± 1.00	39.7	1.29
T9 (S+DW)	7.22 ± 0.02	631.33 ± 1.35	36.0	1.12
T10(S+DW+MA)	6.94 ± 0.10	845.67 ± 2.04	37.8	1.28
T11(S+FYM)	8.31 ± 0.03	723.33 ± 1.71	37.8	1.2
T12(S+FYM+MA)	8.43 ± 0.01	727.67 ± 0.51	39.5	1.3

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3o: Organic carbon, available phosphorus and available sulphur in soil in different treatments after six months of nursery trial of *Toona ciliata*

Treatments	Parameters Analysed (Soil)			
	Org. C (%)	Ava N (%)	Ava P (mg/kg)	Ava S (mg/kg)
T1 (CS)	0.42 ± 0.01	0.023 ± 0.001	19.05 ± 0.47	164.52 ± 15.55
T2 (S+MA)	0.5 ± 0.001	0.028 ± 0.001	26.20 ± 0.29	183.87 ± 7.78
T3 (S+FA)	0.21 ± 0.03	0.011 ± 0.001	33.74 ± 0.57	560.48 ± 9.81
T4 (S+FA+MA)	0.21 ± 0.01	0.012 ± 0.001	35.32 ± 0.09	795.97 ± 3.70
T5 (S+FA+DW)	0.35 ± 0.01	0.020 ± 0.001	27.16 ± 1.21	425.00 ± 9.09
T6 (S+FA+DW+MA)	0.35 ± 0.001	0.020 ± 0.001	30.17 ± 0.44	426.61 ± 9.81
T7 (S+FA+FYM)	0.64 ± 0.01	0.036 ± 0.001	63.25 ± 0.47	838.71 ± 13.28
T8 (S+FA+FYM+MA)	0.94 ± 0.01	0.052 ± 0.001	74.20 ± 0.49	966.94 ± 19.62
T9 (S+DW)	0.70 ± 0.001	0.039 ± 0.001	29.49 ± 0.31	711.29 ± 6.40
T10(S+DW+MA)	0.57 ± 0.01	0.031 ± 0.001	29.88 ± 0.71	742.74 ± 10.55
T11(S+FYM)	1.14 ± 0.01	0.063 ± 0.001	74.52 ± 0.41	548.39 ± 14.06
T12(S+FYM+MA)	1.08 ± 0.001	0.060 ± 0.001	75.84 ± 0.46	610.48 ± 4.49

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.6.1 Nutrition status

In soil: Major elements total phosphorus and potassium were minimum in treatments T6 (18.33 mg/kg) and T5 (577.50 mg/kg) respectively, whereas, total P was maximum in treatment T12 – 329.75 mg/kg and total K was maximum in treatment T11 – 2566.67 mg/kg (Table 3p).

Secondary elements were as follows: Ca was ranging from 15.0 mg/kg (T6) – 2294.17 mg/kg (T11); Mg was ranging from 1127.50 mg/kg (T7) – 5189.17 mg/kg (T9) (Table 3p); Na was having minimum value in treatment T1 – 586.67 mg/kg, whereas, maximum in treatment T6 – 2069.17 mg/kg (Table 3q). Cu was ranging from 0.98 mg/kg (T8) – 3.52 mg/kg (T11); Zn was ranging from 14.37 mg/kg (T9) – 34.00 mg/kg (T11); Mn was having minimum value in treatment T3 – 196.88 mg/kg and was having maximum value in treatment T1 – 290.88 mg/kg; Fe was ranging from 3505.00 mg/kg (T3) – 5205.83 mg/kg (T11) (Table 3q).

In plants: Total P was minimum in roots of *Toona ciliata* (223.33 mg/kg) in treatment T6 and was maximum in leaves, i.e., 3366.0 mg/kg in treatment T8; K was ranging from 6582.50 mg/kg in stem (T8) – 18113.33 mg/kg in leaves (T6); Ca was minimum in stem

(154.17 mg/kg) in treatment T1 and was maximum in leaves (23836.67 mg/kg) in treatment T11 and Mg was ranging from 3634.17 mg/kg in stem (T9) – 13771.67 mg/kg in leaves (T8) (Table 3p). It showed that P, K, Ca and Mg were having their maximum concentration in leaves of *P. deltoides.* Na was ranging from 558.33 mg/kg in root (T2) – 3655.53 mg/kg in root (T7); Cu was ranging from 0.10 mg/kg in stem (T2) – 32.97 mg/kg in root (T10); Zn was minimum in root (14.82 mg/kg) in treatment T1, whereas, it was maximum in leaves (62.77 mg/kg) in treatment T7; Mn was ranging from 28.60 mg/kg in Stem (T7) – 176.47 mg/kg in root (T10) and Fe was minimum in stem (54.17 mg/kg) in T4 and was maximum in root (1003.33 mg/kg) in treatment T9 (Table 3q). It showed that Na, Cu, Mn and Fe were having their maximum concentration in root, whereas, Zn was maximally present in leaves of *T. ciliata.*

Table 3p: Major and secondary elements (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial

Treatments		P	K	Ca	Mg	Na
T1 (CS)	Soil	72.00 ± 23.34	2566.67 ± 33.37	156.67 ± 15.43	3374.17 ± 72.29	586.67 ± 4.64
	Root	471.75 ± 52.05	11754.17 ± 44.26	1386.67 ± 20.43	5588.33 ± 86.61	989.17 ± 106.87
	Stem	967.50 ± 60.36	10671.67 ± 62.49	154.17 ± 4.64	4285.83 ± 75.62	900.83 ± 16.73
	Leaves	1161.00 ± 96.39	16030.83 ± 49.17	5351.67 ± 43.55	10250.00 ± 74.60	3303.33 ± 248.61
T2 (S+MA)	Soil	41.25 ± 11.05	1240.33 ± 84.61	156.67 ± 25.83	2217.50 ± 108.58	870.83 ± 10.24
	Root	240.75 ± 52.02	9430.83 ± 3.63	999.17 ± 16.22	5751.67 ± 4.41	558.33 ± 35.24
	Stem	438.75 ± 54.21	6682.50 ± 72.86	1688.33 ± 13.02	3687.50 ± 157.05	1160.00 ± 4.33
	Leaves	1221.00 ± 40.59	14934.17 ± 44.26	6988.33 ± 78.56	8110.83 ± 6.01	1552.50 ± 151.27
T3 (S+FA)	Soil	84.00 ± 14.39	635.00 ± 10.10	43.33 ± 3.63	1921.67 ± 203.83	1194.17 ± 11.02
	Root	413.75 ± 75.97	7500.00 ± 7.22	2700.83 ± 50.01	6270.83 ± 13.64	2865.83 ± 73.11
	Stem	914.25 ± 116.87	12606.67 ± 55.65	2036.67 ± 30.08	4530.83 ± 32.93	1292.50 ± 7.22
	Leaves	1404.75 ± 90.00	9915.83 ± 7.41	18597.50 ± 183.93	8745.83 ± 13.64	3038.33 ± 89.88
T4 (S+FA+MA)	Soil	34.50 ± 5.61	915.83 ± 35.19	54.17 ± 7.12	3320.00 ± 173.04	1227.50 ± 23.85
	Root	279.50 ± 182.8	7918.33 ± 49.10	3254.17 ± 28.15	6692.50 ± 7.64	3347.50 ± 144.56
	Stem	874.75 ± 168.36	7850.00 ± 33.94	1899.17 ± 27.29	5153.33 ± 16.85	1442.50 ± 22.41
	Leaves	1446.75 ± 41.01	9556.67 ± 40.11	21889.17 ± 78.67	9980.00 ± 85.11	2842.50 ± 71.43
T5 (S+FA+DW)	Soil	61.25 ± 9.37	577.50 ± 7.64	532.50 ± 17.50	3353.33 ± 34.22	1893.33 ± 1.67
	Root	278.33 ± 38.63	10125 ± 27.50	1719.17 ± 40.32	5940.83 ± 24.34	1390.83 ± 137.78
	Stem	513.33 ± 39.06	12411.67 ± 24.17	1969.17 ± 49.84	4692.50 ± 18.43	1693.33 ± 14.60
	Leaves	1351.00 ± 40.59	15920.83 ± 32.29	7871.67 ± 160.16	6970.00 ± 12.99	2425.83 ± 34.80
T6 (S+FA+DW+MA)	Soil	18.33 ± 3.00	783.33 ± 7.27	15.00 ± 1.44	1292.50 ± 159.12	2069.17 ± 10.14
	Root	223.33 ± 31.70	10494.17 ± 38.90	5613.33 ± 59.19	5177.50 ± 23.63	3189.17 ± 178.32
	Stem	515.00 ± 58.97	14385.00 ± 53.05	4720.83 ± 86.24	4248.33 ± 12.28	1922.50 ± 30.24
	Leaves	1420.00 ± 58.47	18113.33 ± 11.67	3219.17 ± 69.62	4910.83 ± 14.81	2710.00 ± 183.93
T7 (S+FA+FYM)	Soil	72.75 ± 7.43	1050.00 ± 2.89	29.17 ± 9.83	1127.50 ± 147.36	618.33 ± 8.21
	Root	2280.0 ± 133.6	7372.50 ± 9.01	6636.67 ± 90.08	4755.00 ± 111.17	3655.83 ± 47.66
	Stem	830.00 ± 104.09	8291.67 ± 13.10	1707.50 ± 20.36	6745.00 ± 19.53	1900.83 ± 11.02
	Leaves	2181.50 ± 224.2	12558.33 ± 43.45	10451.67 ± 145.84	8987.50 ± 17.56	2285.83 ± 78.39
T8 (S+FA+FYM+MA)	Soil	139.50 ± 5.65	880.83 ± 3.00	76.67 ± 2.20	2836.67 ± 145.54	863.33 ± 13.33
	Root	1495.00 ± 98.48	8345.83 ± 34.35	7509.17 ± 57.85	4993.33 ± 12.02	2674.17 ± 114.79
	Stem	328.25 ± 43.58	6582.50 ± 23.85	1844.17 ± 15.43	9752.50 ± 18.76	2057.50 ± 3.82
	Leaves	3366.00 ± 240.2	9439.17 ± 20.02	19126.67 ± 45.83	13771.67 ± 60.32	2888.33 ± 156.50
T9 (S+DW)	Soil	322.25 ± 38.19	1150.00 ± 5.00	622.50 ± 12.83	5189.17 ± 0.83	1410.00 ± 9.01
	Root	463.33 ± 38.25	9960.00 ± 34.49	2075.00 ± 52.82	5567.50 ± 15.28	2028.33 ± 218.72
	Stem	650.00 ± 73.76	8734.17 ± 15.90	1890.00 ± 7.64	3634.17 ± 63.29	787.50 ± 9.47
	Leaves	2315.83 ± 184.7	13630.00 ± 0.00	6548.33 ± 179.83	6358.33 ± 63.42	2871.67 ± 132.86
T10(S+DW+MA)	Soil	24.17 ± 5.83	1253.33 ± 7.41	38.33 ± 4.17	3096.67 ± 95.28	1590.83 ± 5.83
	Root	240.00 ± 31.66	13495.83 ± 24.17	2911.67 ± 31.17	5356.67 ± 89.88	1828.33 ± 110.92
	Stem	666.67 ± 71.22	10648.33 ± 44.26	1566.67 ± 3.63	3860.00 ± 31.26	1063.33 ± 2.20
	Leaves	1304.17 ± 79.67	15871.67 ± 49.17	5140.00 ± 65.26	8150.00 ± 9.47	2718.33 ± 132.62
T11(S+FYM)	Soil	297.50 ± 69.30	1584.17 ± 4.17	2294.17 ± 56.30	3911.67 ± 228.80	903.33 ± 10.14
	Root	549.75 ± 41.41	9844.17 ± 43.65	1435.00 ± 21.26	5717.50 ± 39.39	1218.33 ± 36.10
	Stem	808.75 ± 35.05	12046.67 ± 126.88	5067.50 ± 60.62	4085.83 ± 98.76	2193.33 ± 13.33
	Leaves	1513.0 ± 306	16522.50 ± 96.86	23836.67 ± 473.46	7986.67 ± 97.92	3265.00 ± 90.29
T12(S+FYM+MA)	Soil	329.75 ± 78.49	1932.50 ± 10.90	343.33 ± 4.64	3766.67 ± 84.71	1005.00 ± 12.33
	Root	284.75 ± 32.51	10590.00 ± 62.65	1402.50 ± 10.10	6388.33 ± 57.33	1325.00 ± 86.50
	Stem	698.25 ± 20.78	12620.00 ± 53.05	2000.00 ± 21.80	5258.33 ± 103.49	2392.50 ± 6.29
	Leaves	1974.50 ± 122.5	16700.83 ± 116.92	6028.50 ± 47.85	7850.00 ± 58.15	3264.17 ± 109.02

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3q: Micro nutrients (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial

Treatments		Cu	Zn	Mn	Fe
T1 (CS)	Soil	3.15 ± 0.22	28.70 ± 0.51	290.88 ± 0.18	3964.17 ± 16.41
	Root	5.60 ± 0.73	14.82 ± 0.42	98.85 ± 2.54	450.00 ± 2.89
	Stem	0.23 ± 0.07	15.23 ± 1.24	45.55 ± 0.33	90.83 ± 10.24
	Leaves	9.87 ± 0.77	35.52 ± 0.67	96.85 ± 1.68	162.50 ± 8.66
T2 (S+MA)	Soil	2.77 ± 0.22	29.43 ± 0.27	239.93 ± 1.24	3845.83 ± 68.21
	Root	6.97 ± 0.39	20.02 ± 0.20	62.18 ± 0.62	467.50 ± 11.55
	Stem	0.10 ± 0.10	16.88 ± 0.20	36.25 ± 0.45	68.33 ± 7.95
	Leaves	11.45 ± 0.06	41.47 ± 0.34	85.95 ± 1.22	170.83 ± 1.67
T3 (S+FA)	Soil	1.02 ± 0.07	21.62 ± 0.48	196.88 ± 3.24	3505.00 ± 16.07
	Root	11.42 ± 0.14	23.27 ± 0.42	75.02 ± 0.40	469.17 ± 10.64
	Stem	0.25 ± 0.03	15.87 ± 0.23	32.52 ± 0.47	78.33 ± 4.64
	Leaves	8.40 ± 0.31	44.47 ± 0.37	129.13 ± 0.74	205.00 ± 2.89
T4 (S+FA+ MA)	Soil	1.17 ± 0.10	21.55 ± 0.49	199.97 ± 0.67	3540.00 ± 16.65
	Root	9.02 ± 0.63	18.03 ± 0.41	75.70 ± 1.40	692.50 ± 8.78
	Stem	0.23 ± 0.03	15.67 ± 0.35	31.60 ± 0.39	54.17 ± 10.14
	Leaves	13.85 ± 0.38	39.15 ± 0.48	134.77 ± 0.99	162.50 ± 4.33
T5 (S+FA+ DW)	Soil	2.13 ± 0.09	15.15 ± 0.26	230.37 ± 0.26	3690.83 ± 88.91
	Root	20.02 ± 0.74	23.73 ± 0.41	174.90 ± 2.04	948.33 ± 25.43
	Stem	0.77 ± 0.15	20.25 ± 0.48	43.40 ± 0.54	85.00 ± 2.89
	Leaves	11.62 ± 0.37	54.08 ± 1.17	136.62 ± 1.32	216.67 ± 9.61
T6 (S+FA+ DW+MA)	Soil	1.83 ± 0.03	27.33 ± 0.36	211.13 ± 1.19	3870.00 ± 34.37
	Root	20.32 ± 1.25	34.73 ± 0.15	158.70 ± 1.10	671.67 ± 5.07
	Stem	3.48 ± 0.25	19.12 ± 0.35	35.07 ± 0.38	101.67 ± 4.41
	Leaves	9.63 ± 0.25	43.93 ± 0.26	97.38 ± 0.17	117.50 ± 9.01
T7 (S+FA+ FYM)	Soil	2.03 ± 0.10	23.52 ± 0.46	201.37 ± 1.35	3820.83 ± 13.64
	Root	15.23 ± 0.13	25.17 ± 0.32	80.72 ± 1.88	593.33 ± 9.83
	Stem	2.17 ± 0.11	23.75 ± 0.14	28.60 ± 0.54	111.67 ± 7.27
	Leaves	9.92 ± 0.51	62.77 ± 0.76	126.30 ± 0.93	272.50 ± 9.01
T8 (S+FA+ FYM+MA)	Soil	0.98 ± 0.177	27.20 ± 0.35	203.32 ± 0.47	4125.83 ± 22.38
	Root	13.87 ± 0.61	19.37 ± 0.88	97.83 ± 0.59	523.33 ± 12.02
	Stem	1.42 ± 0.14	17.57 ± 0.39	29.17 ± 0.78	124.17 ± 7.95
	Leaves	5.77 ± 0.19	46.83 ± 0.45	133.30 ± 0.31	249.17 ± 7.27
T9 (S+DW)	Soil	1.90 ± 0.10	14.37 ± 0.23	205.78 ± 0.25	4068.33 ± 19.49
	Root	28.88 ± 0.60	41.72 ± 0.38	173.45 ± 0.79	1003.33 ± 5.83
	Stem	5.83 ± 0.50	24.72 ± 0.28	63.08 ± 0.68	153.33 ± 1.67
	Leaves	14.07 ± 0.53	52.33 ± 0.75	156.87 ± 1.30	151.67 ± 6.01
T10(S+ DW+MA)	Soil	2.22 ± 0.15	27.65 ± 0.48	207.68 ± 0.75	4972.50 ± 21.80
	Root	32.97 ± 0.37	38.85 ± 0.03	176.47 ± 1.16	770.00 ± 15.28
	Stem	6.78 ± 0.47	22.32 ± 0.36	52.23 ± 0.16	76.67 ± 5.46
	Leaves	11.27 ± 0.29	55.37± 0.57	149.28 ± 1.02	165.00 ± 7.64
T11(S+ FYM)	Soil	3.52 ± 0.09	34.00 ± 0.25	260.78 ± 1.54	5205.83 ± 23.38
	Root	27.22 ± 0.76	28.98 ± 0.57	120.10 ± 2.68	809.17 ± 12.28
	Stem	3.13 ± 0.21	17.13 ± 0.24	42.40 ± 0.09	60.83 ± 7.12
	Leaves	4.08 ± 0.12	30.98 ± 0.22	102.98 ± 0.53	120.00 ± 2.50
T12(S+ FYM+MA)	Soil	1.32 ± 0.24	28.98 ± 0.41	249.35 ± 0.38	4176.67 ± 65.30
	Root	23.37 ± 0.80	20.92 ± 0.19	72.58 ± 0.42	656.67 ± 2.20
	Stem	4.08 ± 0.14	17.35 ± 0.41	30.27 ± 0.33	71.67 ± 8.70
	Leaves	5.25 ± 0.12	38.27 ± 0.56	101.13 ± 1.21	175.83 ± 12.28

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

4.2.6.2 Heavy metals

In soil: Heavy metals in soil after six months of nursery trial were as follows: Pb was ranging from 1.30 mg/kg (T4) – 8.80 mg/kg (T10); Cr was ranging from 2.20mg/kg (T1) – 5.43mg/kg (T5) and Cd was having minimum value in treatment T1 – 0.30 mg/kg and maximum value in treatment T11 – 0.67mg/kg. Ni had its minimum concentration intreatment T9 (0.87 mg/kg) and maximum concentration in treatment T12 (7.75 mg/kg). Co was ranging from 1.85 mg/kg (T9) to 5.82 mg/kg (T10) (Table 3r).

In plants: Pb was ranging from 3.08 mg/kg in root (T6) – 19.47 mg/kg in leaves in treatment T7; Cr was maximum in roots of the plant in treatment T10 (8.20 mg/kg) and was minimum in leaves (0.90 mg/kg) in T6; Cd had its maximum concentration (0.87 mg/kg) in leaves of the plant in treatment T6 and minimum in root (0.22 mg/kg) in treatment T2; Ni was ranging from 0.30 mg/kg (T6) in stem to a maximum of 6.58 mg/kg in root in treatment T11 and Co was having its minimum concentration (0.90 mg/kg) in stem (T3) and maximum concentration, i.e., 6.62 mg/kg (T9) in leaves of *T. ciliata*.

4.2.7 Reduction in concentration of heavy metals in soil after completion of nursery trial of T.ciliata (on day 180)

The results showed that the percentage reduction in concentration of Pb was maximum in treatment T4 (94.37%), followed by T3 (88.08%) and was least in control soil (T1), i.e., 58.02% (Table 3s). Concentration of Cr was maximum reduced in control soil (T1) – 68.21%, and was least reduced in T5 – 10.54% only. Treatment T3 showed the maximum reduction potential for the reduction in concentration of Cd (88.77%), as compared to treatment T11 which had the least potential for the reduction in concentration of Cd (67.00%). Concentration of Ni was maximally reduced in treatment T9 (94.37%) and was minimally reduced in T4 (43.56%). The percentage reduction in concentration of Co was ranging from 72.87% (T9) – 02.11% (T4) (Table 3s).

Table 3r: Heavy metals (Mean ± S.E.) (mg/kg) in soil, root, stem and leaves of *Toona ciliata* after six months of nursery trial

Treatments		Pb	Cr	Cd	Ni	Co
T1 (CS)	Soil	5.52 ± 0.12	2.20 ± 0.19	0.30 ± 0.03	1.97 ± 0.36	4.21 ± 0.16
	Root	5.12 ± 0.35	1.55 ± 0.23	0.30 ± 0.06	1.18 ± 0.35	3.72 ± 0.20
	Stem	3.67 ± 0.20	1.00 ± 0.17	0.43 ± 0.07	2.80 ± 0.20	3.07 ± 0.12
	Leaves	7.10 ± 0.24	1.70 ± 0.19	0.53 ± 0.07	1.15 ± 0.22	4.03 ± 0.19
T2 (S+MA)	Soil	4.68 ± 0.54	2.67 ± 0.14	0.38 ± 0.09	2.03 ± 0.18	4.38 ± 0.04
	Root	5.10 ± 0.10	2.32 ± 0.22	0.22 ± 0.04	0.77 ± 0.15	3.20 ± 0.10
	Stem	3.73 ± 0.25	1.12 ± 0.19	0.52 ± 0.09	1.55 ± 0.22	1.50 ± 0.19
	Leaves	7.07 ± 0.10	2.88 ± 0.32	0.65 ± 0.03	1.45 ± 0.32	3.60 ± 0.13
T3 (S+FA)	Soil	2.75 ± 0.08	2.98 ± 0.10	0.43 ± 0.04	5.42 ± 0.09	3.22 ± 0.19
	Root	9.25 ± 0.14	3.42 ± 0.04	0.62 ± 0.10	1.45 ± 0.23	4.50 ± 0.19
	Stem	4.05 ± 0.29	0.95 ± 0.13	0.47 ± 0.03	1.05 ± 0.24	0.90 ± 0.13
	Leaves	7.25 ± 0.25	3.00 ± 0.28	0.53 ± 0.04	1.85 ± 0.09	5.75 ± 0.62
T4 (S+FA+ MA)	Soil	1.30 ± 0.12	4.18 ± 0.25	0.55 ± 0.00	7.10 ± 0.16	4.65 ± 0.06
	Root	9.37 ± 0.22	5.03 ±0.46	0.42 ± 0.04	2.98 ± 0.22	3.33 ± 0.24
	Stem	4.73 ± 0.15	2.05 ± 0.16	0.53 ± 0.06	2.07 ± 0.17	3.90 ± 0.28
	Leaves	8.03 ± 0.26	2.70 ± 0.13	0.48 ± 0.06	2.35 ± 0.26	4.13 ± 0.44
T5 (S+FA+ DW)	Soil	6.17 ± 0.38	5.43 ± 0.32	0.43 ± 0.02	5.85 ± 0.14	3.17 ± 0.33
	Root	5.58 ± 0.19	7.60 ± 0.44	0.32 ± 0.06	1.50 ± 0.40	3.47 ± 0.31
	Stem	5.80 ± 0.35	1.07 ± 0.10	0.57 ± 0.02	0.63 ± 0.13	2.35 ± 0.18
	Leaves	10.58 ± 0.31	1.05 ± 0.20	0.68 ± 0.07	3.15 ± 0.12	2.75 ± 0.16
T6 (S+FA+ DW+MA)	Soil	7.62 ± 0.29	5.05 ± 0.14	0.60 ± 0.03	5.52 ± 0.19	4.20 ± 0.26
	Root	3.08 ± 0.19	6.97 ± 0.50	0.57 ± 0.06	3.98 ± 0.35	5.42 ± 0.29
	Stem	6.68 ± 0.36	1.17 ± 0.07	0.68 ± 0.06	0.30 ± 0.10	2.25 ± 0.13
	Leaves	13.97 ± 0.14	0.90 ± 0.05	0.87 ± 0.04	1.40 ± 0.25	4.92 ± 0.25
T7 (S+FA+ FYM)	Soil	3.30 ± 0.74	4.50 ± 0.25	0.55 ± 0.05	6.87 ± 0.19	4.70 ± 0.14
	Root	3.93 ± 0.16	4.23 ± 0.26	0.48 ± 0.04	4.85 ± 0.36	3.38 ± 0.27
	Stem	3.22 ± 0.31	1.38 ± 0.09	0.55 ± 0.05	2.37 ± 0.41	3.82 ± 0.23
	Leaves	19.47 ± 0.30	2.33 ± 0.09	0.55 ± 0.08	1.62 ± 0.26	4.93 ± 0.28
T8 (S+FA+ FYM+MA)	Soil	3.20 ± 0.46	3.77 ± 0.19	0.48 ± 0.02	6.37 ± 0.16	4.67 ± 0.06
	Root	4.50 ± 0.09	3.93 ± 0.35	0.38 ± 0.07	3.28 ± 0.33	4.07 ± 0.22
	Stem	3.93 ± 0.16	1.13 ± 0.17	0.57 ± 0.04	2.03 ± 0.32	3.75 ± 0.35
	Leaves	16.77 ± 0.16	2.80 ± 0.10	0.37 ± 0.09	2.35 ± 0.31	4.12 ± 0.39
T9 (S+DW)	Soil	7.73 ± 0.41	3.72 ± 0.21	0.47 ± 0.07	0.87 ± 0.27	1.85 ± 0.19
	Root	7.22 ± 0.26	7.67 ± 0.38	0.25 ± 0.03	2.23 ± 0.20	5.68 ± 0.45
	Stem	8.53 ± 0.26	1.92 ± 0.18	0.70 ± 0.03	2.00 ± 0.59	4.65 ± 0.30
	Leaves	16.05 ± 0.39	2.42 ± 0.31	0.68 ± 0.03	2.23 ± 0.17	6.62 ± 0.42
T10(S+ DW+MA)	Soil	8.80 ± 0.15	3.83 ± 0.12	0.60 ± 0.00	5.50 ± 0.24	5.82 ± 0.27
	Root	8.73 ± 0.51	8.20 ± 0.39	0.72 ± 0.07	4.88 ± 0.13	6.37 ± 0.63
	Stem	10.98 ± 0.09	1.87 ± 0.11	0.73 ± 0.02	1.67 ± 0.33	3.47 ± 0.26
	Leaves	14.12 ± 0.36	1.23 ± 0.14	0.52 ± 0.04	2.85 ± 0.30	5.08 ± 0.23
T11(S+ FYM)	Soil	4.72 ± 0.14	4.63 ± 0.24	0.67 ± 0.04	7.22 ± 0.22	4.88 ± 0.09
	Root	7.32 ± 0.48	2.75 ± 0.33	0.62 ± 0.12	6.58 ± 0.31	4.90 ± 0.26
	Stem	5.22 ± 0.13	1.27 ± 0.12	0.53 ± 0.07	1.53 ± 0.26	3.88 ± 0.23
	Leaves	7.63 ± 0.25	1.38 ± 0.06	0.32 ± 0.09	2.03 ± 0.22	4.62 ± 0.15
T12(S+ FYM+MA)	Soil	5.13 ± 0.58	4.87 ± 0.28	0.62 ± 0.03	7.75 ± 0.24	4.28 ± 0.19
	Root	6.48 ± 0.40	4.35 ± 0.55	0.28 ± 0.13	3.13 ± 0.25	5.43 ± 0.33
	Stem	2.63 ± 0.17	2.20 ± 0.16	0.63 ± 0.02	2.80 ± 0.40	2.62 ± 0.37
	Leaves	7.58 ± 0.41	1.28 ± 0.13	0.18 ± 0.03	2.10 ± 0.24	4.85 ± 0.28

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v))

Table 3s: Heavy metal (mg/kg) analysis in soil before plantation and after harvesting of *Toona ciliata* and percentage reduction (R) in concentration of heavy metals in soil

Treatments	Time period	Heavy metals (mg / kg) analysed in Soil									
		Pb		Cr		Cd		Ni		Co	
		In soil	R	In soil	R	In soil	R	In soil	R	In soil	R
T1 (CS)	Day 0	13.15 ± 0.84	58.02 f	6.92 ± 0.28	68.21 a	2.65 ± 0.06	88.68 a	14.97 ± 0.25	86.84 b	5.23 ± 0.08	19.50 d
	Day 180	5.52 ± 0.12		2.20 ± 0.19		0.30 ± 0.03		1.97 ± 0.36		4.21 ± 0.16	
T2 (S+MA)	Day 0	13.15 ± 0.84	64.41 e	6.92 ± 0.28	61.42 b	2.65 ± 0.06	85.66 a	14.97 ± 0.25	86.44 b	5.23 ± 0.08	16.25 e
	Day 180	4.68 ± 0.54		2.67 ± 0.14		0.38 ± 0.09		2.03 ± 0.18		4.38 ± 0.04	
T3 (S+FA)	Day 0	23.08 ± 0.29	88.08 a	4.83 ± 0.31	38.30 d	3.83 ± 0.07	88.77 a	12.58 ± 0.67	56.92 e	4.75 ± 0.10	32.21 b
	Day 180	2.75 ± 0.08		2.98 ± 0.10		0.43 ± 0.04		5.42 ± 0.09		3.22 ± 0.19	
T4 (S+FA+MA)	Day 0	23.08 ± 0.29	94.37 a	4.83 ± 0.31	13.46 f	3.83 ± 0.07	85.64 a	12.58 ± 0.67	43.56 f	4.75 ± 0.10	02.11 g
	Day 180	1.30 ± 0.12		4.18 ± 0.25		0.55 ± 0.00		7.10 ± 0.16		4.65 ± 0.06	
T5 (S+FA+DW)	Day 0	32.92 ± 0.97	81.26 b	6.07 ± 0.67	10.54 g	2.58 ± 0.08	83.33 a	14.17 ± 0.42	58.72 e	4.27 ± 0.20	25.76 c
	Day 180	6.17 ± 0.38		5.43 ± 0.32		0.43 ± 0.02		5.85 ± 0.14		3.17 ± 0.33	
T6 (S+FA+DW+MA)	Day 0	32.92 ± 0.97	76.85 c	6.07 ± 0.67	16.80 f	2.58 ± 0.08	76.74 b	14.17 ± 0.42	61.04 d	4.27 ± 0.20	01.64 g
	Day 180	7.62 ± 0.29		5.05 ± 0.14		0.60 ± 0.03		5.52 ± 0.19		4.20 ± 0.26	
T7 (S+FA+FYM)	Day 0	14.05 ± 0.30	76.51 c	5.30 ± 0.09	15.09 f	1.82 ± 0.07	69.78 c	15.92 ± 0.51	56.85 e	5.57 ± 0.34	1.62 g
	Day 180	3.30 ± 0.74		4.50 ± 0.25		0.55 ± 0.05		6.87 ± 0.19		4.70 ± 0.14	
T8 (S+FA+FYM+MA)	Day 0	14.05 ± 0.30	77.22 c	5.30 ± 0.09	28.87 e	1.82 ± 0.07	73.63 c	15.92 ± 0.51	59.99 d	5.57 ± 0.34	16.16 e
	Day 180	3.20 ± 0.46		3.77 ± 0.19		0.48 ± 0.02		6.37 ± 0.16		4.67 ± 0.06	
T9 (S+DW)	Day 0	32.27 ± 0.60	76.05 c	5.18 ± 0.22	28.19 e	2.08 ± 0.19	77.40 b	15.45 ± 0.24	94.37 a	6.82 ± 0.14	72.87 a
	Day 180	7.73 ± 0.41		3.72 ± 0.21		0.47 ± 0.07		0.87 ± 0.27		1.85 ± 0.19	
T10(S+DW+MA)	Day 0	32.27 ± 0.60	72.73 d	5.18 ± 0.22	26.06 e	2.08 ± 0.19	71.15 c	15.45 ± 0.24	64.40 c	6.82 ± 0.14	14.66 c
	Day 180	8.80 ± 0.15		3.83 ± 0.12		0.60 ± 0.001		5.50 ± 0.24		5.82 ± 0.27	
T11(S+FYM)	Day 0	35.32 ± 0.39	86.64 b	8.70 ± 0.05	46.78 c	2.03 ± 0.09	67.00 d	17.03 ± 0.43	57.60 e	5.37 ± 0.32	09.12 f
	Day 180	4.72 ± 0.14		4.63 ± 0.24		0.67 ± 0.04		7.22 ± 0.22		4.88 ± 0.09	
T12(S+FYM+MA)	Day 0	35.32 ± 0.39	85.48 b	8.70 ± 0.05	44.02 c	2.03 ± 0.09	69.46 c	17.03 ± 0.43	54.49 e	5.37 ± 0.32	20.30 d
	Day 180	5.13 ± 0.58		4.87 ± 0.28		0.62 ± 0.03		7.75 ± 0.24		4.28 ± 0.19	

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v)) Values followed by the same letter within a column are not statistically different

4.3 To study the survival and establishment of molecular tagged microbes in soils amended with solid waste

To study the survival and establishment of molecular tagged microbes in soils amended with solid wastes, *Pseudomonas striata* and *Azotobacter* CBD15 were transformed with plasmid pMMB277 (isolated from *E.coli* 2842) carrying dual marker gene. The growth pattern of all the bacterial strains was monitored before and after transformation. A consortium of both phosphate solubilizing and nitrogen fixing bacteria was inoculated in nursery soil of *Populus deltoides* and *Toona ciliata* with un-inoculated control.

4.3.1 Microbial Growth

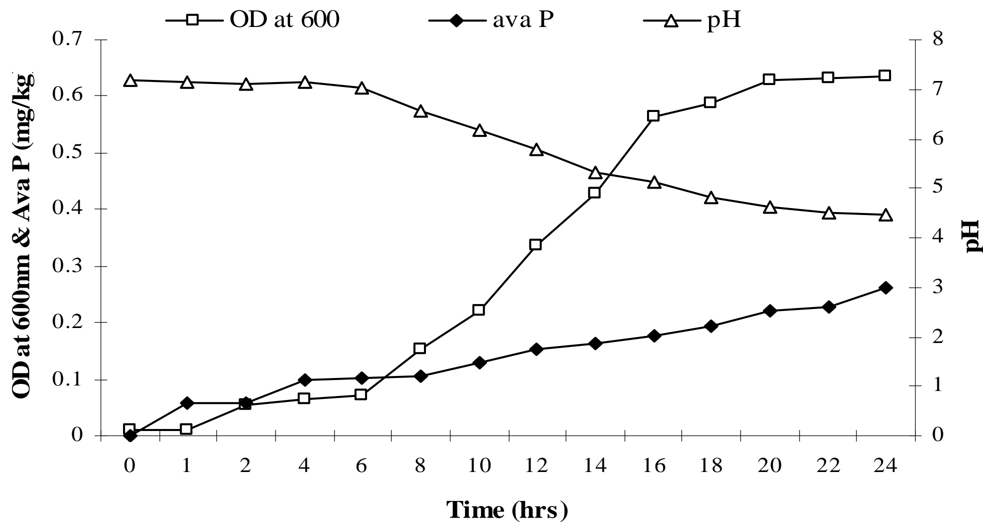
Growth of *Pseudomonas striata* and *Azotobacter* CBD15 in pure culture condition was monitored over a period of 24 hours by taking absorbance at 600nm. The growth of *P. striata* was monitored in Pikovskaya broth supplemented with 0.5% tri calcium phosphate at an interval of 2 hrs over a period of 24 hrs, alongwith changes in the pH of the medium and concentration of available P. The results revealed that the absorbance increased from 0.011 at 0 hr to 0.636 after 24 hr of incubation and concentration of available P increased from 0 mg/kg (0 hr) to 0.262 mg/kg (24 hr), whereas pH decreased from neutral (7.2) to acidic (4.5) (Table 4a , Fig. 2a).

pH of the medium did not show much change till the microorganism was in lag phase, as soon as the bacterial strain entered the log phase the pH of the medium showed a sharp decline till the bacterial strain entered the stationary phase (Fig. 2a). Also, concentration of available phosphorus increased at its maximum during the log phase of the bacterial strain (Fig 2a).

Table 4a: Absorbance of *Pseudomonas striata* at 600nm in Pikovskaya's broth, change in pH and available P

Time(hrs)	OD_{600}	Available P (mg kg^{-1})	pH
0	0.011	0	7.2
1	0.011	0.056	7.2
2	0.055	0.059	7.1
4	0.064	0.099	7.2
6	0.073	0.102	7.0
8	0.152	0.106	6.6
10	0.221	0.128	6.2
12	0.336	0.154	5.8
14	0.427	0.162	5.3
16	0.564	0.176	5.1
18	0.589	0.195	4.8
20	0.628	0.220	4.6
22	0.631	0.229	4.5
24	0.636	0.262	4.5

Fig. 2a: Growth of *Pseudomonas striata* in Pikovskaya's broth, change in pH and available P



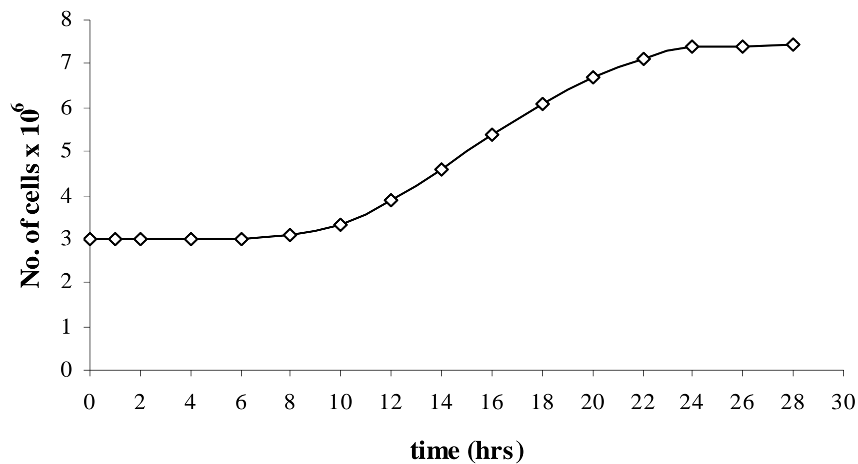
The growth of *Azotobacer* CBD15 in Jensen's broth over a period of 24 hrs (Fig. 2b). The culture came to its log phase in approximately 10-12hrs (Table 4b).

Table 4b: No. of cells of *Azotobacter* CBD15 grown in Jensen's media

Time (hrs)	**No. of cells x 10^6**
0	3.00
1	3.00
2	3.00
4	3.00
6	3.01
8	3.02
10	3.33
12	3.86
14	4.60
16	5.39
18	6.10
20	6.67
22	7.09
24	7.39
26	7.40
28	7.42

The bacterial strain was in lag phase for 8-10 hours and entered its log phase after 10 hours and after 24 hours of growth it entered into its stationary phase (Fig. 2b).

Fig. 2b: Growth of *Azotobacer* CBD15 grown in Jensen's broth



4.3.2 Antibiotic Profiling

Antibiotic profiling of *Psedomonas striata* and *Azotobacter* CBD15 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 µg ml^{-1}), kanamycin (50 µg ml^{-1}), nalidixic acid (50 µg ml^{-1}) and chloramphenicol (10µg ml^{-1}), respectively. *P. striata* showed resistance to streptomycin and nalidixic acid, and was sensitive to chloramphenicol, kanamycin and rifampicin, whereas, *Azotobacter* CBD15 was resistant to all test antibiotics (Table 4c), and kanamycin resistant character was used for its ecological monitoring.

Table 4c: Antibiotic profiling of *P. striata* and *Azotobacter* CBD15

Antibiotics	**Concentration**	***P. striata***	***Azotobacter* CBD15**
Chloramphenicol	10	S	R
Kanamycin	25	S	R
Streptomycin	50	R	R
Nalidixic acid	50	R	R
Rifampicin	50	S	R

S: Sensitive; R: Resistant; Concentration µg ml^{-1}

4.3.3 Bacterial transformation

Bacterial strains *P. striata* and *Azotobacter* CBD15 were transformed with plasmid pMMB277 containing *lacZ* and chloramphenicol resistant gene as molecular markers. Plasmid pMMB277 (Gene, 1997) (Fig. 2c) 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. 2d shows electrophoretogram on 0.7% agarose gel of the plasmid having size 9.237 Kb. The plasmid was purified from low melting agarose (Fig. 2e). *P. striata* and *Azotobacter* CBD15 were transformed with purified pMMB277 plasmid and transformant was obtained only in the case of *P. striata* which was named *P. striata* *($lacZ^+$)*. It was checked for galactosidase expression on chloramphenicol + IPTG + X-gal plates and for plasmid pMMB277 by agarose gel electrophoresis (Fig. 2f). *Azotobacter* CBD15 was not transformed with plasmid pMMB277, therefore, its inherent kanamycin resistant character was used for its ecological monitoring.

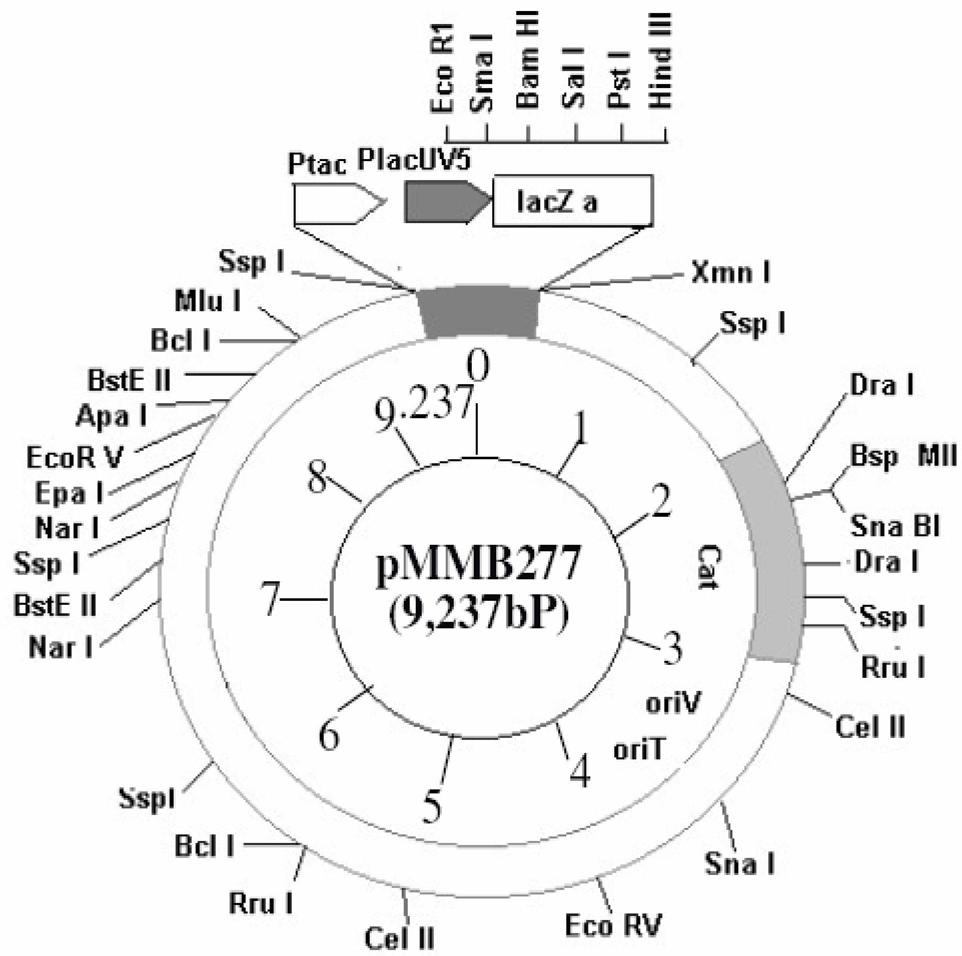


Fig. 2c: Map of pMMB277 plasmid broad host range, artificial cloning vector (Gene, 1997).

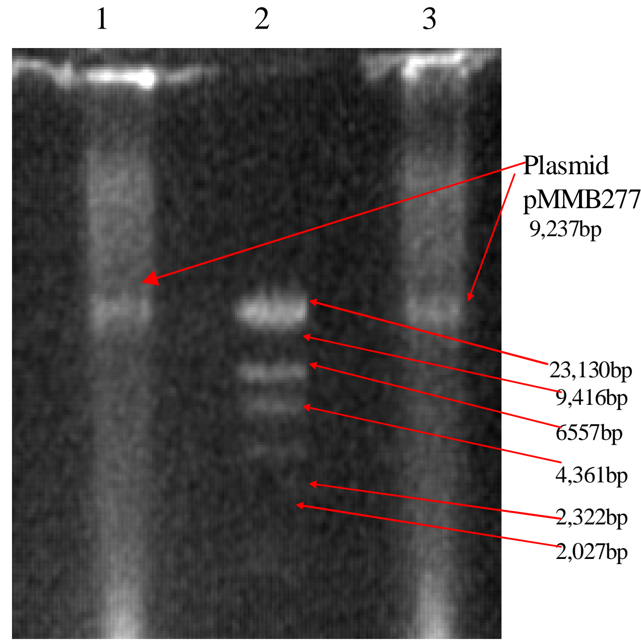


Fig. 2d: Agarose gel (0.7%) electrophoretogram of plasmid pMMB277 isolated by alkali lysis method and Lambda marker cut by Hind III
Lane 1: Plasmid pMMB277 isolated from *E.coli* 2842
Lane 2: Lambda marker cut by Hind III
Lane 3: pMMB277 isolated from *P.striata* (transformant)

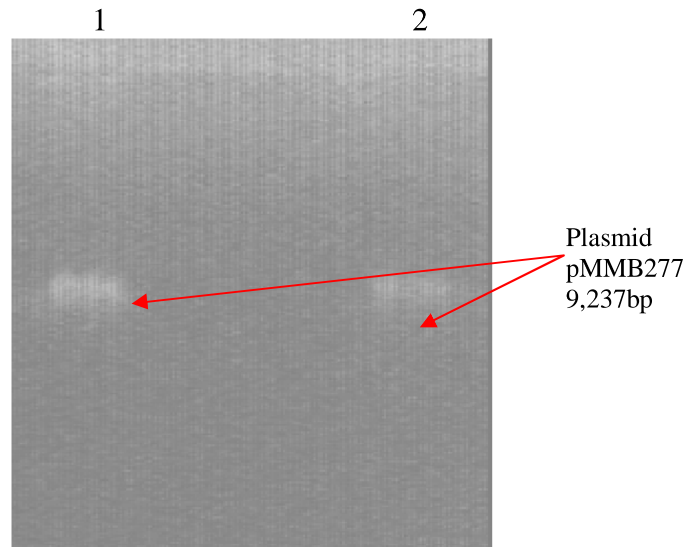


Fig. 2e: Agarose gel (0.7%) electrophoretogram of purified plasmid
Lane 1: Plasmid pMMB277 isolated from *E.coli* 2842
Lane 2: Plasmid pMMB277 isolated from *P. striata (lacZ^{+})*

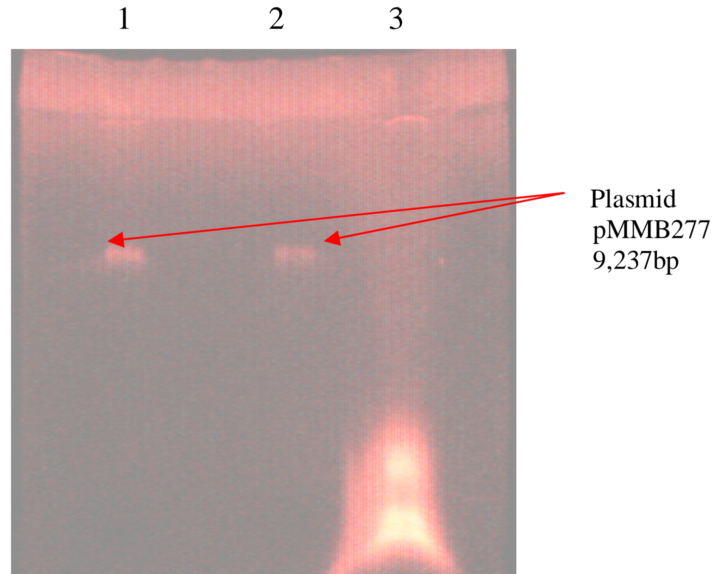


Fig. 2f: Agarose gel (0.7%) electrophoretogram of plasmid pMMB277 isolated by alkali lysis method from *E.coli* 2842 and *Pseudomonas striata* transformed with pMMB27
Lane 1 & 2: Plasmid pMMB277 isolated by alkali lysis method from *E.coli* 2842 and *Pseudomonas striata* transformed with pMMB27
Lane 3: Result of alkali lysis of wild *Pseudomonas striata as control*

4.3.4 Growth of transformed P. striata (lacZ$^+$)

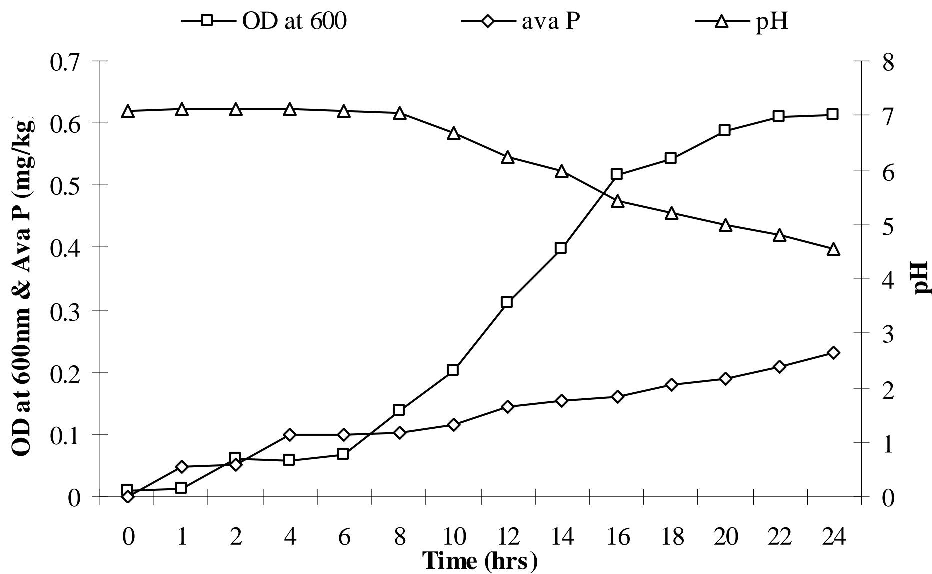
The growth of transformed *P. striata (lacZ$^+$)* was monitored in PKV broth at an interval of 2 hrs over a period of 24 hrs, alongwith changes in pH of the medium and concentration of available P in broth. The results revealed that the absorbance increased from 0.011 at 0 hr to 0.612 at 24 hr, concentration of available P increased from 0 mg/kg (0 hr) to 0.23 mg/kg (24 hr), and pH decreased from neutral (7.1) to acidic (4.6) (Table 4d).

This showed that *P. striata (lacZ$^+$)* phosphate solubilization was comparable to that of *P. striata* and a similar pattern of solubilization of insoluble phosphate and decrease in pH was observed. pH of the medium did not show much change till the microorganism was in lag phase, as soon as the bacterial strain entered the log phase the pH of the medium showed a sharp decline till the bacterial strain entered the stationary phase. Also, concentration of available phosphorus increased at its maximum during the log phase of the bacterial strain (Fig 2g).

Table 4d: Absorbance of *Pseudomonas striata (lacZ⁺)* at 600nm in Pikovskaya's broth, changes in pH and available P

Time(hrs)	OD_{600}	Available P (mg kg^{-1})	pH
0	0.011	0	7.1
1	0.013	0.047	7.1
2	0.062	0.052	7.1
4	0.059	0.099	7.1
6	0.069	0.099	7.1
8	0.139	0.101	7.0
10	0.203	0.117	6.7
12	0.312	0.145	6.2
14	0.398	0.154	6.0
16	0.516	0.162	5.4
18	0.543	0.179	5.2
20	0.589	0.189	5.0
22	0.610	0.210	4.8
24	0.612	0.230	4.6

Fig. 2g: Growth of *P. striata (lacZ⁺)* in Pikovskaya's broth, changes in pH and available Phosphorus



4.3.5 Survival and establishment of molecular tagged microbes in soils amended with solid waste in nursery trial

Ecological monitoring of tagged bacterial strain was done to study survival and establishment of molecular tagged microbes in soils amended with solid waste in nursery trial of *Populus deltoides* and *Toona ciliata*. *P. striata* ($lacZ^+$) and *Azotobacter* CBD15 (kan^R) were mass cultivated and inoculated in soil @ 15 ml /pot in all treatments. The ecological monitoring in terms of their population build-up in soil was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient agar plates and PKV plates separately and kanamycin containing Jensen's media plates. Representative samples were drawn aseptically from each pot after harvesting and analyzed for total viable-cell counts, performed by spread-plating 0.1 ml of dilutions in sterile distilled water on nutrient agar, Pikovskaya,s and Jensen's media plates.

The results showed that from the rhizospheric soil of *P. deltoides* the total culturable bacteria plated on NA (Nutrient agar) plates was found to be maximum in treatment T8 (315 Cfu x 10^6) followed by T7 (290 Cfu x 10^6) and minimum in treatment T9 (117 Cfu x 10^6). The same pattern was observed for phosphate solubilizing bacteria and nitrogen fixing bacteria, i.e., maximum cfu count was observed in treatment T8 – 71 and 24 Cfu x 10^6 respectively, when cultured on PKV and Jensen's media plates respectively. The same was confirmed by analyzing total microbial biomass present in the soil, it revealed that the microbial biomass was highest in treatment T8 (247 µg g^{-1}) followed by treatment T7 (223 µg g^{-1}) and was least in T9 (169 µg g^{-1}) (Table 4e).

After 48 hours of incubation of rhizospheric soil from all treatments of *T. ciliata* the total culturable bacteria on nutrient agar plates was found to be maximum in treatment T8 (295 Cfu x 10^6) and was least in treatment T9 (92 Cfu x 10^6). Both total phosphate solubilizing and nitrogen fixing bacteria were also found to be maximum in no. in treatment T8 (67 & 21 Cfu x 10^6) respectively. The microbial biomass was also maximum in treatment T8 (219 µg g^{-1}) and was minimum in T9 (117 µg g^{-1}) (Table 4e).

Table 4e: Enumeration of phosphate solubilizing and nitrogen fixing bacteria and estimation of microbial biomass in rhizospheric soils of *Populus deltoides* and *Toona ciliata*

Treatments	**Cfu x 10^6** Total culturable bacteria (Nutrient agar)		Total phosphate solubilizing bacteria (Pikovskaya's media)		Total nitrogen fixing bacteria (Jensen's media)		**Microbial biomass (µg g^{-1})**	
	P. deltoides	*T. ciliata*	*P. deltoides*	*T. ciliata*	*P. deltoides*	*T. ciliata*	*P. deltoides*	*T. ciliata*
T1 (CS)	165	140	38	31	07	06	182	145
T2 (S+MA)	220	192	50	45	17	13	206	187
T3 (S+FA)	190	170	39	32	09	08	194	167
T4 (S+FA+MA)	270	255	62	58	22	19	217	192
T5 (S+FA+DW)	152	137	17	12	06	06	197	172
T6 (S+FA+DW+MA)	168	156	38	34	19	17	204	183
T7 (S+FA+FYM)	290	240	45	39	09	07	223	204
T8 (S+FA+FYM+MA)	315	295	71	67	24	21	247	219
T9 (S+DW)	117	92	15	11	05	03	169	117
T10(S+DW+MA)	157	114	34	30	12	09	176	132
T11(S+FYM)	180	152	42	37	09	07	197	169
T12(S+FYM+MA)	260	216	66	59	20	18	219	193

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v)) Values are average of 3 replicates.

Blue coloured lactose positive and chloramphenicol resistant colonies of *P. striata* (*lacZ*$^+$) were counted on Pikovskaya media plates containing 10µg/ml chloramphenicol and after being lightly spreaded with a solution of IPTG (Isopropyl-β-D-thiogalactopyranoside) (0.1g/ml in sterile water), X-Gal (20mg/ml in dimethyl formamide) and sterile water in ratio (8:30:40). 12-18% of the total culturable bacteria showed *lacZ*$^+$ character and were resistant to chloramphenicol, in comparison to 0.02 - 0.05 % total culturable bacteria showing both the traits as background microflora in soil (Table 4f and 4g), their phosphate solubilizing activity was further confirmed on NBRI-BPB media plates. The enumeration of nitrogen fixing bacteria also revealed that their population was maximum in treatment T8. When cultured on NA plates containing kanamycin it was found that the kanamycin resistant character was possessed by some of the bacterial colonies but upon culturing on Jensen's media plates containing kanamycin it was confirmed that very few nitrogen fixing bacteria possessed the trait of being resistant to test antibiotic, so it was easy to monitor *Azotobacter* CBD15 (*kan*^{R}) (Table 4f and 4g).

Table 4f: Enumeration of tagged bacteria in rhizospheric soils of *Populus deltoides* on selective media after 180 days of nursery trial

Treatments	**Cfu x 10^6**			
	NA + IPTG + X-gal + Chl	**NA + Kanamycin**	**PKV + IPTG + X-gal + Chl**	**JM + Kanamycin**
T1 (CS)	06	07	05	01
T2 (S+MA)	96	12	86	08
T3 (S+FA)	03	09	03	01
T4 (S+FA+MA)	106	14	95	09
T5 (S+FA+DW)	06	05	04	01
T6 (S+FA+DW+MA)	119	11	90	10
T7 (S+FA+FYM)	06	09	05	02
T8 (S+FA+FYM+MA)	129	17	107	14
T9 (S+DW)	03	03	02	01
T10(S+DW+MA)	70	05	56	02
T11(S+FYM)	05	09	04	01
T12(S+FYM+MA)	109	14	92	10

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v)) Values are average of 3 replicates.

Table 4g: Enumeration of tagged bacteria in rhizospheric soils of *Toona ciliata* on selective media after 180 days of nursery trial

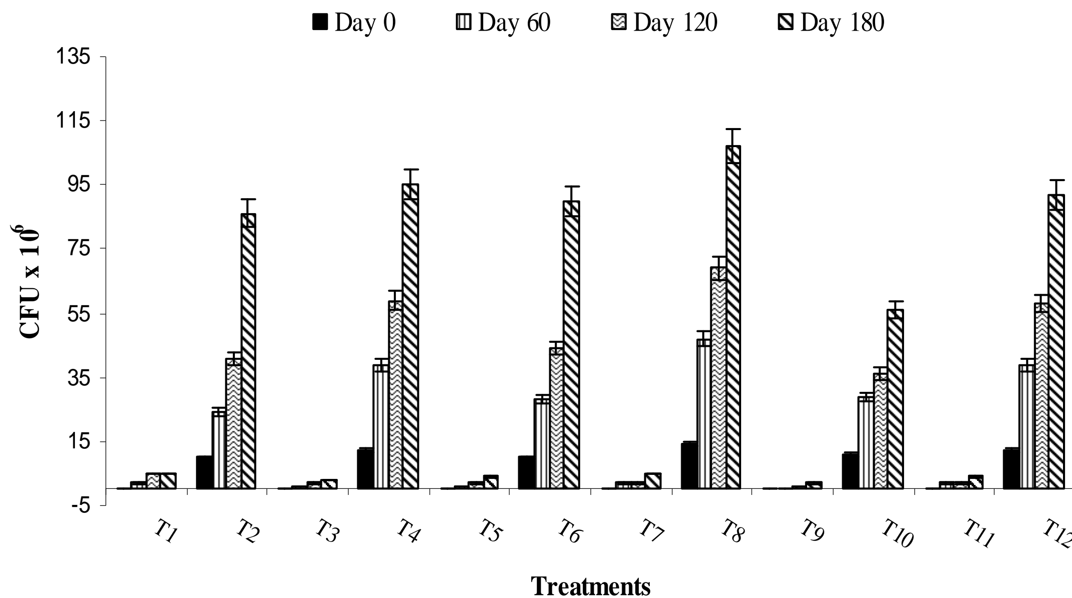
Treatments	**Cfu x 10^6**			
	NA + IPTG + X-gal + Chl	**NA + Kanamycin**	**PKV + IPTG + X-gal + Chl**	**JM + Kanamycin**
T1 (CS)	05	11	03	01
T2 (S+MA)	73	19	56	15
T3 (S+FA)	03	12	03	02
T4 (S+FA+MA)	79	24	65	19
T5 (S+FA+DW)	04	06	03	01
T6 (S+FA+DW+MA)	72	11	58	08
T7 (S+FA+FYM)	04	09	03	02
T8 (S+FA+FYM+MA)	99	29	87	21
T9 (S+DW)	03	07	02	01
T10(S+DW+MA)	71	10	56	07
T11(S+FYM)	04	09	03	01
T12(S+FYM+MA)	89	26	72	19

(CS: Control Soil; S: Soil; MA: Microbial amendment @ 30 ml/pot; FA: Fly ash @ 10% (v/v); DW: Distillery waste @ 05% (v/v) and FYM: Farm yard manure @ 20% (v/v)) Values are average of 3 replicates.

The enumeration of inoculated bacteria on selective media indicated that with the addition of fly ash and farm yard manure there was a positive effect on soil microbial population and an optimum concentration of 10% FA is tolerable for microbes indicating that its amendment provides micronutrients for growth.

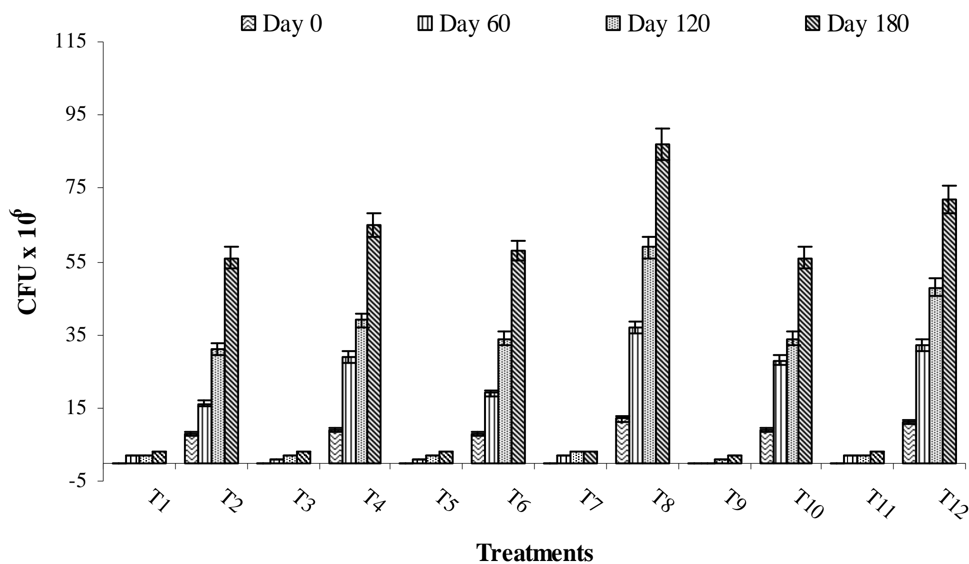
Enumeration of *P. striata ($lacZ^+$)* carried out on PKV plates containing chloramphenicol (10µg ml^{-1}), IPTG and X-gal and the bacteria were identified as blue colonies. On day zero of incubation, bacterial populations was zero cfu g^{-1} soil in uninoculated treatments and $10x10^6$ to $14x10^6$ cfu g^{-1} soil in various inoculated treatments (Fig. 2h) in rhizospheric soil of *P. deltoides*. After 60 days of nursery trial tagged bacterial counts ranged from zero cfu g^{-1} soil to $47x10^6$ cfu g^{-1} soil in treatment amended with fly ash, farmyard manure and microbial consortium (treatment T8) over a period of 180 days of incubation it rose to $107x10^6$ cfu g^{-1} soil again in treatment T8 (amended with fly ash, farmyard manure and microbial consortium) in *P. deltoides*.

Fig. 2h: Enumeration of tagged *P. striata ($lacZ^+$)* in rhizospheric soil of *Populus deltoides* over a period of 180 days



In *T. ciliata* the same trend was observed and the population ranged from zero cfu g^{-1} soil to $12x10^6$ cfu g^{-1} soil on day 0 and after 120 days of nursery trial, in inoculated treatments it ranged from $31x10^6$ cfu g^{-1} soil - $59x10^6$ cfu g^{-1} soil. Over a period of 180 days the tagged bacterial count ranged from $56x10^6$ cfu g^{-1} soil – $87x10^6$ cfu g^{-1} soil (Fig. 2i).

Fig. 2i: Enumeration of tagged *P. striata (lacZ⁺)* in rhizospheric soil of *Toona ciliata* over a period of 180 days



Azotobacter CBD15 counts in uninoculated soil was zero cfu g^{-1} soil and in inoculated it ranged from $1x10^6$ cfu g^{-1} soil - 3 $x10^6$ cfu g^{-1} soil on day zero of nursery trial for *P. deltoides* and ranged from $1x10^6$ cfu g^{-1} soil - 2 $x10^6$ cfu g^{-1} soil in inoculated treatments of *T. ciliata.* After a period of 60 days the tagged bacterial count ranged from 1 $x10^6$ cfu g^{-1} soil - 5 $x10^6$ cfu g^{-1} soil, and over a period of 180 days the bacterial population was ranging from 7 $x10^6$ cfu g^{-1} soil - 21 $x10^6$ cfu g^{-1} soil in inoculated treatments (Fig. 2j and 2k).

Fig. 2j: Enumeration of *Azotobacter* CBD15 *(kan*^{R}*)* in rhizospheric soil of *P. deltoides*

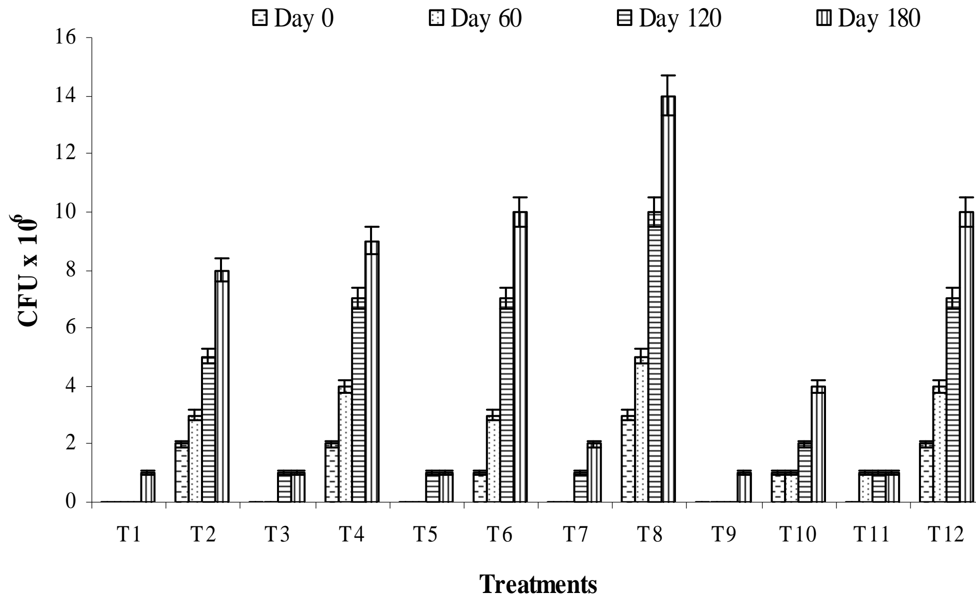
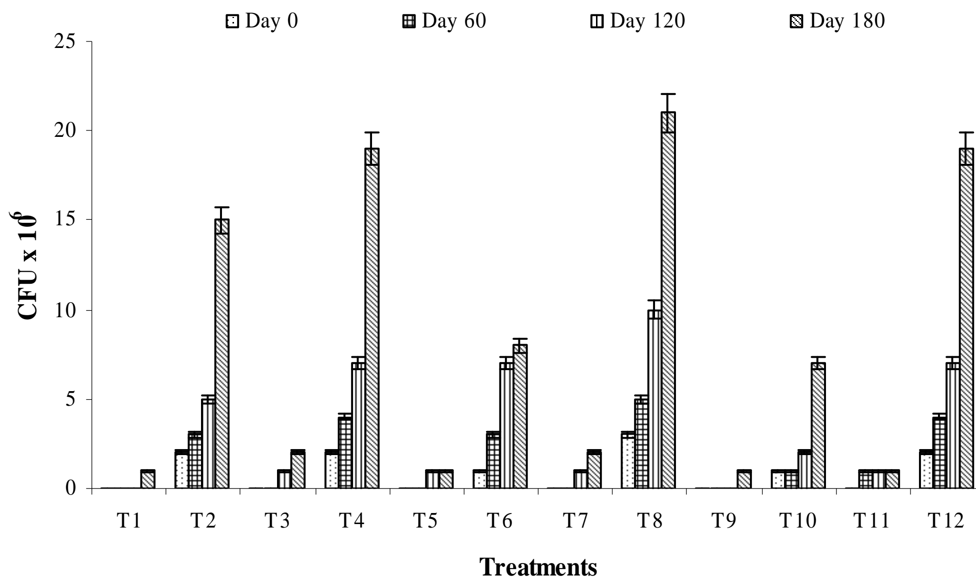


Fig. 2k: Enumeration of *Azotobacter* CBD15 *(kan*^{R}*)* in rhizospheric soil of *Toona ciliata*



Bacterial count and microbial biomass were higher in soil inoculated with phosphate solubilizing and nitrogen fixing bacteria compared to uninoculated soil amended with various amendments, which might be due to better proliferation of the isolate in the amended soil. Improved CFU/g of Phosphate Solubilizing Bacteria (PSB) and nitrogen fixing bacteria in the treatment receiving fly ash @10% (v/v) and farm yard manure @ 20% (v/v) with marked PSB inoculation over a period of 180 days proved that fly ash may be added to soil up to 10% (v/v) level with no adverse effect on inoculated bacteria.

Soil with fly ash and farm yard manure provided the best supporting environment for the total soil microbial population, as well as for inoculated tagged phosphate solubilizing and nitrogen fixing bacteria in the rhizospheric soils of *P. deltoides* and *T. ciliata*.

Chapter 5
Discussion

5.1 To study the effect of beneficial soil microbes and solid waste amendment in soil on percentage survival and growth of nursery seedlings of *Populus deltoides* and *Toona ciliata*

5.1.1 Physico-chemical and microbiological characterization of soil, fly ash, distillery waste and farmyard manure

Physico- chemical characterization of soil, fly ash, distillery waste and farmyard manure revealed that soil had a sandy loam texture and fly ash had loamy sand texture. Distillery waste had high water holding capacity, electrical conductivity, available nitrogen, phosphorus, potassium and sulphur. pH was found to be alkaline for soil, distillery waste and farmyard manure whereas, fly ash had acidic pH of 6.09, therefore, the release of micronutrients was higher (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 hydroxyl ions followed by formation of insoluble hydroxides or oxides. Microbiological characterization revealed that farmyard manure had maximum bacterial count (3.2 x 10^8 cfu gm^{-1}) followed by soil (3.1 x 10^6 cfu gm^{-1}) and distillery waste (2.6 x 10^4 cfu gm^{-1}). Fly ash did not possess any kind of bacterial micro flora and is inert (Jala and Goyal, 2006). Microbial biomass showed the following trend: farmyard manure (276 µg g^{-1}) > distillery waste (208 µg g^{-1}) > soil (172 µg g^{-1}) > fly ash (nil) (Table 2c), FYM is a source of most essential plant nutrients and, hence, is a complete fertilizer for sustaining production of maize and other crops provided that other abiotic and biotic factors are favorable (Negassa *et al.,* 2005).

Moreover, FYM application helps to maintain soil organic matter content and soil biological activity. In other words, the application of FYM continuously could improve the soil physico chemical properties and sustain production and productivity (Negassa *et al.,* 2005). Microbiological activity in terms of bacterial counts and microbial biomass

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).

5.1.2 Percentage survival and Biometric Parameters (plant height, collar diameter and total dry biomass)

The percentage survival of *P. deltoides* was found to be around 75% and for *T. ciliata* it was around 25% in control soil. For both the tree species survival was maximum in treatment T8, soil amended with fly ash and farm yard manure, co-inoculated with microbial consortium which was 93.75% in *P. deltoides* and 83.33% in *T. ciliata* (Table 2d and 2e) showing positive effect of soil amendments on survival percentage as compared to control. Various biometric parameters such as plant height, collar diameter and total dry biomass were studied to determine the impact of different soil amendments (FA, DW and FYM) either singly or in different combinations with and without microbial consortium (*P. striata* and *Azotobacter sp*.) on the growth of two agroforestry tree species, *Populus deltoides* and *Toona ciliata* in nursery. With the addition of fly ash alone the total dry biomass production was increased by 49.4% in *P. deltoides* and 11.58% in *T. ciliata* and infact, the combination of fly ash @ 10% (v/v), farmyard manure @ 20% (v/v) and microbial consortium @ 30 ml/pot led to a further increase in plant height by 43.8%, collar diameter by 40.7% and total dry biomass by 45.1% in *P. deltoides* and *in T. ciliata* plant height was increased by 30.3%, collar diameter by 16.2% and total dry biomass by 14.4%, respectively. These results are in accordance with the application of fly ash at 40 t/ha in conjunction with phosphate solubilizer, *Pseudomonas striata* which improved the bean yield and phosphorus uptake by grain and fly ash had no negative effect on the population of *P. striata* in soil (Gaind and Gaur 2002). Fly ash has shown an increase in the average plant height, root length, yield as well as biomass (Kumar, 2002) of wheat (*Triticum vulgare*), mustard (*Brassica juncea*), pea (*Pisum sativum*) and gram (*Cicer arientinum*). An increase in fresh weight (114.91%) is seen in *Cucumis sativus* (Ajaz and Tiyagi, 2003) when soil is treated with fly ash (25%). Fly ash is a useful ameliorant that improves the physical, chemical and biological properties of problem soils and is a source of readily available plant macro and micro nutrients (Jala and Goyal, 2006).

In conjunction with organic manure, microbial inoculants or biofertilizers, 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. In field trials conducted on degraded soils using ESP fly ash at 18–24% (v/v), 10% increase in the growth of *Eucalyptus tereticornis, Acacia auriculiformis* and *Casuarina equisetifolia* during the initial 6 months was observed (Goyal *et al.,* 2002). Fly ash at 10–20% level (v/v) was also found to be a good potting mix material in nurseries (Goyal *et al.* 2002). Treatments (T7, T11 and T12) having fly ash, farmyard manure and microbial inoculants had a significant impact on plant growth, however it was less pronounced in *T. ciliata*. As indicated in its chemical composition, the applied FA and FYM supplied the crop with considerable amounts of different essential macronutrients and small amounts of micronutrients usually deficient in soils (Negassa *et al.,* 2005).

Two-way Analysis of Variance also showed that the addition of fly ash, farmyard manure had a significant impact on the biometric parameters of *P. deltoides*, and the addition of microbial consortium had significant impact on total dry biomass and collar diameter, whereas, the impact on stem height was non-significant. The interaction between fly ash, farmyard manure and microbial consortium was also significant and had significant impact on all the biometric parameters. The addition of fly ash and farmyard manure had significant impact only on collar diameter and stem height of *T. ciliata*; it did not have a significant impact on total dry biomass of the plant. The addition of microbial consortium had significant impact only on the stem height of the plant and showed non significant impact on the rest of the biometric parameters. Biometric parameters upon analysis indicated that the treatment comprising of fly ash @ 10% (v/v), farmyard manure @ 20% (v/v) and microbial consortium @ 30 ml/pot promoted growth of *P. deltoides*. This could be accounted for improved magnesium, calcium and nitrogen uptake with fly ash and farmyard manure and is in agreement with the findings of Burd *et al.,* (2000), Belimov *et al*., (2004) and Nwaichi *et al*., (2010). They reported that the uptake of different nutrients improves the growth of plants on contaminated soils. The results indicated hat combined addition of fly ash, farm yard manure and microbial inoculants can be used as a good potting mixture for improving survival rates and plant growth in forestry nurseries (Aggarwal and Goyal, 2009).

A combination of fly ash, farmyard manure and microbial amendment had an overall great impact on the plant growth of both the tree species. Treatment with distillery waste however, as one of the soil amending agent showed a decrease in the plant growth which might be due to the high electrical conductivity of 16 mS/cm (Table 2b) leading to toxicity which was not tolerated by the test plants. The locally available non conventional sources of plant nutrients and soil conditioners (industrial wastes, low grade ores, agricultural wastes, sewage sludge, green manures, biofertilizers) can help to improve the environment and ecology as the waste can be utilized for enhancing yield and will also help to solve the disposal problem of wastes (TIFAC publication 2001).

5.2 To study the interaction between beneficial soil microbes and solid waste and uptake of heavy metals (Cr, Zn, Fe, Cd, Pb) by root, stem and leaves.

5.2.1 Interaction between fly ash, farmyard manure and microbial consortium and their effect on biometric parameters

The interaction between fly ash, farmyard manure and microbial consortium was significant and had significant impact on all the biometric parameters of *P. deltoides* with P value < 0.0001 for total dry biomass and stem height and P value $= 0.0496$ for collar diameter. The percentage of variance for interaction between the soil amendments for total dry biomass was 16.58%, for collar diameter it was 4.61% and for stem height it was 4.70% (Table 3a). The interaction between distillery waste and microbial consortium was non significant. The addition of microbial consortium to different treatments also showed a significant impact on total dry biomass and collar diameter (P value < 0.0001), whereas, the impact on height was non-significant with a P value of 1.0.

The interaction between fly ash, farmyard manure and microbial consortium in rhizospheric soil of *T. ciliata* was non significant and the percentage of variance of interaction was 1.17% for total dry biomass, 0.63% for collar diameter and 0.30% for stem height (Table 3b). The addition of microbial consortium had significant impact only on the stem height of the plant and showed non significant impact on the rest of the biometric parameters, viz. total dry biomass and collar diameter. Two-way ANOVA for *T. ciliata* showed that the addition of fly ash and farmyard manure had significant impact

only on collar diameter and stem height with a P value of <0.0001 and 0.0032 respectively, it did not have a significant impact on total dry biomass of the plant. The addition of distillery waste had non significant impact on the biometric parameters. Ocampo *et al*. (1975) found that *Azotobacter* and phosphobacteria in the rhizospheric soils were more when plants were inoculated with consortium of these organisms in comparison of individual inoculation. Addition of 2% farmyard manure to the richer soil enhanced this effect. Statistical analysis revealed that the N/P fertilizers and FYM significantly ($p < 0.05$) increased grain yield in all locations except for Walda in 1997. Interactions of FYM and NP fertilizer rates were significant ($p \leq 0.05$) at all locations. The application of FYM alone at rates of 4, 8, and 12 t ha^{-1} produced average grain yields of 5.76, 5.61 and 5.93 t ha^{-1}, respectively, compared to 3.53 t ha^{-1} for the control treatment. There were significant residual effects of FYM and NP fertilizers applied in 1997 on maize grain yields in 1998 (Negassa *et al*., 2005). Pearl millet production was sustainable over the years with the application of FYM (sheep/goat manure) @ 10 t ha^{-1} once in two years compared to 40 kg N ha^{-1} as urea (Rao and Shaktawat, 2002; Balakrishnan *et al.,* 2010).

5.2.2 Physico-chemical analysis of soil on completion of nursery trial

In *P. deltoides* the results showed that distillery waste had a marked effect on pH and electrical conductivity of the soil, while addition of fly ash @10% (v/v) improved the water holding capacity of the soil. Water holding capacity of treatment T3 was found to be increased by 17.86% as compared to day zero of the nursery trial. Bulk density was ranging from 1.03 gm/cm^3 (T9) - 1.20 gm/cm^3 (T3) (Table 3h). Organic carbon was maximum in treatments T6 & T10 due to the amendment with distillery waste, as distillery waste is rich in organic carbon. Available N and P were found in their maximum concentration in treatments amended with fly ash, whereas S had its maximum concentration in treatment T10 amended with distillery waste and microbial consortium.

Distillery waste also had a marked effect on pH and electrical conductivity of the soil in nursery trial of *Toona ciliata*, while addition of fly ash @10% (v/v) and farmyard manure @20% (v/v) improved the water holding capacity of the soil by 5.2%. In *T. ciliata*

organic carbon content and available nitrogen were maximum in treatment T11 followed by T12 amended with FYM, as FYM is a rich source of organic carbon, whereas, available phosphorus was maximum in treatment T12 followed by T11 and available sulphur showed its maximum concentration in treatment T8 amended with fly ash, farmyard manure and microbial inoculants, which in turn showed that available P and S were more in treatments amended with microbial consortium as compared to their respective controls (Table 3i and 3o).

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; Elseewi *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; Elseewi and Page, 1984). Several more studies on effect of crops grown in variety of soils (Ajaz and Tiyagi, 2003; Mittra *et al.,* 2003; Garg *et al.,* 2005; Ram *et al.,* 2007) have reported increase in nutrient component of soil and crop yield with use of fly ash (Singh *et al.,* 1994). The application of fly ash increased Si, P and K uptake by the rice plants, but did not result in an excessive uptake of heavy metals in the submerged paddy soil. In conclusion, fly ash could be a good supplement to other inorganic soil amendments to improve the nutrient balance in paddy soils (Lee *et al.,* 2006). The study has also indicated that fly ash can be more beneficial when used in combination with organic amendments (Rautaray *et al.,* 2003; Dhadse *et al.,* 2008). Compared to traditional soil conditioning materials as asbestos, fly ash seems to be more advantageous as it is an environmental safe material, contains plant nutrients and can be used in biological cultivation (EU dir 889/08). Additionally, it is a low cost material and thanks to its granular composition, is readily applicable (Chassapis *et al.,* 2010). Application of farm yard manure for improving the availability of nutrients and physical properties is an age old practice in Indian agriculture (Negassa *et al.,* 2005).

5.2.3 Uptake of heavy metals by root, stem and leaves

Results showed that major nutrients P and K were having their maximum concentration in leaves of *P. deltoides*. Secondary elements Ca and Mg showed their maximum uptake by leaves of *P. deltoides*, whereas, Na had its maximum concentration in roots of the

plant. In case of *T. ciliata* leaves of the plant showed the maximum uptake of major nutrients (P and K) and secondary nutrients (Ca and Mg), whereas, Na had its maximum concentration in the roots of *T. ciliata*.

Lead

Pb showed its maximum concentration in stem (T9) of *P. deltoides*, and in leaves of *T. ciliata* in treatment T7 (Table 3l and 3r) respectively. Many researchers have reported that a decrease in the solution pH causes increased Pb desorption from soil constituents, thereby increasing its concentration in soil solution (Harter, 1983; Yang *et al*., 2006) and uptake by plant. The study by Cui *et al*. (2004) showed that the presence of elemental S significantly enhanced the solubilization of heavy metals (Pb, Zn). Pb availability to plants is very limited (Raskin *et al.*, 1997), due to its rapid reaction with mineral and organic fractions of soil (McBride, 1994; Blaylock *et al.*, 1997; Shen *et al.*, 2002). Natural phytoextraction that utilizes hyperaccumulator plant species and use of high biomass producing plant species without the application of mobilizing agents have been reported to achieve little success in the case of Pb contaminated soils (Meers *et al.*, 2008). Huang *et al.* (1997) reported over 100-fold increase in Pb concentration in plant shoots following the application of EDTA. Wheat plants treated with the highest rate of citric acid (30 mmol kg−1) resulted in significantly higher Pb phytoextraction compared to that with EDTA or Sulphur. Application of citric acid at higher rates increased not only the Pb concentration in plant shoots but also resulted in high Mn concentration in plant shoots (Ghafoor *et al.,* 2010).

Chromium

Cr showed its maximum concentration in leaves (T6) of *P. deltoides*, and in roots (T10) of *T. ciliata.* Large amounts of the applied Cr were removed from the hydroponic solution in the presence of the plants. Significantly faster removal of Cr (III) than Cr (VI) was achieved by hybrid willows from the hydroponic solutions at all temperatures ($P < 0.01$) (Yu *et al.,* 2010). Phytoextraction of both chemical forms of Cr by various

plants is well documented. One conclusive point is that Cr (VI) is much more mobile and soluble than Cr (III) and the internal concentrations of Cr in plants were many fold higher than those of plants exposed to Cr (III) (Mei *et al*. 2002; Shahandeh and Hossner 2000). Mesquite (*Prosopis* spp.), which is an indigenous desert plant species, can remove Cr from the environment via active transport systems to the aerial portions of the plant. The study was performed by growing mesquite on solid media (agar) at Cr (VI) concentrations of 75 and 125 ppm. The accumulation found in the leaves under the present conditions indicated that mesquite could be classified as a hyperaccumulator of chromium (Aldrich *et al.,* 2003).

Cadmium

Cd showed its maximum concentration in stem (T10) of *P. deltoides*, and in leaves of *T. ciliata* (T6). Previous investigations (Zacchini *et al.*, 2009) showed that almost all selected willow clones, used to extract Cd, accumulated half of the absorbed metal in the aerial parts exhibiting a translocation factor twice respect to poplar clones. To avoid the different implications on ecological and productivity levels that the high Cd accumulation either in willow leaves or in poplar roots would bring about, researchers aimed to select poplar clones with higher tolerance level and Cd accumulation preferentially in stem tissues. Significant variability for high translocation of absorbed Cd to woody parts exists among poplar clones as well as certain variability for reduced accumulation both in roots and in leaves (Pietrini *et al.,* 2010). A three month microcosm study regarding interaction of micro-organisms with metals showed that Sorghum (*Lepidium sativum*) plants were highly tolerant to metal pollution and capable of reaching high biomass, values of shoot Cd concentration were higher than 100 mg/kg than the threshold value for hyperaccumulators. Microbial parameters showed higher values in planted pots than in control unplanted pots (Epelde *et al.,* 2009). Among a total of 36 plant species from 16 families, four species (*Chromolaena odoratum, Gynura pseudochina, Impatiens violaeflora and Justicia procumbens*) could be considered as Cd hyperaccumulators since their shoot Cd concentrations exceeded 100 mg Cd /kg dry mass and they showed a translocation factor > 1 (Phaenark *et al.,* 2009). Vassilev *et al.,* (2002) indicated that the use of low Cd–accumulating genotypes of crops (Archambault *et al.,* 2001), both non-

food crops and cereals (Vassilev *et al.,* 1996; Zheljazkov and Nielsen, 1996) offers a potential strategy for the successful management of Cd–contaminated agricultural soils. The high biomass producing crops, such as *Brassica juncea*, *Helianthus annuus*, *Nicotiana tabacum* are also considered as suitable species for phytoremediation as they could compensate lower Cd accumulation with much higher biomass yields (Vassilev *et al*., 2002).

Nickel and Cobalt

Ni was present in its maximum concentration in roots of both *P. deltoides* (T9) and *T. ciliata* (T11); leaves of both *P. deltoides* (T5) and *T. ciliata* (T9) showed the maximum uptake of Co. Nickel is concerned to be one of the small heavy metals having the ability to accumulate in generative plant organs at high concentrations. Maize and poplar leaves were those where elevated nickel contents due to sewage application were found as compared to the control (Badora and Filipek, 2004), our study showed a deviation which might be due the difference in cultivars and presence of multi metals. Microbial metal mobilization from particulate fly ash by *Acidithiobacilli* resulted in cadmium, copper and zinc mobilization of > 80%, whereas, lead, chromium and nickel were mobilized by 2, 11 and 32% respectively (Seidel *et al.,* 2001). The relationship between plant consortia, consisting of 1-4 *Metallicolous pseudometallophytes* with different metal tolerance studies (*Thlaspi caerulescens,* hyperaccumulator) and their rhizosphere microbial communities were studied in a mine spoil polluted soil with high levels of Cd, Pb and Zn which showed that the plant was very tolerant to metal stress and most suitable for metal phytoextraction, soil microbial properties had a stronger effect on plant biomass (35.2%) (Epelde *et al.,* 2010). Fly ash amendment had a significant role in reducing the soil pH (from 8.43 to 7.06), which may have influenced Cu, Pb and Cd solubilities and mobilities. This observation agreed with the Santillan-Metrano and Jurinak (1975) findings that increasing soil acidity led to increase in Pb and Cd solubilities and mobilities (Adewole *et al.,* 2010).

Uptake of micronutrients by root, stem and leaves

Manganese, Copper, Zinc and Iron

In case of micro nutrients Mn, Cu and Zn were having their maximum concentration in leaves of *P. deltoides*, whereas, Fe had its maximum concentration in roots of the plant. In *Toona ciliata* Cu, Mn and Fe were maximally retained by roots of the plant, whereas, Zn showed its maximum uptake by the leaves of *T. ciliata.* (Table 3p and 3q). These results suggest that phytoremediation of Fe, Cd and Cu contaminated sites is feasible. These results are comparable to up to 22% phytoextraction of soil-exchangeable Cu from a contaminated site (Gerrard *et al.,* 2000) by *T. caerulescens* and showed remarkable Cu tolerance. A study on uptake of macro and micro nutrients into leaf, stem and root of *Populus* after irrigation with landfill leachate showed the maximum concentration of Cu and Mn in the leaves of plant and Fe concentration to be maximum in roots (Zalesny *et al.,* 2008). In aboveground plant parts (leaves and branches) harvested at the end of the experiment, Zn concentrations did not exceed 2,250 $\mu g\ g^{-1}$ Zn in the dry leaf tissue, or 900 $\mu g\ g^{-1}$ in the woody branches, on a dry weight basis (Negri and Hinchman, 2000). Among a total of 36 plant species from 16 families, only *Justicia procumbens* could be considered as a Zn hyperaccumulator (Zn concentration in its shoot more than 10,000 mg Zn /kg dry mass with the translocation factor > 1) (Phaenark *et al.,* 2009). Zn is an essential micronutrient for normal plant growth at low concentrations. However, at high concentrations Zn induces a strong phytotoxicity and retards plant growth (Ait Ali *et al*., 2002; Mendelssohn *et al.,* 2001). The ability of amendments to modify the soil properties and influence plants to immobilize Cu and Zn was studied in a naturally contaminated, additionally spiked podzolic soil. *Lolium perenne* L. (perennial rye grass), *Festuca rubra* L. (creeping red fescue) and *Poa pratensis* L. (Kentucky blue grass) were tested in a pot study in the presence of soil amendments (lime, phosphate and compost individually and in combination) to assess the effect of soil-plant amendment interaction on phytostabilisation. Application of lime significantly reduced the exchangeable fraction of Zn, whereas phosphate application had an accelerating effect on exchangeable Cu. With combined application of amendments, the plant metal concentration decreased by more than 40% for Cu and 70% for Zn compared to soils receiving no amendments. Combined application of amendments, in conjunction with growth of *Festuca* and *Poa*,

can be recommended for phytostabilisation of Cu and Zn in moderately contaminated acidic soils of Southwest British Columbia (Padmavathiamma and Li, 2010).

The heavy metal uptake analysis by the plant showed that the uptake was more pronounced in the treatments amended with distillery waste followed by amendment with fly ash. The pattern of uptake of different heavy metals by *P. deltoides* was Cr > Pb > Ni > Co > Cd. Leaves of *Populus deltoides* in the present study showed the maximum concentration of Cr, Zn and Co and was in accordance (except for Pb and Cd) with earlier studies by Madejon *et al.,* (2004) which reported that on comparing the plant organs, leaves accumulated a higher concentration of trace elements than stems. Our results was a deviation from earlier reported findings to some extent, this dissimilar trend may possibly be due to differences in cultivars / species used. The heavy metal uptake by the plant organs followed an order of leaves > stem > roots.

T. ciliata showed the maximum uptake of Pb > Cr > Co > Ni > Cd by various plant parts. Leaves > root > stem showed the maximum affinity for the uptake of heavy metals during the 180 day nursery trial. Leaves of *T. ciliata* can also be used for the monitoring of heavy metal pollution in the soil. Leaves of *Populus sp.* have been selected widely for biomonitoring of trace element pollution (Djingova *et al.,* 1995, 1996, 1999, 2001; Bargagli, 1998; Robinson *et al.,* 2000; Madejon *et al.,* 2004). Fly ash and farmyard amendments increased biomass production, plant height and phytoextraction potentials identified with test plant *P. deltoides*; good biomass yield and high accumulation in harvestable plant parts are essential key factors for efficient phytoextraction (McGrath and Zhao, 2003; Vassilev *et al*., 2004; Nwaichi *et al*., 2010).

5.2.4 Reduction in concentration of heavy metals in soil

Treatments amended with microbial inoculants as an amendment had shown potentially more reduction in concentration of heavy metal in soil. The uptake of heavy metals was more pronounced in treatments amended with fly ash and microbial consortium. Several lines of evidence suggest that soil microorganisms possess mechanisms capable of altering environmental mobility of metal contaminants with subsequent effects on the potential for root uptake (Lasat, 2002). Microbes are also known to alter chemical

properties of the rhizosphere soil with subsequent effects on the environmental mobility of metal contaminants. For example, chemolithotropic bacteria have been shown to enhance environmental mobility of metal contaminants via soil acidification, or in contrast, to decrease their solubility due to precipitation as sulfides (Kelley and Tuovinen, 1988). In the present investigation, the mixed application of fly ash and farmyard manure had also shown promising results for carrying out reduction in concentration of heavy metal content in soil. An appreciable change in the soil physicochemical properties, rising of pH and increased crop yield was obtained by mixed application of fly ash, paper factory sludge and farmyard manure (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. Presence of organic matter has an 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; Pitchel and Hayes, 1990; Jala and Goyal, 2006), also it effectively raised the pH of the growing medium, resulting in suboptimal conditions for uptake of Pb, Zn and Cu (Pulford and Watson, 2003).

An overall, 65 – 95% reduction in concentration of Pb, 3 – 100% of Cr, 5 – 72% of Cd, 26 – 84% of Ni and 43 – 92% of Co (Table 3m) in rhizospheric soil of *P. deltoides* was estimated in different treatments, whereas, the reduction in concentration of heavy metals in rhizospheric soil of *T. ciliata* was as: Pb had a reduction in concentration of 58 – 94%, Cr: 10 – 68%, Cd: 67 – 88%, Ni: 43 – 94% and Co: 2 – 72% (Table 3s). The maximum Zn uptake was found in *Thlaspi caerulescens* (*T. caerulescens*) accumulating 2000-8000 mg Zn kg^{-1}. *T.caerulescens* was shown to accumulate 1000-4000 mg kg^{-1} Cd (Brooks *et al.,* 1998). *Sedum alfredii* Hance has been identified as a new Zn and Cd hyperaccumulating plant species (Yang *et al.,* 2004). Zn concentration in its shoot can reach over 20 g kg^{-1} when grown at 80 mg Zn l^{-1} in nutrient solution without showing any toxic symptoms (Yang *et al*., 2002). Cd concentration in leaves and stem of *S. alfredii* increased with increasing Cd supply levels, and reached a maximum upto 9000 – 65000 mg kg^{-1} dry weight (Yang *et al*., 2004). Seedlings of *Sesbania drummondii* can hyperaccumulate Pb in a controlled hydroponic environment (Barlow *et al*., 2000). High

Cu concentration has been found in *Betula* roots (Kozlov *et al*., 1995; Maurice and Lagerkvist, 2000) as well as in *Salix* roots (Punshon and Dickinson, 1997).

Logically, higher phytoextraction of metals should result in a low metal concentration in the soil (Purakayastha *et al.,* 2008) which was further established in the present study. Some tree sps. mainly *Salix* spp. and *Populus* spp. exhibit these traits and are already in use in phytoremediation programmes and for Cd phytoextraction from lightly polluted agricultural soils (Landberg and Greger*,* 1994). About 150 clones of different *Salix* sps. have been screened for uptake, transport of metals to shoots and tolerance to Cd, Zn and Cu (Landberg and Greger, 1996; Landberg and Greger, 1994). Some reports by Grant and Bailey (1997), Griga *et al.* (2003), Yankov *et al.* (2000) and Yankov and Tashin (2001) showed that crops for fiber or oil production could be used for profitable crop production accompanied by phytoextraction of metal from polluted soils.

Populus deltoides and *Toona ciliata* extracted a good amount of Pb, Cr, Cd, Ni and Co from soil and there was a great depletion in total heavy metal concentration in rhizosphere soil. The roots of the established hyperaccumulators absorb metal elements from the soil and translocate them to the above ground shoots, where they get accumulated in high concentration (Prasad and Freitas, 2003). It is also based on high biomass producing plants used together with chemical agents enhancing both metal solubility and uptake by plants (Huang, 1997; Blaylock *et al.,* 1997).

5.3 To study the survival and establishment of molecular tagged microbes in soils amended with solid waste

5.3.1 Antibiotic Profiling and Bacterial Transformation

Antibiotic profiling of the isolates was carried out on media containing streptomycin (50 $\mu g\ ml^{-1}$), kanamycin (25 $\mu g\ ml^{-1}$), nalidixic acid (50 $\mu g\ ml^{-1}$), chloramphenicol (10$\mu g\ ml^{-1}$) and rifampicin (50 $\mu g\ ml^{-1}$) respectively. *P. striata* showed resistance to streptomycin and nalidixic acid, and was sensitive to chloramphenicol, kanamycin and rifampicin, whereas, *Azotobacter* CBD15 was resistant to all test antibiotics (Table 4a), and its inherent kanamycin resistant character was used for ecological monitoring. 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). The kanamycin resistance gene was one of the first markers to be developed and was available from many laboratories. It is widely used as a selectable marker in the transformation of organisms as diverse as bacteria, yeast, plants and animals (Conner, 1998).

Among *P.striata* and *Azotobacter* CBD15, *P. striata* was successfully transformed with plasmid pMMB277 (carrying *lac Z* and chloramphenicol marker) 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 alkali lysis method and to confirm the size of 9,237 bp it was followed by agarose gel electrophoresis. The growth of transformed *P. striata* *(lac* Z^{+}*)* was monitored in PKV broth at an interval of 2 hrs over a period of 24 hrs, along with changes in pH of the medium and concentration of available P in broth. The results revealed that the absorbance increased from 0.011 at 0 hr to 0.612 at 24 hr, concentration of available P increased from 0 mg kg^{-1} (0 hr) to 0.23 mg kg^{-1} (24 hr), and pH decreased from neutral (7.1) to acidic (4.6) (Table 4d). This showed that phosphate solubilization by *P. striata (lac* Z^{+}*)* was comparable to that of *P. striata* (native) and a similar pattern of solubilization of insoluble phosphate and decrease in pH was observed. Rashid *et al*., (2004) found that phosphate solubilization of the added tri-calcium phosphate (TCP) in broth was in the range of 0.04%- 0.14%. Phosphate solubilizing microbes solubilize insoluble phosphates and also promote plant growth (Rodrignez and Fraga, 1999). It also resulted in reduction in pH from 7.1 – 4.4. Several researchers had reported the drop in pH in liquid cultures (Cunningham and Kuiack, 1992; Illmer *et al*., 1995; Bar Yosef *et al*., 1999; Rashid *et al*., 2004).

5.3.2 Survival and establishment of molecular tagged microbes in soils amended with solid waste in nursery trial

Transformant *P. striata (lac* Z^{+}*)* and *Azotobacter* CBD15 *(*kan^{R}*)* @ 30ml consortium per pot were inoculated in soil amended with fly ash, distillery waste and farm yard manure along with uninoculated control. Ecological monitoring in terms of population buildup was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient

agar plates, PKV plates and kanamycin containing Jensen's media plates. β-galactosidase positive activity of the indigenous population ranged from 20 to 40% of aerobic heterotrophic microorganisms, including fungi, cultured on nutrient agar media containing X-gal and IPTG (Flemming *et al.*, 1994). Reporter gene systems are indispensable tools for understanding gene regulation in prokaryotes and eukaryotes (Loper and Lindow, 1997), such as *lac Z* is most popular on account of easy detectability, sensitivity, specific activity and rapidity (Bronstein *et al.*, 1994; Manafi *et al.*, 1991; Zhang *et al.*, 1991). The *lac Z* reporter gene from *E.coli* has become a standard tool for following localized transgene expression in many organisms, including transgenic animals. Detection of the encoded β-galactosidase activity is achieved by *in*-situ visualization using chromogenic substrate X-gal, as it forms an insoluble blue precipitate upon hydrolysis by β-galactosidase, X-gal permits *in-situ* detection of *lac Z* transgene expressing cells in cultures and facilitates spatial determination of reporter gene expression (Tsuchida *et al.,* 2004).

The analysis of representative samples over regular time interval over a period of 180 days was carried out to find best compatibility of a particular treatment for favorable proliferation of introduced bacteria. The analysis showed increase in the bacterial count with due course of time. The technique of monitoring tagged bacteria in natural environment by direct plating of soil sample on selective media was found superior to conventional plating technique. It allowed unambiguous selection of introduced strains while eliminating parent or native strains. *Tn5-lac Z* reporter gene was introduced as a molecular tag for ecological monitoring of phosphobacteria in soyabean rhizosphere. 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 i*s the most commonly used reporter gene in microbial ecology (Slauch and Silhavy, 1991). *E.coli lac Z* has proved to be a highly versatile reporter gene in *Sacchromyces cerevisiae* and has been used to study many aspects of signal-transduction pathways and other cellular processes (Guarente and Ptashne, 1981; Rose *et al.,* 1981; Guarente, 1983; Burns *et al.,* 1994; Uhl and Johnson, 2001). The *lac* marker alone was not found to be suitable for monitoring bacteria in soil since 20-42% of cultured, aerobic, heterotrophic soil bacteria possessed *lac* Z^+ characteristic. The combination of introduced *lac Z* expression

and chloramphenicol resistance provided a stable and precise reporter system, which made its ecological monitoring easy on selective media. Reporter genes provide easily detectable phenotypes to microbial cells and are therefore valuable tools for the study of microorganisms in the environment (Jansson, 2003).

Phosphate solubilizing and free-living nitrogen-fixing bacteria in rhizospheric soil collected from established nursery plantation of *P. deltoides* and *T. ciliata* were screened on Pikovskaya's and Jensen's media plates where the population of the latter was less in rhizosphere soil of both tree species, which indicated the better adaptability of phosphate-solubilizing bacteria to fly ash and farm yard amended soil. Overall populations of phosphate-solubilizing bacteria ranged from $17x10^6$ to $71x10^6$ cfu g^{-1} soil and nitrogen-fixing bacteria were comparatively less, varying from $05x10^6$ to $24x10^6$ cfu g^{-1} soil in rhizosphere soil of *P. deltoides* and *T. ciliata* (Table 4e). Results showed that available nitrogen and phosphorus were more in treatments amended with microbial consortium as compared to their respective controls (Table 3i and 3o). 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). 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).

P. striata (lac Z^+*)* was ranging from 02 x 10^6 cfu g^{-1} soil – 107 x 10^6 cfu g^{-1} soil in rhizosphere of *P. deltoides*, whereas, in *T. ciliata* its total count was ranging from 03 x 10^6 cfu g^{-1} soil – 87 x 10^6 cfu g^{-1} soil. *Azotobacter* CBD15 *(*kan^R*)* was ranging from 01 x 10^6 cfu g^{-1} soil – 14 x 10^6 cfu g^{-1} soil (T8) in rhizosphere of *P. deltoides*, and in rhizosphere of *T. ciliata* it was found to be ranging from 01 x 10^6 cfu g^{-1} soil - 21 x 10^6 cfu g^{-1} soil (Fig. 2g and 2h). Treatment T8 amended with fly ash and farmyard manure upon inoculation with microbial consortium showed 19.6% increase in population of transformed *P.striata (lac* Z^+*)* and 42.6% increase in the population of *Azotobacter* CBD15 *(*kan^R*)* as compared to inoculated control in the rhizospheric soil of *P. deltoides.*

The same treatment showed marked effect on the population of tagged bacterial strains in the rhizospheric soil of *T. ciliata*, in case of transformed *P. striata (lac* Z^+*)* the increase was 35.6 % and for *Azotobacter* CBD15 *(*kan^R*)* the increase was 28.6% as compared to inoculated control. Rippon and Wood (1975) attributed the increased microbial population with fly ash addition to the release of nutrients from fly ash with time. Surridge *et* al. (2009) have reported that fly ash addition has a liming effect on the soil leading to increased mobility of calcium and hydroxide ions, ultimately causing an increase in bacterial species richness.

When fly ash is added at levels more than 10%, a decline in microbial activity was observed (Kohli and Goyal, 2010), which may be due to a decrease in substrate availability associated with accumulation of persistent lignite-derived organic carbon compounds (Rumpel *et al.* 1998). Gaind and Gaur (2004) found that *Azotobacter chroococcum*, *Azospirillum brasilense* and *Bacillus circulans* showed their maximum viability in fly ash alone whereas *Pseudomonas striata* proliferated most in soil-fly ash (1:1) combination. Schutter and Fuhrmann (2001) have indicated that fly ash amendment may benefit fungi and gram-negative bacteria relative to other components of the soil microbial community. On the whole, the effects of the level of fly ash application on soil aggregation coupled with the influence of the growing plants effects on soil microbial diversity could be favourable for plant growth and soil resurgence (Kohli and Goyal, 2010).Soil with fly ash and farm yard manure provided the best supporting environment for the total soil microbial population, as well as for inoculated tagged phosphate solubilizing and nitrogen fixing bacteria in the rhizospheric soils of *P. deltoides* and *T. ciliata*.

Improved Cfu g^{-1} of Phosphate Solubilizing Bacteria (PSB) and nitrogen fixing bacteria in the treatment receiving fly ash @10% (v/v) and farm yard manure @ 20% (v/v) with marked PSB inoculation over a period of 180 days proved that fly ash may be added to soil up to 10% (v/v) level with no adverse effect on inoculated bacteria. The enumeration studies of *lac-cat* marked PSB population in inoculated and un-inoculated soil samples showed the establishment of the introduced strain. These bacteria, finding soil and fly ash environment most suitable for their proliferation, contributed toward the enhanced availability of soil P (Gaind and Gaur, 1991).

Microbial biomass is a sensitive indicator for the assessment of soil and gives a good insight into the complexities of the nutrient profile in soil and therefore was examined to study the effect of various amendments on microbial activity along with bacterial count. The results revealed that the total microbial biomass in the rhizosphere soil of *P. deltoides* was ranging from 169 µg g^{-1} in treatment T9 – 247 µg g^{-1} in treatment T8 (Table 4b). The similar pattern was observed for the rhizosphere soil of *T. ciliata*, i.e., microbial biomass was ranging from 117 µg g^{-1} (T9) – 219 µg g^{-1} in T8 respectively (Table 4c). The chloroform extraction method offers advantages such as easy execution and analytical ability and its applicability to various soils (Brookes *et al.,* 1985; 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 and nitrogen fixing bacteria compared to uninoculated soil amended with various amendments, which might be due to better proliferation of the isolate in the amended soil. Soil dehydrogenase activity and microbial biomass were greatest at 10% level of fly ash amendment since fly ash amendment at moderate levels provides nutrients to the micro-organisms for carrying out various metabolic activities without any adverse effect (Wong and Wong 1986, Saffigna *et al.* 1989). The rhizosphere of plants creates a more aerobic environment in soil that stimulates microbial activity which enhances oxidation of organic chemical residues (Anderson *et al.*1993, Schnoor *et al.* 1995, Narayanan *et al.* 1998, Jones *et al.* 2004, Kirk *et al.* 2005). Fly ash application at 10% level was found to be optimum for soil microbiological parameters such as number of bacteria, dehydrogenase activity and microbial biomass (Kohli and Goyal, 2010).

Results showed that the combination of reporter genes are suitable for tracking microorganisms in soil, because presence of both genes are potentially innocuous to the indigenous gene pool, assayed easily and rapidly. It was found convenient, however, to use an antibiotic marker with some phenotypic marker, where the number of microorganisms becomes critically low in soil.

Chapter 6
Conclusions

1. The percentage survival of *P. deltoides* was found to be around 75% and for *T. ciliata* it was around 25% in control soil. For both the tree species survival was maximum in treatments T8 (soil amended with fly ash and farm yard manure, co-inoculated with microbial consortium in T8), i.e., for *P. deltoides* it was 93.75% and for *T. ciliata* it was 83.33% respectively.

2. With the addition of fly ash alone the total dry biomass production was increased by 49.4% in *P. deltoides* and 11.58% in *T. ciliata* and infact, the combination of fly ash @ 10% (v/v), farmyard manure @ 20% (v/v) and microbial consortium @ 30 ml/pot led to a further increase in plant height by 43.8%, collar diameter by 40.7% and total dry biomass by 45.1% in *P. deltoides* and *in T. ciliata* plant height was increased by 30.3%, collar diameter by 16.2% and total dry biomass by 14.4%, respectively.

3. Two-way Analysis of Variance showed that the addition of fly ash, farmyard manure had a significant impact on the biometric parameters of *P. deltoides*, and the addition of microbial consortium had significant impact on total dry biomass and collar diameter, whereas, the impact on stem height was non-significant. Treatment with distillery waste however, as one of the soil amending agent showed a decrease in the plant growth which might be due to the high electrical conductivity leading to toxicity which was not tolerated by the test plants.

4. The interaction between fly ash, farmyard manure and microbial consortium had significant impact on all the biometric parameters that is plant height, collar diameter and total dry biomass.

5. The pattern of uptake of different heavy metals by *P. deltoides* was Cr > Pb > Ni > Co > Cd. Leaves of *Populus deltoides* in the present study showed the maximum concentration of Cr, Zn and Co.

6. The heavy metal uptake by the plant parts of *Populus deltoides* followed an order of leaves > stem > roots.

7. *Toona ciliata* showed maximum uptake of Pb > Cr > Co > Ni > Cd. Maximum affinity for the uptake of heavy metals during the 180 day nursery trial was in leaves followed by root and stem of the plant.

8. Leaves of *P. deltoides* and *T. ciliata* can also be used for the monitoring of heavy metal pollution in the soil as they showed the maximum uptake of heavy metals in both the tree species.

9. Treatments amended with microbial inoculants as an amendment had shown potentially more reduction of heavy metal concentration in soil as compared to their un-inoculated control treatments.

10. *Populus deltoides* and *T. ciliata* extracted a good amount of Pb, Cr, Cd, Ni and Co from soil and there was a considerable reduction in concentration of heavy metal in rhizospheric soil.

11. The *lac* marker alone was not found to be suitable for monitoring bacteria in soil since 20-42% of cultured, aerobic, heterotrophic soil bacteria possessed *lacZ*$^{+}$ characteristic. The combination of introduced *lacZ* expression and chloramphenicol resistance provided a stable and precise reporter system, which made its ecological monitoring easy on selective media. For *Azotobacter* CBD15 inherent kanamycin resistance was used as marker gene for its ecological monitoring.

12. Soil with fly ash and farm yard manure provided the best supporting environment for proliferation of the total soil microbial population, as well as for inoculated tagged phosphate solubilizing and nitrogen fixing bacteria in the rhizospheric soils of *P. deltoides* and *T. ciliata*. The results indicated that combined addition of fly

ash, farm yard manure and microbial inoculants can be used as a good potting mixture for improving survival rates and plant growth in forestry nurseries, leading to gainful utilization of fly ash.

Chapter 7
Bibliography

1. Abe Y, Sagawa T, Sakai K and Kimura S 1987 Enzyme-linked immunosorbent assay (ELISA) for human epidermal growth factor (hEGF). Clinica Chimica Acta, 168, 87-95.

2. Adewole MB, Sridhar MKC and Adeoye GO 2010 Removal of heavy metals from soil polluted with effluents from a paint industry using *Helianthus annuus* L. and *Tithonia diversifolia* (Hemsl.) as influenced by fertilizer applications. Bioremediation Journal, 14, 169-179.

3. Adholeya A, Bhatia NP, Kanwar S and Kumar S 1998 Fly ash source and substrate for growth of sustainable agro-forestry system. In: Proceedings of Regional Workshop cum Symposium on Fly Ash Disposal and Utilization. Organized by Kota Thermal Power Station, RSEB, Kota, Rajasthan, India.

4. Adler T 1996 Botanical cleanup crews. Science News, 150, 42-43.

5. Adriano DC, Page AL, Elseewi AA, Chang AC and Straughan I 1980 Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review. Journal of Environmental Quality, 9, 333–344.

6. Adriano DC, Woodford TA, Ciravolo TG 1978 Growth and elemental composition of corn and bean seedlings as influenced by soil application of coal ash. Journal of Environmental Quality, 7, 416-421.

7. Aggarwal H and Goyal D 2007 Phytoremediation of some heavy metals by agronomic crops. Concepts and Applications in Environmental Geochemistry (Developments in Environmental Science 5), pp 79-98. (Sarkar D, Datta R and Hannigan R, Eds.) Elsevier.

8. Aggarwal H and Goyal D 2009 Impact of addition of soil amendments and microbial inoculants on nursery growth of *Populus deltoides* and *Toona ciliata*. Agroforestry Systems, 75, 167-173.

9. Agostini E, Coniglio MS, Milrad SR, Tigier HA and Giulietti AM 2003 Phytoremediation of 2,4-dichlorophenol by Brassica napus hairy root cultures. Biotechnology and Applied Biochemistry, 37, 139-144.

10. Ait Ali N, Bernal MP and Ater M 2002 Tolerance and bioaccumulation of copper in *Phragmites australis* and Zea mays. Plant Soil, 239, 103–111.

11. Aitken RL, Campbell DJ and Bell LC 1984 Properties of Australian fly ash relevant to their agronomic utilization. Australian Journal of Soil Research, 22, 443-453.

12. Ajaz S and Tiyagi SA 2003 Efefcts of different concentration of fly ash on the growth of cucumber plant, *Cucumis sativus.* Archieve in Agronomy Soil Science, 49, 457-461.

13. Aldrich MV, Gardea-Torresdey JL, Peralta-Videa JR and Parsons JG 2003 Uptake and reduction of Cr(VI) toCr(III) by Mesquite (*Prosopis* spp.) : Chromate plant interaction in hydroponics and solid media studied using XAS. Environmental Science and Technology, 37, 1859-1864.

14. Alkorta I, Allica JH, Becerril JM, Amezaga I, Albizu I, Onaindia M and Garbisu C 2004 Chelate-enhanced phytoremediation of soils polluted with heavy metals. Critical Reviews in Environmental Science and Biotechnology, 3, 55-70.

15. Anandakumar R 2005 Environment legislation and fly ash utilization. Fly ash India, New Delhi.

16. Anderson CWN, Brooks RR, Stewart RB and Simcock R 1998 Harvesting a crop of gold in plants. Nature, 395, 553-554.

17. Anderson TA, Guthrie EA and Walton BT 1993 Bioremediation. Environmental Science and Technology, 27, 2630-2636.

18. Archambault DJ, Marentes E, Buckley W, Clarke J and Taylor GJ 2001 A rapid, seedlingbased bioassay for identifying low cadmium–accumulating individuals of durum wheat (*Triticum turgidum* L.). Euphytica, 117, 175–182.

19. Atlas RM 1992 Molecular methods for environmental monitoring and containment of genetically engineered microorganisms. Biodegradation, 3, 137-146.

20. Atta-Krah K, Kindt R, Skilton J N, and Amaral W 2004 Managing biological and genetic diversity in tropical agroforestry. In: New Vistas in Agroforestry: A Compendium for the 1st World Congress of Agrofrestery, (eds. Nair PKR, Rao MR and Buck LE) Netherlands, Kluwer Academic Publishers, pp. 183-194.

21. Badora A and Filipek T 2004 The influence of applying purified municipal sewage on nickel content in plants. Polish Journal of Environmental Studies, 13, 435-437.

22. Badri M and Springuel I 1994 Biogeochemical prospecting in the south-easter desert of Egypt. Journal of Arid Environment, 28, 257-264.

23. Baker AJM and Walker PL 1990 Ecophysiology of metal uptake by tolerant plants. *In*: Heavy metal tolerance in plants: evolutionary aspects. Eds. Shaw AJ pp.155-177. CRC Press, Boca-Raton, FL.

24. Baker AJM, McGrath SP, Sidoli CMD and Reeves RD 1994 The possibility of in situ heavy metal decontamination of polluted soils using crops of metal accumulating plants. Resources Conservation and Recycling, 11, 41-49.

25. Baker AJM, Reeves RD and McGrath SP 1991 In situ decontamination of heavy metal polluted soils using crops of metal-accumulating plants-a feasibility study. *In* In situ bioreclamation. Eds. Hinchee RE and Olfenbuttel RF pp. 600-605. Butterworth-Heinemann, Boston.

26. Balakrishnan V, Venkatesan K, Sanjiviraja, K, Indrajith A and Ravindran KC 2010 Application of an organic halophytic manure on yield characteristics of *Arachis hypogea* Linn. International Journal of Engineering Science and Technology, 2, 134-143.

27. Banuelos GS, Ajwa HA, Mackey LL, Wu C, Cook S and Akohoue S 1997b Evaluation of different plant species used for phytoremediation of high soil selenium. Journal of Environmental Quality, 26, 639-646.

28. Banuelos GS, Ajwa HA, Terry N and Downey S 1997a Phytoremediation of Selenium laden effluent. Fourth International in situ and on-site bioremediation symposium, April 28 - May 1, 1997, New Orleans, LA. 3:303.

29. Bargagli R 1998 Trace Elements in Terrestrial Plants: An Ecophysiological Approach to Biomonitoring and Biorecovery. Springer-Verlag, Berlin.

30. Barlow R, Bryant N, Andersland J and Sahi S 2000 Lead hyperaccumulation by *Sesbania drummondii*. Proceedings of the 2000 Conference on Hazardous Waste Research. pp.112-114.

31. Bar-Yosef B, Rogers RD, Wolfran JH and Richman E 1999 *Pseudomonas cepacia* – mediated rock phosphate solubilization in kaolinite and montmorillonite suspensions. Soil Science Society of America, 63, 1703-1708.

32. Basu M, Pande M, Bhadoria PBS and Mahapatra SC 2009 Potential fly ash utilization in agriculture. A global review. Progress in Natural Science. 19, 1173-1186.

33. Baumann A 1885 Das Verhalten von Zinksatzen gegen Pflanzen und im Boden. Landwirtsch. Vers.-Statn. 31, 1-53.

34. Beetz A 2002 Agroforestry overview. Horticulture systems guide. (ed, Earles R) http://www.attra.ncat.org/attra-pub/PDF/agrofor.pdf.

35. Belimov AA, Kunakova AM, Safronova VI, Stepanok VV, Yudkin LY, Alekseev YV and Kozhemyakov AP 2004 Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. Microbiology, 73, 99-106.

36. Belkin HE, Finkelman RB and Zhang BS 1999 Arsenic Poisoning caused by residential coal combustion in Guizhou Province, China. Pan-Asia-Pacific Conference on Fluoride and Arsenic Research, Shenyang.

37. Bennet LE, Burkhead JL, Hale KL, Terry N, Pilon M and Pilon-Smits EAH 2003 Analysis of transgenic Indian mustard plants for phytoremediation of metal-contaminated mine tailings. Journal of Environmental Quality, 32, 432-440.

38. Berti WR and Cunningham SD 1997 In-place inactivation of Pb in Pb-contaminated soils. Environmental Science and Technology, 31, 1359-1364.

39. Berti WR and Cunningham SD 2000 Phytostabilization of metals. In: Phytoremediation of toxic metals: using plants to clean up the environment. (eds. Raskin I and Ensley BD), John Wiley & Sons, New York, pp.71-88.

40. Bhalla BK 1989 Agroforestry for meeting the industrial requirements. Wimco Ltd., Indian Mercantile Chambers, Bombay, India.

41. Birnboim HC and Doly J 1979 Isolation of bacterial plasmid by alkali lyses method. Nucleic Acids Research, 7, 1513-1523.

42. Black CA, Evans DD, White JL, Ensmingher LE, Clarke FE and Dinauer RC 1965 Methods of Soil Analysis. Part 2-Chemical and Microbiological Properties , 9 Series Agronomy. American Society of Agronomy, Inc., Madison, Wisconsin, USA

43. Blaylock MJ and Huang JW 2000 Phytoextraction of metals. In: Phytoremediation of toxic metals: using plants to clean up the environment. (eds. Raskin I and Ensley BD), John Wiley & Sons, New York, pp.53-70.

44. Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD and Raskin I. 1997. Enhanced accumulation of Pb in Indian mustard by soil–applied chelating agents. Environmental Science and Technology, 31, 860-865.

45. Boivin C, Carnut S, Malpica CA, Truchet G and Rosenberg C 1990 *Rhizobium meliloti* genes encoding catabolism of trigonelline are induced under symbiotic conditions. Plant Cell, 2, 1157- 1170.

46. Boominathan R, Saha-Chaudhury N M, Sahajwalla V and Doran PM 2004 Production of nickel bio-ore from hyperaccumulator plant biomass: applications in phytomining. Biotechnology and Bioengineering, 86, 243-250.

47. Bouyoucos GJ 1962 Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54, 464–465.

48. Bowen GC and Rovira AD 1991 The rhizosphere- the hidden half of the hidden half. In: Plant Roots-The Hidden Half (eds. Waisel Y, Eshel A and Kaffkaffi U). New York, Marcel Dekker. pp. 641-669.

49. Brandl H and Farmarzi MA 2006 Applying microbe metal interaction for the biotechnological treatment of mineral waste. China Particuology, 4, 93-97.

50. Bray RH and Kurtz LT 1945 Determination of total, organic and available forms of phosphorous in soils. Soil Science, 59, 39-45.

51. Broadhurst CL, Chaney RL, Angle JS, Maugel TK, Erbe EF and Murphy CA 2004 Simultaneous hyperaccumulation of nickel, manganese, and calcium in *Alyssum* leaf trichomes. Environmental Science and Technology, 38, 5797-5802.

52. Bromilow RH, Chamberlain K 1995 Principles governing uptake and transport. In: Contamination: Modeling and Simulation of Organic Chemical Processes. (eds. Trapp S and McFarlane JC), Lewis, Boca Raton, FL, USA, pp 37–68.

53. Bronstein I, Fortin J, Stanley PE, Stewart GSAB and Kricka LJ 1994 Chemiluminescent and bioluminescent reporter gene assays. Analytical Biochemistrty, 219, 169-181.

54. Brookes PC, Landman A, Pruden G and Jenkinson DS 1985 Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass in soil. Soil Biology and Biochemistry, 17, 837-842.

55. Brooks RR 1977 Copper and cobalt uptake by *Haumaniastrum* species. Plant Soil. 48, 541-544.

56. Brooks RR 1983 Biological methods of prospecting for minerals. Wiley-Interscience, New York, pp.313.

57. Brooks RR 1998 Plants that hyperaccumulate heavy metals. Wallingford CAB International, pp. 380.

58. Brown SL, Chaney RL, Angle JS and Baker AJM 1995 Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge amended soils. Environmental Science and Technology, 29, 1581-1585.

59. Buoyoucos GJ 1962 Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54, 464.

60. Burd GI, Dixon DG and Glick BR 2000 Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Canadian Journal of Microbiology, 43, 237-245.

61. Burken JG and Schnoor JL 1997 Uptake and metabolism of atrazine by poplar trees. Environmental Science and Technology, 31, 1399-1406.

62. Burns N, Grimwade B, Ross-Macdonald PB, Choi EY, Finberg K, Roeder GS and Snyder M 1994 Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae.* Genes and Development, 8, 1087-1105.

63. Bustamante MA, Paredes C, Marhuenda-Egea FC, Perez-Espinosa A, Bernal MP and Moral R 2008 Co-composting of distillery wastes with animal manures: Carbon and nitrogen transformations in the evaluation of compost stability. Chemosphere, 72, 551-557.

64. Cappuccino JG and Sherman N 1987 Microbiology. A laboratory manual. Benjamin Cummings Publications.

65. Carlson CL and Adriano DC 1993 Environmental impacts of coal combustion residues. Journal of Environmental Quality, 22, 227-247.

66. Chaney RL 1983 Plant uptake of inorganic waste constitutes. In: Land treatment of hazardous wastes. (eds. Parr JF, Marsh PB and Kla JM), Noyes Data Corp., Park Ridge, NJ, pp.50-76.

67. Chaney RL, Malik KM, Li YM, Brown SL, Brewer EP and Angle JS 1997 Phytoremediation of soil metals. Current Opinion in Biotechnology, 8, 279-284.

68. Chang AC, Lund LJ, Page AL and Warneke JE 1977 Physical properties of fly ash amended soils. Journal of Environmental Quality, 6, 267-270.

69. Chassapis K, Roulia M, Vrettou E, Fili D and Zervaki M 2010 Biofunctional Characteristics of Lignite Fly AshModified by Humates: A New Soil Conditioner. Bioinorganic Chemistry and Applications, *In Press*. doi:10.1155/2010/457964.

70. Chaturvedi AN 1982 Poplar Farming in Uttar Pradesh (India). Uttar Pradesh Forest Bulletin No. 45. Nainital, Uttar Pradesh Forest Dept.

71. Chaudhry TM, Hayes WJ, Khan AG and Khoo CS 1998 Phytoremediation-Focusing on accumulator plants that remediate metal-contaminated soils. Australian Journal of Ecotoxicology, 4, 37-51.

72. Chesnin L and Yien CH 1950 Turbid metric method for determination of sulphur. Proceedings of Soil Science Society of America, 14, 149–151.

73. Chopra SL and Kanwar JS 1991 Analytical agricultural chemistry. Kalyani Publishers, New Delhi.

74. Ciravolo TG and Adriano DC 1979 Utilization of coal ash by crops under greenhouse conditions. In: Ecology and Coal Resource Development (ed. Wali MK), Vol 2. Permagon press, New York, pp. 958-966.

75. Citterio S, Santagostino A, Fumagalli P, Prato N, Ranalli P and Sgorbati S 2003 Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. Plant Soil, 256, 243-252.

76. Comis D 1996 Green remediation: Using plants to clean the soil. Journal of Soil and Water Conservation, 51, 184-187.

77. Conger RM and Portier R 1997 Phytoremediation experimentation with herbicide bentazon. Remediation, 7, 19-37.

78. Conner AJ, Keizer LCP, Mlynárová L and Stiekema WJ 1998 Evaluation of diallel analysis using β-glucuronidase activity from transgenes in Nicotiana tabacum. Euphytica, 102,161–168.

79. Cope F 1962 The development of a soil from an industrial waste ash. In: Soil Science and Society. In Trans. Comm. IV, V. International Society of Soil Science, Palmerstown, New Zealand, pp. 859-863.

80. Creman H, Velikonja-Bolta S, Vodnik D, Kos B and Lestan D 2001 EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant Soil, 235, 105-114.

81. Cui Y, Wang Q, Dong Y, Li H and Christie P 2004 Enhanced uptake of soil Pb and Zn by Indian mustard and winter wheat following combined soil application of elemental sulphur and EDTA. Plant Soil, 261, 181–188.

82. Cunningham JE and Kuiack C 1992 Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. Applied and Environmental Microbiology, 58, 1451-1458.

83. Cunningham S D and Ow D W 1996 Promises and prospects of phytoremediation. Plant Physiology, 110, 715-719.

84. Cunningham SD, Berti WR and Huang JW 1995 Phytoremediation of contaminated soils. Trends in Biotechnology, 13, 393-397.

85. Cunningham SD, Shann JR, Crowley DE and Anderson TA 1997 Phytoremediation of Contaminated Water and Soil. In: Phytoremediation of Soil and Water Contaminants. (eds. Kruger EL, Anderson TA and Coats JR), ACS Symposium Series No. 664. American Chemical Society, Washington, DC.

86. Davey HM and Kell DB 1996 Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. Microbiological Reviews, 60, 641-696.

87. Davis LC and Erickson LE 2002 A review of the potential for phytoremediation of the volatile contaminants ethylene dibromide, ethylene dichloride, and carbon tetrachloride. Proceedings of the Conference on Application of Waste Remediation Technologies to Agricultural Contamination of Water Resources. Kansas State University, pp. 205-220.

88. Davis LC, Erickson LE, Narayanan N and Zhang Q 2003 Modeling and design of phytoremediation. In: Phytoremediation: Transformation and Control of Contaminants. (eds. McCutcheon SC and Schnoor JL), Wiley, New York, pp.663-694.

89. Davison RL, Natusch DFS, Wallace JR and Evans CA Jr 1974 Trace elements in fly ash: Dependence of concentration on particle size. Environmental Science and Technology, 8, 1107-1113.

90. Degirmenci N 2008 Utilization of phosphogypsum as raw and calcined material in manufacturing of building products. Construction and Building Materails, 22, 1857-1862.

91. Dermatas D, Meng X 2003 Utilization of fly ash for stabilization/solidification of heavy metal contaminated soils. Engineering Geology, 70, 377-394.

92. Dhadse S, Kumari P and Bhagia LJ 2008 Fly ash characterization, utilization and government initiatives in India – A review. Journal of Scientific and Industrial Research, 67, 11-18.

93. Dierberg F E, Debusk T A and Goulet J R 1987 Removal of copper and lead using a thin-film technique. *In* Aquatic plants for water treatment and resource recovery. pp.497-504. Mangolia Publisher.

94. Dirilgen N 1998 Effects of pH and chelator EDTA on Cr toxicity and accumulation in *Lemna minor*. Chemosphere, 37, 771-783.

95. Djingova R, Ivanova J, Wagner G, Korhammer S and Markert B 2001 Distribution of lanthanoids, Be, Bi, Ga, Te, Tl, Th and U on the territory of Bulgaria using *Populus nigra* ‘‘Italica’’ as an indicator. Science of Total Environment, 280, 85-91.

96. Djingova R, Wagner G and Kuleff I 1999 Screening of heavy metal pollution in Bulgaria using *Populus nigra* ‘‘Italica’’. Science of Total Environment, 234, 175-184.

97. Djingova R, Wagner G, Kuleff I and Peshev D 1996 Investigations on the time-dependant variations in metal concentration in the leaves of *Populus nigra* ‘‘Italica’’. Science of Total Environment, 184, 197-202.

98. Doran JW and Martens DC 1972 Molybdenum availability as influenced by application of fly ash to soil. Journal of Environmental Quality, 1, 186-189.

99. Douglas JS and Hart RA 1985 Food Production using tree and other forest resources. Forrest Farming.Intermediate Technology Publications, New York, NY, pp. 207.

100. Drew AP, Guth RL and Greatbatch W 1987 Variation in heavy metal accumulation by hybrid poplar clones on sludge amended soil. Poplar culture to the year 2000. Proceedings of the Poplar Councils of the USA and Canada Joint Meeting. 109-117.

101. Dushenkov S and Kapulnik Y 2000 Phytofiltration of metals. *In* Phytoremediation of toxic metals: using plants to clean up the environment. Eds. Raskin I and Ensley B D. pp.89-106. John Wiley & Sons, New York.

102. Dushenkov V, Kumar PBAN, Motto H and Raskin I 1995 Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environmental Science and Technology, 29, 1239 -1245.

103. Eapen S, Suseelan KN, Tivarekar S, Kotwal SA and Mitra R 2003 Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Journal of Environmental Research, 91, 127-33.

104. Ebbs S, Brady D and Kochian L 1998 Role of uranium speciation in the uptake and translocation of uranium by plants. Journal of Experimental Botany, 49, 1183-1190.

105. Ebbs SD, Lasat MM, Brandy DJ, Cornish J, Gordon R and Kochian LV 1997 Heavy metals in the environment: Phytoextraction of cadmium and zinc from a contaminated soil. Journal of Environmental Quality, 26, 1424-1430.

106. Eberts S M, Harvey G J, Jones S A and Beckman S W 2003 Multiple-process assessment for a chlorinated-solvent plume. *In* Phytoremediation-Transformation and control of contaminants (eds. McCutcheon SC and Schnoor JL) Wiley, Hoboken, N.J. pp. 589–633.

107. Elfving DC, Bache CA, Gutenmann WH and Lisk DJ 1981 Analysis of crops grown on waste amended soils. Biocycle, 12, 44-47.

108. El-Mogazi D, Lisk DJ and Weinstein LH 1988 A review of physical and chemical and biological properties of fly ash and effects on agricultural ecosystems. Science of Total Environment, 74, 1-37.

109. El-Mogazi D, Lisk DJ and Weinstein LH 1988. A review of physical, chemical and biological properties of fly ash and effects on agricultural ecosystems. Science of Total Environment, 74, 1-37.

110. Elseewi AA and Page AL 1984 Molybdenum enrichment of plants grown on fly ash treated soils. Journal of Environmental Quality,13, 394-398.

111. Elseewi AA, Bingham FT and Page AL 1978 Availability of sulfur in fly ash to plants. Journal of Environmental Quality, 7, 69-73.

112. Elseewi AA, Grimm SR, Page AL and Straughan IR 1981 Boron enrichment of plants and soils treated with coal ash. Journal of Plant Nutrition, 3, 409-427.

113. Elseewi AA, Page AL and Grimm SR 1980a Chemical characterization of fly ash aqueous systems. Journal of Environmental Quality, 9, 424-428.

114. Elseewi AA, Straughan IR and Page AL 1980b Sequential cropping of fly ash amended soils. Effects on soil chemical properties and yield and elemental composition of plants. Science of Total Environment, 15, 247-259.

115. El-Shaushouny AR, Hassan Ma and Ghaffar BAA 1989 Synergistic effects of vesicular arbuscular mycorrhizae and *A. chroococcum* on the growth and the nutrient contents of tomato plants. Phyton (Horn), 29, 203-212.

116. Emery JJ and Matchett DB 1979 A waste management strategy for major industries. Conservation and Recycling, 3, 439-446.

117. EPA 1997 Electrokinetic laboratory and field processes applicable to radioactive and hazardous mixed waste in soil and groundwater. EPA/402/R-97/006. Environmental Protection Agency, Washington, DC.

118. EPA 2000 Introduction to phytoremediation. EPA/600/R-99/107. Environmental Protection Agency, Washington, DC.

119. Epelde L, Becerril JM, Barrutia O, González-Oreja JA and Garbisu C 2010 Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environmental Pollution, 158, 1576-1583.

120. Epelde L, Mijangos I, Becerril JM and Garbisu C 2009 Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biology and Biochemisrty, 41, 1788-1794.

121. Ernst WHO 1996 Bioavailability of heavy metals and decontamination of soils by plants. Applied Geochemistry, 11, 163-167.

122. Evans GM and Furlong JC 2003 Environmental Biotechnology: theory and applications. John Wiley and Sons, West Sussex, UK.

123. FAO 1986 Natural resources and the human environment for food and agriculture in Africa, pp. 88, FAO Environment and Energy Paper No. 6.

124. Finkelman RB, Belkin HE, Zhang BS and Centeno JA 2000 Arsenic Poisoning caused by residential coal combustion. In Proc.of the 31st Intl. Geol. Cong. Guizhou Province, China.

125. Flathman PE and Lanza GR 1998 Phytoremediation: current views on an emerging green technology. Soil and Sediment Contamination, 7, 415-432.

126. Flemming CA, Lee H and Trevors JT 1994 Bioluminescent Most-Probable-Number method to enumerate *lux*-marked *Pseudomonas aeruginosa* UG2Lr in soil. Applied and Environmental and Microbiology, 60, 3458-3461.

127. Gaind S and Gaur AC 2002 Impact of fly ash and phosphate solubilising bacteria on soybean productivity. Bioresource Technology, 85, 313-315.

128. Gaind S and Gaur AC 2003 Quality assessment of compost prepared from fly ash and crop residues. Bioresource Technology, 87, 125-127.

129. Gaind S and Gaur AC 2004 Evaluation of fly ash as a carrier for diazotrophs and phosphobacteria. Bioresource Technology, 95, 187-190.

130. Garbisu C, Hernandez-Allica J, Barrutia O, Alkorta I and Becerril J M 2002 Phytoremediation: A technology using green plants to remove contaminants from polluted areas. Environmental Health, 17, 75-90.

131. Gardea-Torresdey J L, Parsons J G, Gomez E, Peralta-Videa J, Troiani H E, Santiago P and Jose Y M 2002 Formation and growth of Au nanoparticles inside live alfa-alfa plants. Nano Letters, 2, 397-401.

132. Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G and Parsons JG 2005. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coordination Chemistry in Reviews, 249, 1797-1810.

133. Garg RN, Pathak H, Das DK and Tomar RK 2005 Use of fly ash and biogas slurry for improving wheat yield and physical properties of soil. Environmental Monitoring Assessment, 107, 1-9.

134. Gaur AC 1985 Phosphate solubilizing bacteria as biofertilizer. Proceedings National Seminar on Development and Use of Biofertilizers, Ministry of Agriculture, New Delhi.

135. Gaur AC 1990 Organic Manures, ICAR, New Delhi, pp. 159.

136. Geebelen W, Vangronsveld J, Adriano DC, Van Poucke LC and Clijsters H 2002 Effects of Pb-EDTA and EDTA on oxidative stress reactions and mineral uptake in *Phaseolus vulgaris*. Plant Physiology, 115, 377-384.

137. Gerard E, Echevarria G, Sterckeman T and Morel JL 2000 Cadmium availability to three plant species varying in cadmium accumulation pattern. Journal of Environmental Quality, 29, 1117-1123.

138. Ghafoor SA, Zia MH, Murtaza G, Waraich EA, Bibi S and Srivastava P 2010 Comparison of Organic and Inorganic Amendments for Enhancing Soil Lead Phytoextraction by Wheat (*Triticum aestivum L.*). International Journal of Phytoremediation, 12, 633-649.

139. Giachetti G and Sebastiani L 2007 Effects of tannery waste on growth dynamics and metal uptake in *Salix alba* L. Plant Biosystems, 141, 22-30.

140. Glass DJ 2000 Economic potential of phytoremediation. *In* Phytoremediation of toxic metals: using plants to clean up the environment (eds. Raskin I and Ensley BD). John Wiley & Sons, New York. pp.15-32.

141. Goyal D 2001 Biofetilizers: Exploitation potential and commercialization prospects. In: Business Opportunities in Biotechnology (ed. Tiwari S), Publ. Confederation of Indian Industry (CII), New Delhi, pp. 79-87.

142. Goyal D, Kaur K, Garg R, Vijayan V, Nanda SK, Nioding A, Khanna S, Ramamurthy V 2002 Industrial fly ash as a soil amendment agent for raising forestry plantations. In: Taylor PR (ed) EPD Congress and Fundamental of Advanced Materials for Energy Conversion. TMS Publication, Warrendale, PA, pp. 251–260

143. Goyal D, Sharma RK, Ramamurthy V and Kothari RM 2001 An integrated biotech approach for the sustainable development in agroforestry systems. In: Innovative Approaches in Microbiology (eds. Maheshwari DK and Dubey RC), Publ. BSMP Singh, Dehradun, pp. 111-120.

144. Goyal SK, Singh BV, Nagpal V and Marwaha TS 1997 An improved method for production of algal biofertilizer. Indian Journal of Agricultural Science, 67, 314.

145. Grant CA and Bailey LD 1997 Effects of phosphorus and zinc fertilizer management on cadmium accumulation in flaxseed. Journal of Science Food Agriculture, 73, 307-314.

146. Griga M, Bjelkova M and Tejklova E 2003 Potential of flax (*Linum usitatissimum*) for heavy metal extraction and industrial processing of contaminated biomass – a review. *In* Risk assessment and sustainable land management using plants in trace element-contaminated soils. Eds. Mench M and Mocquot B. pp.173-179. COST Action 837, 4th WG2 Workshop, Bordeaux 2002. INRA Centre Bordeaux-Aquitaine, Villenave d, Ornon.

147. Guarente L 1983 Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. Methods in Enzymology, 101, 181-191.

148. Guarente L and Ptashne M 1981 Fusion of *Escherichia coli lacZ* to the cytochrome c gene of *Sacchromyces cerevisiae*. Proceedings of the National Academy of Sciences of the United States of America, 78, 2199-2203.

149. Gustafsson K and Jansson JK 1993 Ecological risk assessment of the deliberate release of genetically modified microorganisms. Ambio, 22, 236-242.

150. Hammer D, Kayser A and Keller C 2003 Phytoextraction of Cd and Zn with *Salix viminalis* in field trials. Soil Use Management, 19, 187-192.

151. Hart BR, Hayden DB and Powell MA 2003 Evaluation of pulverized fuel ash mixed with organic matter to act as a manufactured gowth medium. International Ash Utilization Symposium, Centre for Applied Energy Research, University of Kentucky, Paper#119.

152. Harter RD 1983 Effect of soil pH on adsorption of lead, copper, zinc and nickel. Soil Science Society of American Journal, 47, 47–51.

153. Hartman WJ Jr 1975 An evaluation of land treatment of municipal wastewater and physical siting of facility installations. US Department of Army, Washington, DC.

154. Hasselgren K 1999 Utilisation of sewage sludge in short-rotation energy forestry: a pilot study. Waste Management Research, 17, 251-262.

155. Hildebrandt G and Schott W 2001 Comparison of direct colony count methods and the MPN-method for quantitative detection of Listeria in model and field conditions. Berl Munch Tierarztl Wochenschr. 114, 453-464.

156. Hill MJ and Lamp CA 1980 Use of pulverized fuel ash from Victorian brown coal as a source of nutrients for pasture species. Australian Journal Experiments in Agriculture and Animal Husbandary, 20, 377-84.

157. Huang J, Blaylock M, Kapulnik Y and Ensley B 1998 Phytoremediation of uranium contaminated soils: role of organic acids in trigerring uranium hyperaccumulation in plants. Environmental Science and Technology, 32, 2004-2008.

158. Huang JW, Chen R, Berti WR and Cunningham SD 1997 Phytoremediation of lead contaminated soils: role of synthetic chelators in lead phytoextraction. Environmental Science and Technology, 31, 800-805.

159. Huxley P 1999 Tropical Agroforestry. Oxford, UK, Blackwell Science Ltd, pp. 371.

160. Illmer P and Schinner F 1992 Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biology and Biochemistry, 24, 389-395.

161. Illmer P and Schinner F 1995 Solubilization of inorganic calcium phosphates, solubilization mechanisms. Soil Biology and Biochemistry, 27, 257-263.

162. Illmer P, Barbato A and Schinner f 1995 Solubilization of hardly soluble $AIPO_4$ with P-solubilizing microorganisms. Soil Biology and Biochemistry, 27, 260-270.

163. Isebrands JG and Karnosky DF 2001 Environmental benefits of poplar culture. In: Poplar Culture in North America NRC-CNRC Press, Ottawa, Ontario, Canada, pp 207-218.

164. Jackson ML 1967 Soil Chemical Analysis. Asia Publishing House, Bombay

165. Jacques MA, Linda LK and Morris CE 1995 Population sizes, immigration, and growth of epiphytic bacteria on leaves of different ages and positions of field grown endive (*Cichorium endivia var. latifolia*). Applied Environmental Microbiology, 61, 899-906.

166. Jain SK and Singh P 2000 Economic analysis of industrial agroforestry: Poplar (*Populus deltoides*) in Uttar Pradesh (India). Agroforestry Systems, 49, 255-273.

167. Jala S and Goyal D 2006 Fly ash as a soil ameliorant for improving crop production- A review. Bioresource Technology, 97, 1136-1147.

168. Jamwal N 2003 Is it all grey? Down to earth, 30: 38-41.

169. Jansson JK 2003 Marker and reporter genes: illuminating tools for environmental microbiologists. Current Opinion in Microbiology, 6, 310-316.

170. Jenkinsen DS 1988 Determination of microbial biomass carbon and nitrogen in soil. In: Advances in nitrogen cycling in agricultural ecosystems (ed. Wilson JR) CAB international, Willingford. pp. 368-386.

171. Jensen HL 1954 The Azotobacteriaceae. Journal of Bacteriology, 18, 195-214.

172. Jones R, Sun W, Tang CS and Robert FM 2004 Phytoremediation of petroleum hydrocarbons in tropical coastal soils. II. Microbial response to plant roots and contaminant. Environmenatl Science and Pollution Research, 11, 340-346.

173. Joshi HC, Pathak H, Choudhary A and Kalra N, 1996 Fertilizer News, 41, 41.

174. Kanshik RC 1961 Problems of Irrigated Forest Plantations. In proceedings of the 10th Silvicultural Conference, IFRI, Dehra Dun, India, 1: 469–477.

175. Katupitiya S, Millet J, Vesk M, Viccars L, Zeman A, Lidong Z, Elmerich C and Kennedy IR 1995a A mutant of *Azospirillum brasilense* Sp7 impaired in flocculation with a modified colonization pattern and superior nitrogen fixation in association with wheat. Applied and Environmental Microbiology, 61, 1987-1995.

176. Katupitiya S, New PB, Elmerich C and Kennedy IR 1995b Improved N_2 fixation in 2,4-D treated wheat roots associated with *Azospirillum lipoferum*: studies of colonization using reporting genes. Soil Biology and Biochemistry, 27, 447-452.

177. Kay SH, Haller WT and Garrard LA 1984 Effect of heavy metals on water hyacinth [*Eichhornia crassipes* (Mart.) Solms]. Aquatic Toxicology, 5, 117-128.

178. Kayser A, Wegner K, Keller A, Attinger W, Felix HR, Gupta SK and Schulin R 2000 Enhancement of phytoextraction of Zn, Cd and Cu from calcareous soil: the use of NTA and sulfur amendments. Environment Science & Technology, 34, 1778-1783.

179. Keeling S M, Stewart R B, Anderson C W and Robinson B H 2003 Nickel and cobalt phytoextraction by the hyperaccumulator *Berkheya coddii*: implications for polymetallic phytomining and phytoremediation. International Journal of Phytoremediation, 5, 235-244.

180. Kelley BC and Tuovinen OH 1988 Microbial oxidation of minerals in mine tailings. In: Chemistry and biology of solid waste (eds. Solomons W and Foerstner V). Springer Verlag, Berlin, pp. 33-53.

181. Khilji S and Bareen F 2008 Rhizofiltration of heavy metals from the tannery sludge by the anchored hydrophyte, *Hydrocotyle umbellatal*. African Journal of Biotechnology. 7, 3711-3717.

182. Khullar S and Chahal VPS 1977 Effect of *Azotobacter* inoculation on carrot. Indian Journal of Microbiology, 17, 45.

183. Kirk J, Klironomos J, Lee H and Trevors JT 2005 The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. Environmental Pollution, 133, 455-465.

184. Kishor P, Ghosh AK and Kumar D 2010 Use of flyash in agriculture: A way to improve soil fertility and its productivity. Asian Journal of Agricultural Research, 4: 1-14.

185. Klose S, Wernecke KD and Makeschin F 2004 Microbial activities in forest soils exposed to chronic depositions from a lignite power plant. Soil Biology and Biochemistry, 36, 1913-1923.

186. Klubek B, Carlson CL, Oliver J and Adriano DC 1992 Characterization of microbial abundance and activity from three coal ash basins. Soil Biology and Biochemistry, 24, 1119-1125.

187. Kluepfel DA 1993 The behavior and tracking of bacteria in the rhizosphere. Annual review of phytopathology, 31, 441-472.

188. Kohli SJ and Goyal D 2010 Effect of fly ash application on some soil physical properties and microbial activities. Acta Agrophysica, *In Press.*

189. Komossa D C, Langebartels and Sanderman H Jr. 1995 Metabolic processes for organic chemicals in plants. *In* Plant contamination: modeling and simulation of organic chemical processes (eds. Trapp S and McFarlane JC). Lewis Publishers, Boca Raton, FL.

190. Kopp RF, Smart LB, Maynard CA, Isebrands JG, Tuskan GA, Abrahamson LP 2001 The development of improved willow clones for eastern North America. The Forestry chronicle, 77, 287-292.

191. Kos B, Greman H and Lestan D 2003 Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil and Environment, 49, 548-553.

192. Kourtev PS, Ehrenfield JG and Huang WZ 2002 Emzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. Soil Biology and Biochemistry, 34, 1207-1218.

193. Kozlov MV, Haukioja E, Bakhtiarov AV and Stroganov DN 1995 Heavy metals in Birch leaves around a nickel-copper smelter at Monchegorsk, north western Russia. Environmental Pollution, 90, 291-299.

194. Kumar N 2002 Growth response in crops raised in fly ash amended soil. Pollution Research, 21, 409-416.

195. Kumar PBAN, Dushenkov V, Motto H and Raskin I 1995a Phytoextraction: the use of plants to remove heavy metals from soils. Environmental Science and Technology, 29, 1232-1238.

196. Kumar PBAN, Motto H and Raskin I 1995b Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environmental Science and Technology, 29, 1239-1245.

197. Kundu BS and Gaur AC 1980 Establishment of nitrogen fixing and phosphate solubilizing bacteria in rhizosphere and their effect on yield and nutrient uptake of wheat crop. Plant and Soil, 57, 223-230.

198. Lai KM, Ye DY and Wong JWC 1999 Enzyme activities in a sandy soil amended with sewage sludge and coal fly ash. Water, Air and Soil Pollution, 113, 261-272.

199. Landberg T and Greger M 1994 Can heavy metal tolerant clones of Salix be used as vegetation filters on heavy metal contaminated land? *In Willow* vegetation filters for municipal wastewaters and sludges. A biological purification system. Eds. Aronsson P and Perttu K. pp.133-144. Report 50, Swedish University of Agricultural Science, Uppsala.

200. Landberg T and Greger M 1996 Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Applied Geochemistry, 11, 175-180.

201. Lasat M 2000 Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. Journal of Hazardous Materials, 2, 5-25.

202. Lasat M 2002 Phytoextraction of toxic metals: A review of biological mechanisms. Journal of Environmental Quality, 31, 109-120.

203. Lee H, Ha HS, Lee CH, Lee YB and Kim PJ 2006 Fly ash effect on improving soil properties and rice productivity in Korean paddy soils. Bioresource Technology, 97, 1490-1497.

204. Lee M and Yang M 2010 Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L.) to remediate uranium contaminated groundwater. Journal of Hazardous Materials, 173, 589-596.

205. Leij FAAM, Sutton EJ, Whipps JM, Fenlon JS and Lynch JM 1995 Impact of Field Release of Genetically Modified *Pseudomonas fluorescens* on Indigenous Microbial Populations of Wheat. Applied and Environmental and Microbiology, 3443–3453

206. Lerche C and Khan AS 1967 Economic Management of Irrigated Plantations in West Pakistan. Pakistan Forest Institute, Pakistan, pp. 38.

207. Lewis BG, Johnson CM and Delwiche CC 1966 Release of volatile selenium compounds by plants. Collection procedures and preliminary observations. Journal of Agriculture and Food Chemistry, 14, 638-640.

208. Licht LA and Isebrands JG 2005 Linking phytoremediation pollutant removal to biomass economic opportunities. Biomass Bioenergy, 28: 203-218.

209. Licht LA and Schnoor JL 1993 Tree buffers protect shallow ground water at contaminated sites. EPA Ground Water Currents, Office of Solid Waste and Emergency Response. EPA/542/N-93/011.

210. Lindow SE, Knudsen GR, Seidler RJ, Walter MV, Lambou VW, Amy PS, Schmedding D, Prince V and Hern S 1988 Aerial dispersal and epiphytic survival of *Psuedomonas*

syringae during a pretest for the release of genetically engineered strains into the environment. Applied Environmental Microbiology, 54, 1557-1563.

211. Lindsay WL and Norvell WA 1978 Development of DTPA soil test for Zinc, Iron, Manganese and Copper. Soil Sci. Soc. Am. J., 42, 421-428.

212. Loper JE and Lindow SE 1997 Manual of Environmental Microbiology (ed. in chief Hurst CJ; eds. Koudsen GR, McInerney MJ, Stetzenbach LD and Walter MV) ASM Press Washington, D.C. pp 482-492.

213. Madejon P, Maranon T, Murillo JM and Robinson B 2004 White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests. Environmental Pollution, 132, 145-155.

214. Mains D, Craw D, Rufant CG and Smith CMS 2006 Phytostabilization of gold mine tailings, New Zealand. Part 1: Plant establishment in alkaline saline substrate. International Journal of Phytoremediation. 8, 131-147.

215. Malairajan P, Gopalkrishnan G, Narasimhan S, Veni KJK and Kavimani S 2007 Anti-ulcer activity of crude alcoholic extract of *Toona ciliata* Roemer (heart wood). Journal of Ethnopharmacology, 110, 348-351.

216. Manafi M, Kneifel W and Bascomb S 1991 Photogenic and chromogenic substrates used in bacterial diagnostics. Microbiology Review, 55, 335-348.

217. Manohari PL 2002 Organic farming for sustainable agriculture. Manage Extension Research Review, vol. III.

218. Martens DC 1971 Availability of plant nutrients in fly ash. Compost Science, 12, 15-19.

219. Masson L, Comeau Y, Brousseau R, Samson R and Greer C 1993 Construction and application of chromosomally integrated *lac-lux* gene markers to monitor the fate of a 2, 4-dichlorophenoxyacetic acid-degrading bacterium in contaminated soils. Microbiology Releases, 1, 209-216.

220. Mattigod SV, Rai D, Eary LE and Ainsworth CC 1990 Geochemical factors controlling the mobilization of inorganic constituents from fossil fuel combustion residues: I. Review of the major elements. Journal of Environmental Quality, 19, 188–201.

221. Maurice C and Lagerkvist A 2000 Using *Betula pendula* and *Telephora caryophyllea* for soil pollution assessment. Journal of Soil Contamination, 9, 31-50.

222. McBride MB 1994 Environmental Chemistry of Soils. New York: Oxford University Press, pp. 416.

223. McCutcheon SC 1996 Phytoremediation of organiccCompounds: science validation and field-testing. In: Workshop on phytoremediation of organic wastes (eds. Kovalick WW and Olexsey R) Worth, TX. An EPA unpublished meeting summary (http://www.rtdf.org).

224. McCutcheon SC and Schnoor JL 2003 Overview of phytotransformation and control of wastes. In: Phytoremediation-Transformation and control of contaminants (eds. McCutcheon SC and Schnoor JL). Wiley, Hoboken, N.J. pp. 3–58.

225. McGrath S, Sidoli C, Baker AJM and Reeves R 1993 The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: Integrated Soil and Sediment Research: A basis for proper protection (eds. Eijsackers H and Hamers T). Kluwer, Dordrecht. pp.673-676.

226. McGrath SP and Zhao F 2003 Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology, 14, 277-282.

227. Mcinnes BIA, Dunn C, Cameron EM and Kameko L 1996 Biogeochemical exploration for gold in tropical rain forest regions of Papua New Guinea. Journal of Geochemical Exploration, 57, 227-243.

228. McIntyre TC 2003 Databases and protocol for plant and microorganism selection: hydrocarbons and metals. In: Phytoremediation: Transformation and Control of Contaminants (eds. McCutcheon SC and Schnoor JL). Wiley, New York. pp.887-904.

229. Meagher RB 2000 Phytoremediation of toxic elemental and organic pllutants. Current Opinion in Plant Biology, 3, 153-162.

230. Meers E, Tack FMG, Van SS, Ruttens A, Vangronsveld J and Verloo MG 2008 Chemically assisted phytoextraction: A review of potential soil amendments for increasing plant uptake of heavy metals. International Journal of Phytoremediation, 10, 390-414.

231. Mei B, Purytar JD and Newton RJ 2002 Assessment of chromium tolerance and accumulation in selected plant species. Plant Soil, 247, 223-231.

232. Mendel M and Higa A 1970 Calcium chloride method for bacterial transformation. Journal of Molecular Biology, 53, 159-162.

233. Mendelssohn IA, McKee KL and Kong T 2001 A comparison of physiological indicators of sublethal cadmium stress in wetland plants. Environmental Experimental Botany, 46, 263–275.

234. Menon MP 1993 Elements in Coal and Coal Ash Residues and their Potential for Agricultural Crops. Trace Elements in Coal and Coal Combustion Residues. Lewis Publishers, Florida, USA, pp. 259–285.

235. Merwin HD and Peech M 1951 Exchangeability of soil potassium in sand, silt and clay fractions, as influenced by the nature of the complimentary exchangeable cations. Proceedings of Soil Science Society of America, 15, 125-158.

236. Metting FB 1992 Structure and physiological ecology of soil microbial communities. In: Soil Microbial Ecology (ed. Metting Jr FB). Marcel Dekker, New York, pp 3-25

237. Meyers RJK 1994 The biological management of tropical soil fertility (ed. Woomer PL and Swift MJ), John Wiley and Sons, West Sussex, U.K., pp. 81-116.

238. Minguzzi C and Vergnano O 1948 Il contento di nichel nelli ceneri di Alyssum bertlonii Desv. Atti della Societa Toscana di Science Naturali, Mem Ser A 55, 49-77.

239. Mirck J, Isebrands JG, Verwijst T, Ledin S 2005 Development of short rotation willow coppice systems for environmental purposes in Sweden. Biomass Bioenergy, 28, 219-228

240. Mishra PK 2004 Plant species for Restoration of Degraded Land. Challenges in Sustainable Development. 166-171.

241. Mittra BN, Karmakar S, Swain DK and Ghosh BC 2003 Fly ash – A potential source of soil amendment and a component of integrated plant nutrient supply system (Centre for Applied Energy Research, University of Kentucky) 1447-1451.

242. Mo SC, Choi DS and Robinson JW 1989 Uptake of mercury from aqueous solution by duckweed: the effect of pH, copper and humic acid. Journal of Environmental Health, 24, 135-146.

243. Molliner AM and Street JJ 1982 Effect of fly ash and lime on growth and composition of corn (*Zea mays* L.) on acid sandy soils. Proceedings of Soil Crop Science and Society, FL, 41, 217–220.

244. Mullis and Kary Dancing Naked in the Mind Field, ISBN 0679774009.

245. Murugaragavan R 2002 Distillery spentwash on crop production in dryland soils. M. Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

246. Narayanan M, Davis LC and Erickson LE 1995 Fate of volatile chlorinated organic compounds in a laboratory chamber with alfalfa plants. Environmental Science and Technology, 29, 2437–2444.

247. Narayanan MT, Tracy JC, Davis LC and Erickson LE 1998 Modeling the fate of toluene in achamber with alfalfa plants 2. Numerical results and comparison study. Journal of Hazardous Substance Research, 1, 1-28.

248. Natusch DSF and Wallace JR 1974 Urban aerosol toxicity: the influence of particle size. Science, 186, 695.

249. Negassa W, Gebrekidan H and Friesen DK 2005 Integrated use of farmyard manure and NP fertilizers for Maize on farmers' fields. Journal of Agriculture and Rural Development in the Tropics and Subtropics, 106, 131-141.

250. Negri CM and Hinchman RR 2000 The use of plants for the treatment of radionuclides. In: Phytoremedation of toxic metals: Using plants to clean up the environment. Eds. Raskin I, pp. 107-132. Wiley Interscience, John Wiley & Sons Inc. New York, NY.

251. Newman LA and Reynolds CM 2004 Phytodegradation of organic compounds. Current Opinion in Biotechnology, 15, 225-230.

252. Newman LA, Bod C, Cortellucci R, Domroes D, Duffy J, Ekuan G, Fogel D, Heilman P, Muiznieks IA, Newman T, Ruszaj M, Strand S and Gordon MP 1997b Abstract: Results

from a Pilot Scale Demonstration: Phytoremediation of trichloroethylene and carbon tetrachloride. 12th Annual Conference on Contaminated Soils.

253. Newman LA, Strand SE, Choe N, Duffy J and Ekuan G 1997a Uptake and biotransformation of trichloroethlene by hybrid poplars. Environmental Science and Technology, 31, 1062-1067.

254. Nikado H 1994 Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science, 264, 382-388.

255. Nowak DJ, McHale PJ, Ibarra M, Crane D, Stevens J and Luley C 1998 Modeling the effects of urban vegetation on air pollution. *In*: Air Pollution Modeling and Its Application XII. Eds. Gryning S and Chaumerliac N. pp.399-407. Plenum Press, New York.

256. Nwaichi EO, Onyeike EN and Wegwu MO 2010 Comparison of chicken manure and urea fertilizers as potential soil amendments for enhanced phytoextraction of heavy metals. Bioremediation Journal, 14, 180-188.

257. Oblinger JL and Koburger JA 1975 Understanding and Teaching the Most Probable Number Technique. Journal of Milk Food Technology, 38, 540-545.

258. Ocampo JA, Barea JM and Montoya, E 1975 Interaction between *Azotobacter* and phosphobacteria and their establishment in the rhizosphere as affected by soil fertility. Canadian Journal of Microbiology, 21, 1160-1165.

259. Olsen SR, Cole CV, Watanabe FS and Dean LA 1954 Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circular U. S. Department of Agriculture, 939.

260. Padmavathiamma PK and Li LY 2010 Effect of Amendments on Phytoavailability and Fractionation of Copper and Zinc in a Contaminated Soil. International Journal of Phytoremediation, 12, 697-715.

261. Page AL 1982 Methods of Soil Analysis Part II. Soil Sci. Society of America, Madison, Wisconsin.

262. Page AL, Elseewi AA, Straughan IR 1979 Physical and Chemical properties of fly ash from coal-fired power plants with special reference to environmental impacts. Residue Review, 71: 83-120.

263. Pal R and Rai JPN 2010 The phytoextraction potential of water hyacinth (*Eichhornia crassipes*), removal of selenium and copper. Chemistry and Ecology, 26, 163-172.

264. Parekh BK and Goldberger WM 1976 An assessment of technology for possible utilization of Bayer process Muds. EPA 600/2-76-301, Washington D.C.

265. Paterson S, Mackay D, Tam D and Shiu WY 1990 Uptake of organic chemicals by plants: A review of processes, correlations and models. Chemosphere, 21, 297-331.

266. Pawlowska TE, Chaney RL, Chin M and Charvat I 2000 Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal contaminated landfill. Applied Environmental Microbiology, 66, 2526-2530.

267. Petruzzelli G 1989 Recycling wastes in agriculture: heavy metal bioavailability. Agriculture Ecosystem and Environment, 27, 493–503.

268. Phaenark C, Pokethitiyook P, Kruatrachue M and Ngernsansaruay C 2009 Cd and Zn accumulation in plants from the padaeng zinc mine area. International Journal of Phytoremediation, 11, 479-495.

269. Phung HT, Lam HV, Lund LJ and Page AL 1979 The practice of leaching Boron and salts from fly ash amended soils. Water, Air and Soil Pollution, 12, 247-254.

270. Pierzynski GM, Schnoor JL, Youngman A, Licht L and Erickson LE 2002 Poplar trees for phytostabilization of abandoned zinc-lead smelter. Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management. 6, 177-183.

271. Pietrini F, Zacchini M, Iori V, Pietrosanti L, Bianconi D and Massacci A 2010 Screening of Poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. International Journal of Phytoremediation, 12, 105-120.

272. Pikovskya RI 1948 Mobilization of phosphorous in soil in connection with the vital activity of some microbial species. Mikrobiologiya, 17, 362-370.

273. Pilon-Smits E 2005 Phytoremediation. Annual Review of Plant Biology, 56, 15-39.

274. Pilon-Smits E, de Souza MP, Hong G, Amini A and Bravo RC 1999 Selenium volatilization and accumulation by twenty aquatic plant species. Journal of Environmental Quality, 28, 1011-1017.

275. Piper CS 1960 Soil and Plant Analysis. Hans Publishers, Bombay

276. Pitchel JR 1990 Microbial respiration in fly ash/sewage sludge amended soils. Environmental Pollution, 63, 225–237.

277. Pitchel JR and Hayes JM 1990 Influence of fly ash on soil microbial activity and populations. Journal of Environmental Quality, 19, 593–597.

278. Plank CO, Martens DC and Hallock DL 1975 Effect of soil application of fly ash on chemical composition and yield of corn (*Zea mays* L.) and on chemical composition of displaced soil solutions. Plant and Soil, 42, 465-476.

279. Prasad MNV and Freitas HMO 2003 Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology. Electronical Journal of Biotechnology, 6, 285-321.

280. Prasad PM and Sharma JM 1986 Characterization of and applications for an Indian red mud. Proceedings of Electrometallurgy, pp. 12-23.

281. Prosser JI 1994 Molecular marker systems for detection of genetically engineered micro-organisms in the environment. Microbiology, 140, 5-17.

282. Pulford ID and Watson C 2003 Phytoremediation of heavy metal contaminated land by trees – a review. Environment Internationsl, 29, 529-540.

283. Punshon T and Dickinson NM 1997 Acclimation of *Salix* to metal stress. New Phytology, 137, 303-314.

284. Purakayastha TJ, Viswanath S, Bhadraray S, Chhonkar PK, Adhikari PP and Suribabu K 2008 Phytoextraction of zinc, copper, nickel and lead from a contaminated soil by different species of *Brassica*. International Journal of Phytoremediation, 10, 61-72.

285. Rabinow and Paul Making PCR : A Story of Biotechnology, ISBN 0226701468.

286. Rajukkannu K and Manickam TS, Proceedings of the Sixth National Symposium on Environment, Tamil Nadu Agricultural University, Coimbatore, India, 1997, 286-290.

287. Ram LC, Srivastava NK, Jha SK, Sinha AK, Masto RE and Selvi VA 2007 Management of lignite fly ash for improving soil fertility and crop productivity. Environmental Management, 40, 438-452.

288. Ramana S, Biswas AK and Singh AB 2002 Bioresource Technology 84, 295.

289. Rao AV and Sharma RL 1981 Effect of *Azotobacter* on wheat and tomato. Indian Journal of Microbiology, 21, 25-27.

290. Rao KV 1996 Biostatistics. A Manual of Statistical Methods for Use in Health, Nutrition and Anthropology. Jaypee Brothers, Medical Publishers (Pvt.) Limited. New Delhi.

291. Rao SS and Shaktawat MS 2002 Effect of organic manure, phosphorus and gypsum on groundnut (*Arachis hypogaea*) production under rainfed condition. Indian Journal of Agronomy, 47, 234-241.

292. Rashid M, Khalil S, Ayub N, Alam S and Latif F 2004 Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under *in vitro* conditions. Pakistan Journal of Biological Sciences, 7, 187-196.

293. Raskin I and Ensley BD 2000 Phytoremediation of toxic metals using plants to clean up the environment. Wiley, New York. pp. 316.

294. Raskin I, Kumar PBAN, Dushenkov S and Salt DE 1994 Bioconcentration of heavy metals by plants. Current Opinion in Biotechnology, 5, 285-290.

295. Raskin I, Smith RD and Salt DE 1997 Phytoremediation of metals: Using plants to remove pollutants from the environment. Current Opinion in Biotechnology, 8, 221–226.

296. Rautaray SK, Ghosh BC and Mittra BN 2003 Effect of fly ash, organic wastes and chemical fertilizers on yield, nutrient uptake, heavy metal content and residual fertility in a rice-mustard cropping sequence under acid lateritic soils. Bioresource Technology, 90, 275-283.

297. Rees WI and Sidrak GH 1956 Plant nutrition on fly ash. Plant and Soil, 8, 141–153.

298. Reeves RD and Baker AJM 2000 Metal-accumulating plants. *In* Phytoremediation of toxic metals: using plants to clean up the environment. Eds. Raskin I and Ensley BD. pp.193-230. John Wiley & Sons, New York.

299. Reynolds KA, Kruger RA and Rethman NFG 1999 The manufacture and evaluation of an artificial soil prepared from fly ash and sewage sludge. Proceedings of International Ash Utilization Symposium, Lexington, Kentucky, U.S.A, pp. 378-385.

300. Riekerk H 1984 Coal- ash effects on fuelwood production and runoff water quality. Southern Journal of Applied Forestry, 8, 99-102.

301. Rippon JE and Wood MJ 1975 Microbiological aspects of pulverized fuel ash. In Chadwick MJ and Goodman GT (ed.). The ecology of resource degradation and renewal. John Wiley and Sons, New York, 331-349.

302. Rizvi R and Khan AA 2009 Response of Eggplat (*Solanum melongena* L.) to fly ash and brick kiln dust amended soil. Biology and Medicine, 1, 20-24.

303. Robinson BH, Leblanc M, Petit D, Brooks RR, Kirkman JH and Gregg PEH 1998 The potential of *Thlaspi caerulescenss* for phytoremediation of contaminated soils. Plant and Soil, 203, 47-56.

304. Robinson BH, Mills TM, Petit D, Fung LE, Green SR and Clothier BE 2000 Natural and induced cadmium accumulation in poplar and willow: Implications for phytoremediation. Plant Soil, 227, 301-306.

305. Rockwood DL, Cardellino R, Alker G, Lin C, Brown N, Spriggs T, Tsangaris S, Isebrands J, Hall R, Lange R, Nwokike B 2004 Fast-growing trees for heavy metal and chlorinated solvent phytoremediation. In: Magar VS, Kelley ME (eds) Proceedings of the Seventh International In Situ and On-Site Bioremediation Symp., Orlando, FL, June 2-5, 2003, Paper F-12, Battelle Press, Columbus, OH

306. Rodriguez E, Peralta Videa JR, Parsons JG, Jimenez GC, Gonzalez JR, Salcido BES, Saupe GB, Gardea MD and Gardea Torresday JL 2007 Phytomining using XAS to determine gold reduction and nanoparticle formation within plant tissues. International Journal of Phytoremediation, 9, 133-147.

307. Rodriguez H and Fraga R 1999 Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances, 17, 319-339.

308. Rose M, Casadaban MJ and Botstein D 1981 Yeast genes fused to β-galactosidase in *Escherichia coli* can be expressed normaly in yeast. Proceedings of the National Academy of Sciences of the United States of America, 78, 2460-2464.

309. Ross DJ 1990 Estimation of soil microbial carbon C by a fumigation method: influence of seasons, soils and calibration with the fumigation incubation procedure. Soil Biology and Biochemistry, 22, 295-300.

310. Rumpel C, Knicker H, Kogel-Knaber I, Skjiemstad JO and Huuetti RF 1998 Types and chemical composition of organic matter in reforested lignite-rich mine soils. Geoderma, 86, 123-142.

311. Russell D and Franzel S 2004 Trees of prosperity: agroforestry, markets, and the African smallholder. In: NewVistas in Agroforestry: A Compendium for the 1st World Congress of Agrofrestery, (eds. Nair PKR, Rao MR and Buck LE) Netherlands, Kluwer Academic Publishers, pp. 345-356.

312. Saffigna PG, Powlson DS, Brookes PC and Thomas GA 1989 Influence of sorghum residues and tillage on soil organic matter and soil microbial biomass in an Australian vertisol. Soil Biology and Biochemistry, 21, 759-765.

313. Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I and Raskin I 1995 Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 13, 468-474.

314. Salt DE, Smith RD and Raskin I 1998 Phytoremediation. Annual Review of Plant Physiology, 49, 643-668.

315. Sambrook and Russell 2001 Molecular cloning [A lab manual], 3rd edition. Cold Spring Harbor Laboratory Press.

316. Santilan-Metrano J and Jurinak JJ 1975 The chemistry of lead and cadmium in soils: Solid phase formation. Soil Science Society of America Proceedings, 29, 851-856.

317. Schnappinger MG Jr, Martens DC and Plank CO 1975 Zinc availability as influenced by application of fly ash to soil. Environmental Science and Technology, 9, 258-261.

318. Schneegurt MA and Kulpa CF Jr. 1998 The application of molecular techniques in environmental biotechnology for monitoring microbial systems. Biotechnology and Applied Biochemistry, 27, 73-79.

319. Schnoor JL 2000 Phytostabilization of metals using hybrid poplar trees. *In* Phytoremediation of toxic metals: using plants to clean up the environment. Eds. Raskin I and Ensley BD. pp.133-150. John Wiley & Sons, New York.

320. Schnoor JL 2004 Demonstration project of phytoremediation and rhizodegradation of perchlorate in groundwater at the Longhorn Army Ammunition Plant. University of Iowa, department of civil and environmental engineering.

321. Schnoor JL, Licht LA, McCutcheon SC, Wolfe NL and Carreira LH 1995 Phytoremediation of organic and nutrient contaminants. Environmental Science and Technology, 29, 318-323.

322. Schutter ME and Fuhrmann JJ 2001 Soil microbial community responses to fly ash amendment as revealed by analyses of whole soils and bacterial isolates. Soil Biology and Biochemistry, 33, 1947-1958.

323. Seeling B and Junjk A 1992 VDLUFA-Seheriftemeihe, 35, 147-150.

324. Seidel A, Zimmels Y, Armon R 2001 Mechanism of bioleaching of coal fly ash by *Acidithiobacillus tiooxidans*. Chemical Engineering Journal, 83,123–130.

325. Sell J, Kayser A, Schulin R and Brunner I 2005 Contribution of ectomycorrhizal fungi to cadmium uptake of poplars and willows from a heavily polluted soil. Plant and Soil, 277, 245-253.

326. Sen T and Mishra U 2010 Usage of industrial waste products in village road construction. International Journal of Environmental Science and Development, 1, 122-126.

327. Shahandeh H and Hossner IR 2000 Plant screening for chromium phytoremediation. International Journal of Phytoremediation, 2, 31-51.

328. Shapiro HM 1990 Flow cytometry in laboratory microbiology: New directions. ASM News. 56, 584-588.

329. Sharma BM, Aggarwal RK and Kumar P 1990 Water retention and nutrient availability in a fly ash amended desert sandy soil: A study in vitro. Arid Soil Research and Rehabilitation, 4, 53-58

330. Sharma NK, Samra JS and Singh HP 2001 Influence of boundary plantation of poplar (*Populus deltoides* M.) on soil-water use and water use efficiency of wheat. Agricultural Water Management, 51, 173-185.

331. Shen ZG, Li XD, Wang CC, Chen HM and Chua H 2002 Lead phytoextraction from contaminated soil with high biomass plant species. Journal of Environmental Quality, 31, 1893-1900.

332. Sikka R and Kansal BD 1994 Characterization of thermal power-plant fly ash for agronomic purposes and to identify pollution hazards. Bioresource Technology, 50: 269-273

333. Singh GB and Dwivedi BS 1996 Integrated nutrient management for sustainability. Indian Farming, 46, 9-15.

334. Singh N, Singh SN, Yunus M and Ahmad KJ 1994 Growth response and element accumulation in *Beta vulgaris* L. raised in fly ash amended soils. Ecotoxicology, 3, 287-298.

335. Sinhal VK, Srivastava A and Singh VP 2010 EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). Journal of Environmental Biology, 255-259.

336. Slauch JM and Silhavy TJ 1991 Genetic fusions as experimental tools. Methods in Enzymology, 204, 213-248.

337. Smith RAH and Bradshaw AD 1992 Stabilization of toxic mine wastes by the use of tolerant plant populations. Transaction of the Institution of Mining and Metallurgy. 81, A230-A237.

338. Smith WH 1990 Air pollution and forests. pp.618. Springer-Verlag, New York.

339. Sparling GP and West AW 1989 Importance of soil water content when estimating soil microbial C, N and P by the fumigation-extraction methods. Soil Biology and Biochemistry, 21, 245-253.

340. Sthiannopkao S and Sreesai S 2009 Utilization of pulp and paper industrial wastes to remove heavy metals from metal finishing wastewater. Journal of Environment Management, 90, 3283-3289.

341. Subbiah BV and Asija GL 1956 A rapid procedure for the determination of available nitrogen in soils. Current Science, 25, 259-260.

342. Sun B, Zhao FJ, Lombi E and McGrath SP 2001 Leaching of heavy metals in contaminated soils using EDTA. Environental Pollution, 113, 111-120.

343. Surridge AKJ, Merwe A and Kruger R 2009 Preliminary microbial studies on the impact of plants and South African fly ash on amelioration of crude oil polluted soils. In World of Coal Ash (WOCA) Conference, May 4-7.

344. Suryan S, Jala S, Goyal D, Singh J, Vijayan V, Nioding A and Khanna S 2004 Impact of fly ash addition in soil on heavy metal accumulation by Eucalyptus tereticornis. In Biotechnological approaches for sustainable development (eds. Reddy MS and Khanna S). Allied Publishers, New Delhi. pp. 305-308.

345. Tandon HLS 1992 Fertilizers, organic manures, recyclable wastes and biofertilizers: Components of integrated plant nutrition. New Delhi: Fertilizer Development and Consultation Organization

346. Tate KR, Ross DJ and Feltham CW 1988 A direct extraction method to estimate soil microbial biomass C: effects of experimental variables and some different calibration procedures. Soil Biology and Biochemistry, 20, 329-335.

347. Tejwani KG 1994 Agroforestry in India. New Delhi, India: Concept Publishing Co. pp. 222.

348. Thompson PL, Ramer LA and Schnoor JL 1998 Uptake and transformation of TNT by hybrid poplar trees. Environmental Science and Technology, 32, 975-980.

349. Tichy R, Fajtl J, Kuzel S and Kolar L 1997 Use of elemental sulphur to enhance a cadmium solubilization and its vegetative removal from contaminated soil. Nutrient Cycling in Agroecosystems, 46, 249-255.

350. TIFAC, Technology linked Business Opportunity Publications, Non Conventional Sources of Plant Nutrient & Soil Conditioners to Enhance Agricultural Productivity 2001 Code no. TMS1551.

351. Tiwari VN, Pathak AN and Lehri KK 1988 Manurial value of compost enriched with rock phosphate and microbial inoculants to green gram. Journal of Indian Society of Soil Science, 36, 280-283.

352. Tsuchida T, Berlin WK and Sauer B 2004 Dual chromogenic reporter gene detection in mammalian cells with *lacZ* and arabinofuranosidase. Biotechniques, 37, 896-897.

353. U.S. EPA. 2000. Introduction to Phytoremediation. EPA/600/R-99/107. Office of Research and Development, Washington, DC.

354. Uhl MA and Johnson AD 2001 Development of *Streptococcus thermophilus lacZ* as a reporter gene for *Candida albicans.* Microbiology, 147, 1189-1195.

355. Utsunamyia T 1980 Japanese Patent Application No. 55-72959.

356. Valliappan K 1998 Recycling of distillery spentwash and ecofriendly effective reclamation technology for soils, Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

357. Van NM and Ong CK 1999 Can the ecosystem mimic hypotheses be applied to farms in African savannahs? Agroforestry Systems. 45: 131-158.

358. Vance ED, Brookes PC and Jenkinson DS 1987 An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19, 703-707.

359. Vandenhove H, Merckx R, Wilmots H and Vlasak K 1991 Survival of *Psuedomonas fluorescens* inocula of different physiological stages in soil. Soil Biology and Biochemistry, 23, 1133-1142.

360. Vangronsveld J, van Assche F and Clijsters H 1995 Reclamation of a bare industrial area contaminated by non-ferrous metals: in situ metal immobilization. Environmental Pollution, 87, 51-59.

361. Vassilev A, Kerin V and Atanassov P 1996 Effect of cadmium pollution of soil upon productivity and seedling qualities of two barley (*H. vulgare* L.) cultivars. Bulgarian Journal of Agricultural Science, 2, 333-340.

362. Vassilev A, Schwitzguebel JP, Thewys T, van der Lelie D and Vangronsveld J 2004 The use of plants for remediation of metal-contaminated soils. Journal of Science World, 4, 9-34.

363. Vassilev A, Vangronsveld J and Yordanov I 2002 Cadmium phytoextraction: present state, biological backgrounds and research needs. Bulgarian Journal of Plant Physiology, 28, 68-95.

364. Verma OP and Shende ST 1993 *Azotobacter* a biofertilizer for vegetable crops. Biofertilizer Newsletter, 1, 6-10.

365. Vesquez P, Holguin ME, Puente A, Cortes L and Bashan Y 2000 Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal lagoon. Biology and Fertility of Soils, 30, 460-468.

366. Walkley A and Black IA 1934 Estimation of soil organic carbon by chromic acid titration method. Soil Science, 37, 29–38.

367. Wallace A, Alexander GV, Soufi SM and Mueller RT 1980. Micronutrient supplying power of pyrite and fly ash. Journal of Plant Nutrition, 2, 147-153.

368. Weinstein LH, Osmeloski JF, Rutzke M, Beers AO, McCahan JB, Bache CA and Lisk DJ 1989 Elemental analysis of grasses and legumes growing on soil covering coal fly ash landfill sites. Journal of Food Safety 9, 291-300.

369. Whiting SN, Neumann PM and Baker AJM 2003 Nickel and zinc hyperaccumulation by Alyssum murale and Thlaspi caerulescens (Brassicaceae) do not enhance survival and whole plant growth under drought stress. Plant and Cell Environment, 26, 351-360.

370. William ST and Davies FL 1965 Use of antibiotics for selective isolation and enumeration of Actinomycetes in soil. Journal of General Microbiology, 38, 251-261.

371. Wilson DM and Lindow SE 1993 Release of recombinant microorganisms. Annual Review of Microbiology, 47, 913-944.

372. Wilson K, Hughes JSG and Jeferson RA 1992 The *Escherichia coli gus* operon: Introduction and expression of the *gus* operon in *E. coli* and the occurrence and use of *GUS* in other bacteria. In: *GUS* protocols: using the *GUS* gene as a reporter of gene expression (ed. Gallagher SR). Academic Press, Inc., New York. pp. 7-22.

373. Wong MH and Wong JWC 1986 Effects of fly ash on soil microbial activity. Environmental Pollution, 40, 127-144.

374. World Agroforestry Centre 1993 http://www.worldagroforestrycentre.org/sea/publications.

375. World Bank 1996 Pollution Prevention and Abatement: Iron and Steel Manufacturing". Draft Technical Background Document. Environment Department, Washington, D.C.

376. Yang XE, Li TQ, Long XX, Xiong YH, He ZL and Stoffella PJ 2006 Dynamics of zinc uptake and accumulation in the hyperaccumulating and non-hyperaccumulating ecotypes of Sedum alfredii Hance. Plant Soil, 284, 109–119.

377. Yang XE, Long XX, Ni WZ and Fu CX 2002 *Sedum alfredii* H: A new Zn hyperaccumulating plant first found in China. Chinense Science Bulletin, 47, 1634-1637.

378. Yang XE, Long XX, Ye HB, He ZL, Calvert DV and Stofella PJ 2004 Cadmmium tolerance and hyperaccumulation in a new Zn-hyperaccumumlating plant species (*Sedu alfredii* Hance). Plant and Soil, 259, 181-189.

379. Yankov B and Tashin N 2001 Accumulation and distribution of Pb, Cu, Zn and Cd in sunflower (*Helianhus annuus* L.) grown in an industrially polluted region. Helia. 24, 131-136.

380. Yankov B, Delibaltova V and Bojinov M 2000 Content of Cu, Zn, Cd and Pb in the vegetative organs of cotton cultivars grown in industrially polluted regions. Plant Science, 37, 525-531.

381. Young A 1989 Agroforestry for Soil Conservation. ICRAF: Science and Practice of Agroforestry, 4, UK, CAB Int, pp. 276.

382. Yu XZ, Peng XY and Xing LQ 2010 Effect of temperature on phytoextraction of hexavalent chromium by hybrid willows. Ecotoxicology, 19, 61-68.

383. Zacchini M, Pietrini F, Scarascia MG, Jori V, Pietrosanti L and Massacci A 2009 Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. Water, Air and Soil Pollution, 197, 23-34.

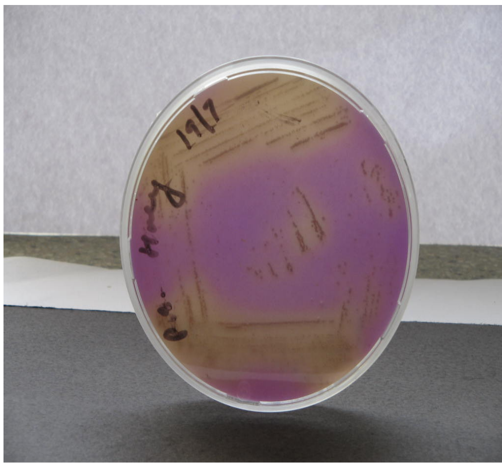
384. Zalesny JA, Zalesny Jr RS, Wiese AH, Sexton BT and Hall RB 2008 Uptake of macro- and micro-nutrients into leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. Journal of Sustainable Forestry, 27, 303-327.

385. Zhang GY, Dou Z, Toth JD and Ferguson J 2004 Use of fly ash as environmental and agronomic amendments. Journal of Environmental Geochemistry and Health, 26, 129-134.

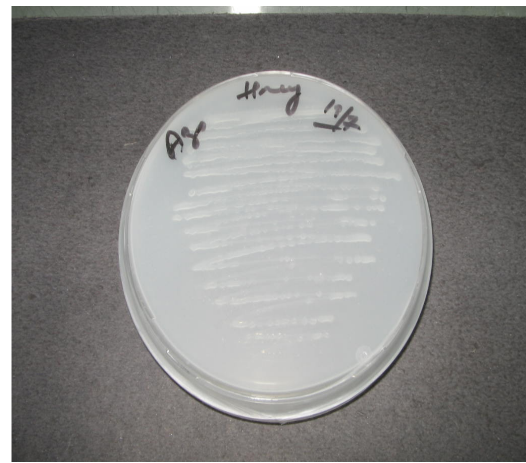
386. Zhang YZ, Naleway JJ, Larison KD, Huang Z and Haugland RP 1991 Detecting *lac Z* expression in living cells with new lipophilic, fluorogenic - galactosidase substrates. FASEB Journal, 5, 3108-3113.

387. Zheljazkov V and Nielsen N 1996 Effect of heavy metals on peppermint and cornmint. Plant Soil. 178, 59–66.

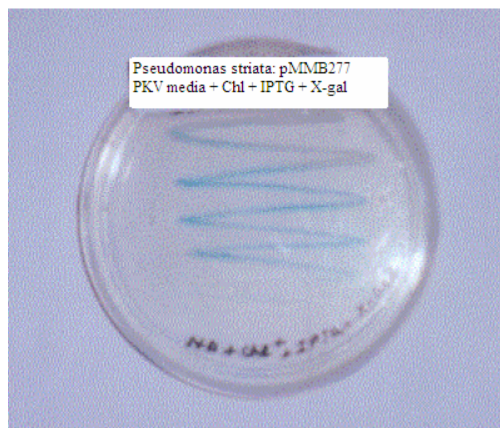
388. Zhu YL, Zayed AM, Quian JH, De Souza M and Terry N 1999 Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. Journal of Environmental Quality, 28, 339-344.



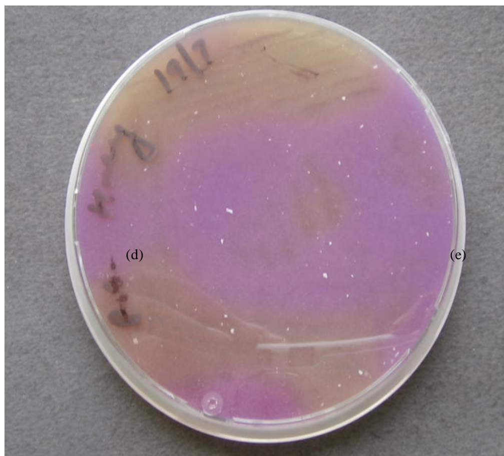
(a)



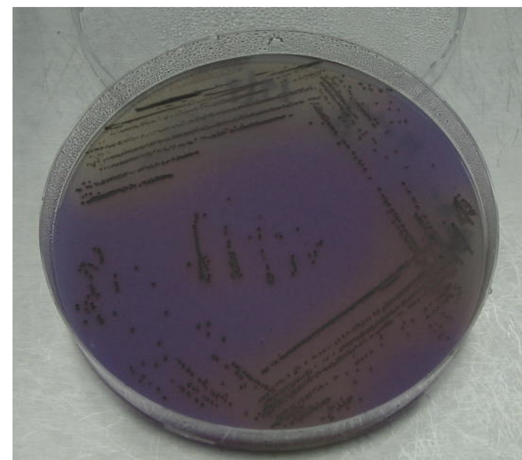
(b)



(c)



(d)



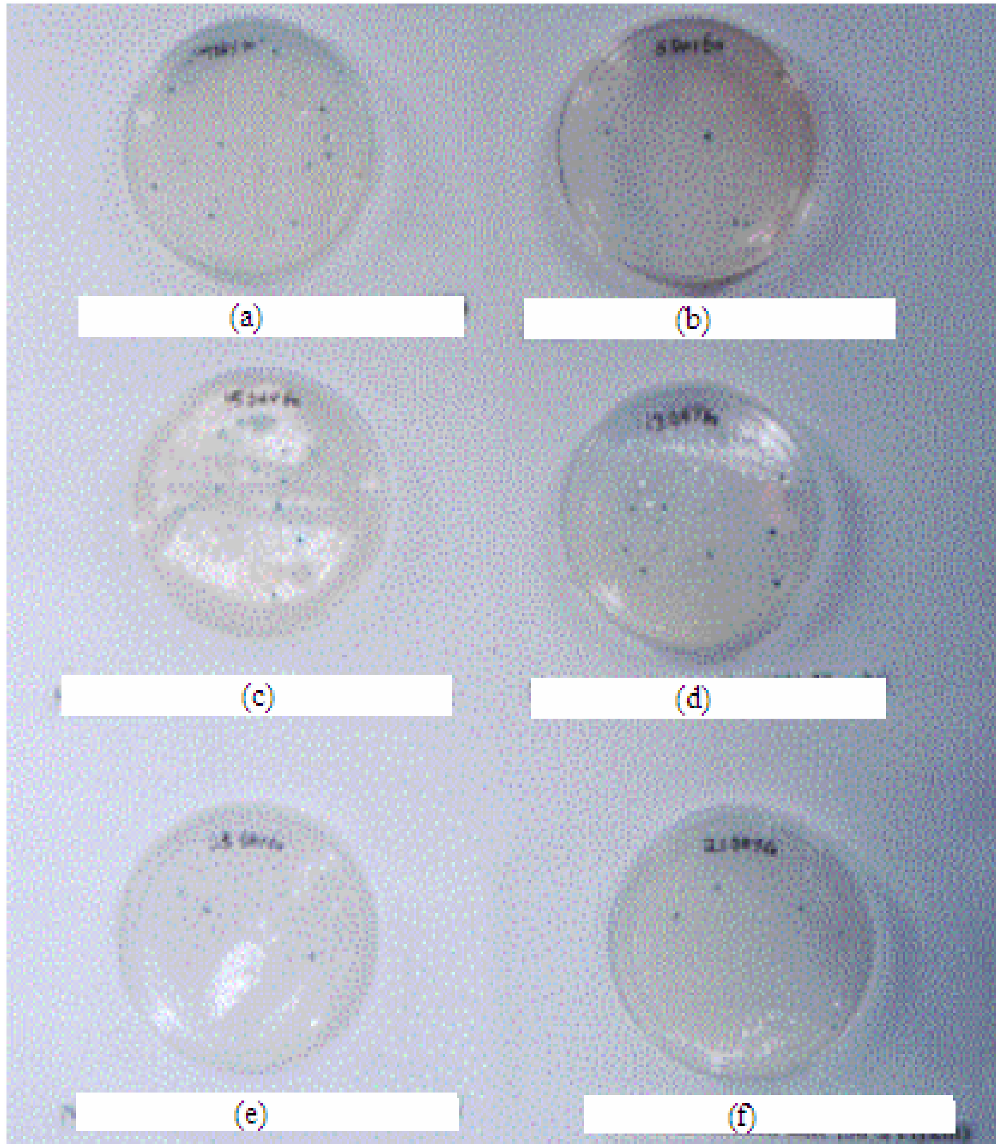
(e)

(a) *Pseudomonas striata* on NBRI-BPB media (b) *Azotobacter* CBD15 on Jensen's media

(c) Transformed *P.striata (lacZ$^+$)* on Pikovskaya's media + chloramphenicol + IPTG + Xgal

(d) and (e) *P.striata (lacZ$^+$)* on NBRI-BPB media

Plate 1



Pseudomonas striata (lacZ⁺) (a - f) Enumeration of inoculated *P.striata (lacZ⁺)* on Pikovskaya's media containing chloramphenicol + IPTG + X-gal from different soil treatments ameneded with fly ash, farmyard manure, distillery waste and microbial inoculants

Plate 2

(a)

(b)

(c)

(d)

Populus deltoides (a) Nursery trial of *Populus deltoides* in 12 different treatments (b-d) *Populus deltoides* in alkaline soil amended with microbial inoculants, fly ash and farmyard manure during nursery trial

Plate 3

(a)

(b)

(c)

(d)

Populus deltoides (a-d) *Populus deltoides* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial

Plate 4

(a)

(b)

(c)

(d)

Toona ciliata (a-b) Nursery trial of *Toona ciliata* in 12 different treatments (c-d) *Toona ciliata* in alkaline soil amended with microbial inoculants and fly ash during nursery trial

Plate 5

(a)

(b)

(c)

(d)

Toona ciliata (a-d) *Toona ciliata* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial

Plate 6

(a)

(b)

(c)

(d)

Toona ciliata (a-b) *Toona ciliata* in alkaline soil amended with microbial inoculants, fly ash, farmyard manure and distillery waste during nursery trial (c) Roots of *Toona ciliata* in treatments amended with fly ash and microbial inoculant (T4) alongwith its control and (d) in treatments amended with fly ash, farmyard manure and microbial inoculant (T8) alongwith its control

Plate 7

ANNEXURE II

List of Research Publications and Conference Proceedings

PUBLICATION

International

1. **Aggarwal, H.** and Goyal, D. Phytoremediation of some heavy metals by agronomic crops. *Concepts and Applications in Environmental Geochemistry (Developments in Environmental Science 5)*, Eds. Sarkar, D., Datta, R. and Hannigan, R. Elsevier. pp 79-98, 2007.
2. **Aggarwal, H.** and Goyal, D. Impact of addition of soil amendments and microbial inoculants on nursery growth of *Populus deltoides* and *Toona ciliata*. *Agroforestry Systems*, 167-173, Vol 75/2, 2009.
3. **Aggarwal, H.** and Goyal, D. Upatke of heavy metals (Pb, Cr, Zn and Co) by *Populus deltoides. Bioremediation Journal.* (Communicated)
4. **Aggarwal, H.** and Goyal, D. Phytoextraction of heavy metals by *Populus deltoides* from soil contaminated with ESP fly ash. *International Journal of Phytoremediation.* (Communicated)

CONFERENCE PROCEEDINGS

International

1. **Aggarwal, H.,** Jaj, N. and Goyal, D. Heavy metal and coliforms contamination in green leafy vegetables. *Botany and Plant Biology 2007 Joint Congress, held at Hilton Chicago, Chicago, Illinois, USA* from July 7-11, 2007, abstract ID: 570.

National

1. **Aggarwal, H.** and Goyal, D. Impact of addition of soil amendments and microbial inoculants on nursery growth of *Populus deltoides*. *Proc. of 46th Annual conference of Association of Microbiologist of India*, held at Osmania University, Hyderabad (India) from Dec. 8-10, 2005, pp.183.

2. **Aggarwal, H.** and Goyal, D. Impact of inoculation of phosphate solubilizing bacterium on the growth of *Populus deltoides* in nursery plantation. *Proc. of 47th Annual conference of Association of Microbiologist of India*, held at Barkhatullah University, Bhopal (India) from Dec. 6-8, 2006, pp. 138.

3. **Aggarwal, H.**, Jala, S. and Goyal, D. *In*-vitro phosphate solubilization by microbes. *Souvenier of* 3rd National workshop on Reorientation in Life Sciences, held at Dolphin (PG) college of life sciences, Chunni Kalan, Distt. Fatehgarh Sahib, Punjab (India) from Jan. 20-21, 2007, pp. 60.

4. **Aggarwal, H.** and Goyal, D. Impact of microbial inoculation and soil amendment on nursery seedlings of *Toona ciliata.* Accepted for presentation at *48th Annual conference of Association of Microbiologist of India*, to be held at Indian Institute of Technology Madras, Chennai (India) from December 18 – 21, 2007.

5. **Aggarwal, H.,** Jaj, N. and Goyal, D. Heavy metal analysis in green leafy vegetables. *Proc. of National Conference on Multidisciplinary Approach in Frontier Areas of Environmental Science and Engineering (MAFAESE-2011),* held at Guru Jambeshwar University of Science & Technology, Hisar, Haryana (India) from March 4-5, 2011.