Monitoring of *lacZ* marked *Azotobacter* and *Pseudomonas* in wheat nursery soil treated with fly ash

Submitted in partial fulfillment of the requirements

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Submited By:

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Candidate's Declaration

I, hereby declare that the work presented in the dissertation entitled **"Monitoring of *lacZ* marked *Azotobacter* and *Pseudomonas* in wheat nursery soil treated with fly ash"** in partial fulfillment of the requirement for the award of the degree of Masters in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is an authentic record of my own work during the period of six months from January 2011 to June 2011, 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

Date: 30.06.2011

(Veni Gupta)

Certificate

This is to certify that the thesis entitled "**Monitoring of *lacZ* marked *Azotobacter* and *Pseudomonas* in wheat nursery soil treated with fly ash**" submitted by Ms. Veni Gupta in partial fulfillment of the requirements for the award of Degree of Masters of Science in Biotechnology to Thapar University, Patiala, is a record of student's own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

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Abstract

Soil amended with different proportions of fly ash, a solid waste generated from coal-fired thermal power plants, was evaluated as a soil conditioner and nutrient supplement on the growth of wheat (***Triticum aestivum)*** nursery trial. Present study evaluated the growth of wheat in nursery soil treated with 0%, 5% and 10% fly ash and microbial consortium of *Pseudomonas striata* and *Azotobacter sp.* in different combination under six different treatments. pH and organic carbon (OC) content did not increased significantly in fly ash amended soil, but significant increase in electrical conductivity, available phosphorus and available nitrogen content of soil was observed after harvest. Soil amended with 5% fly ash and microbial consortium increased available nitrogen by 26% and available phosphorus by 48% as compared to control soil. Fly-ash alone and in combination with *lacZ*$^{+}$ marked *Pseudomonas* and *Azotobacter* was evaluated for bio-efficacy on wheat and their population buildup in fly ash treated soil. Overall 5% fly ash amendment in soil increased the percentage wheat germination. It was observed that, soil treated with fly ash and microbial consortium comprising of *Pseudomonas* and *Azotobacter* significantly enhanced crop yield. Soil amended with 5% fly ash increased the grain yield by 18% and shoot biomass by 15% as compared to control soil. However treatment comprising of 5% fly ash and microbial consortium increased the grain yield by 59% and shoot biomass by 30% as compared to control soil. Soil amended with 10% fly ash had nearly same impact as control soil which shows that it's addition is not inhibitory for wheat growth. The results indicated that combined addition of fly ash and microbial inoculants can be used for improving survival rates and crop growth and fly ash at 5% dose can be used on a large scale to boost soil fertility and crop productivity. Owing to the heterogeneous nature of fly ash it can be applied in soil only in conjunction with microbial inoculants, which can be used to design a soil benefaction strategy.

CONTENTS

ABBREVIATIONS

cm	Centimetre
rpm	Revolution per minute
g	Gram
mg	Milligram
µg	Microgram
L	Litre
$\mu S\ cm^{-1}$	Microsiemens per cm
$mS\ cm^{-1}$	Millisiemens per cm
ml	Millilitre
µl	Microlitre
$mg\ ml^{-1}$	Milligrams per millilitre
$\mu g\ ml^{-1}$	Micrograms per millilitre
%	Percentage
mg/kg (ppm)	Milligrams per kilogram (parts per million)
$\mu g\ g^{-1}$	Microgram per gram
$g\ l^{-1}$	Grams per litre
$g\ cm^{-3}$	Grams per centimetre cube
nm	nanometre
V/cm	Volt per centimetre
□ C	Degree centigrade
M	Molar
N	Normal
($x10^{6}$ cfu g^{-1} soil)	$x\ 10^{6}$ colony forming unit per gram soil
V	Volume of the solution
mm	Millimeter
C	Carbon
N	Nitrogen
P	Phosphorous
K	Potassium
S	Sulphur
Pb	Lead
Cr	Chromium
Zn	Zinc

Fe	Iron
Ni	Nickel
Mn	Manganese
As	Arsenic
Se	Selenium
Mo	Molybdenum
Cu	Copper
Al	Aluminium
Ca	Calcium
Mg	Magnesium
Na	Sodium
B	Boron
Cd	Cadmium
Co	Cobalt
NA	Nutrient Agar
PKV	Pikovskaya Media
JM	Jensens Media
Chl^{+}	Chloramphenicol
X-gal	5-Bromo-4-chloro-3 indolyl-β-D-thiogalactopyranoside
IPTG	Isopropyl-β-D-thiogalactopyranoside
TBE	Tris borate EDTA
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
WHC	Water holding capacity
OC	Organic carbon
EC	Electrical condutivity
PSB	Phosphate solubilizing bacteria
FA	Fly ash
MA	Microbial amendment
S	Sterile
NS	Non-sterile

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Introduction

Since the late 19th and early 20th centuries inorganic compounds containing nitrogen, potassium and phosphorus (NPK) were synthesized and used as fertilizers. Due to the growth in human population fertilizers have been used to increase crop production and meet the rising demands for food. Increase in the production cost, and the hazardous nature of chemical fertilizers for the environment has led to a resurgence of interest in the use of biofertilizers for enhanced environmental sustainability, lower cost of production and good crop yields.

Preparations of live microorganisms (bacteria) utilized for improving plant growth and crop productivity are generally referred to as biofertilizers or microbial inoculants and research in the this area has resulted in the development of different kinds of biofertilizers including nitrogen fixing bacteria, phosphate solubilizing microorganisms, vesicular arbuscular mycorrhizae (VAM). The use of biofertilizers is steadily on rise in agriculture and offers an attractive way to partly replace chemical fertilizers, pesticides and supplements. Free-living soil bacteria beneficial to plant growth are usually referred to as plant growth promoting rhizobacteria (PGPR), capable of promoting plant growth by colonizing the plant root. The plant–microbe interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization of nutrients from a limited nutrient pool, and subsequently uptake of essential nutrients by plants to realize their full genetic potential.

The mode of action of plant growth promoting rhizobacteria in increasing plant growth can be either indirectly or directly. The direct mechanisms of plant growth promotion may involve the synthesis of substances by bacterium or facilitation of the uptake of nutrients from environment by (1) Biological nitrogen fixation (2) Increasing the availability of nutrients in rhizosphere (solubilization of phosphorus) and (3) inducing phytohormones production such as auxins, cytokinins, gibberellins. The indirect mechanism of plant growth occurs when they lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or increasing the natural resistance of host.

Wheat (*Triticum aestivum L.*) is a widely cultivated cereal crop and is grown under various climatic conditions. In India, wheat is cultivated in an area of about 26,121.8 thousand-hectares with production of about 2493 kg ha^{-1}. Most of the studies on the effect of N_2 fixing, PSMs have been performed in either sterilized soil or in nonsterile soil, using these organisms either alone or in combination. However, the effect of co-inoculation of N_2 fixing bacteria with PSMs have rarely been tested in disturbed soil. Therefore in this study effect of co-inoculation of two beneficial soil micro-organisms *Pseudomonas* and *Azotobacter* on growth of wheat was monitored. *Pseudomonas* and *Azotobacter* colonize plant roots and enhance plant growth by wide variety of mechanism like phosphate solubilization and N_2 fixation respectively.

1.1 Fly ash

Coal is an exhaustible energy source, meeting the ever increasing energy demands of countries around the world. Combustion of coal in thermal power stations produces a variety of residues one of such residue is fly ash which is produced more than 100 mt per annum. It is regarded as problematic solid waste as its conventional disposable method degrades soil and contaminates groundwater with heavy metals.

Fly ash is an amorphous mixture of ferroaluminosilicate minerals generated from combustion of ground or powdered coal at temperatures ranging from 400-1500^{o}C. 90-99% of fly ash consists of Si, Al, Fe, Ca, Mg, Na and K. Major matrix elements in fly ash are Si and Al together with significant percentage of K, Fe, Ca and Mg. Fly ash contains all naturally- occurring elements and is substantially rich in trace elements like lanthanum, terbium, mercury, cobalt, chromium.

The presence of almost all essential plant nutrients in ionic form and the ameliorating effect on the physical, chemical and microbial nature of soil makes fly ash an important input for biomass production, especially on variously degraded soils and wastelands. Therefore, development of proper technologies for disposal of this solid waste in an eco-friendly manner becomes essential to derive maximum benefit from its heterogeneous nature, since it is a storehouse of readily available plant macro and micronutrients.

India generates more than 130 million tones of fly ash per annum and is dumped on open land, as landfill material or in ash ponds affecting the soil microbial ecosystem and ecological development which otherwise could be put for raising forestry plantations etc.

Soils are central to the sustainability of our ecosystem. The applications of wastes to soil as a recycling option can only be sustained if there are demonstrable ‘ecological benefits’ which is usually justified in terms of elevated organic carbon and its effect on soil conditions and stimulation of microbial activity and nutrient supply and this is sustainable only if threshold levels of pollutants does not exceed. Bacterial population can influence carbon or mineral cycles and have the ability to colonize harsh environments. The analysis of microbial communities is potentially a sensitive way of detecting changes in soil functioning and could therefore be employed to evaluate the effectiveness of soil protection policies. However, little efforts have been made in studying the microbial ecology of such soils. In such soils or sites affected with fly ash introduction of beneficial soil microorganisms and their establishment, colonization and survival along with their role in improving soil fertility and interaction with plant roots will reveal more information on developing strategies for faster remediation of such sites.

1.2 Monitoring of tagged microbes.

To monitor the behaviour of these soil beneficial microbes in nature, it is necessary to have a sensitive and reliable means of specifically detecting and quantifying them. Reporter gene systems (such as use of *lacZ* markers) provide an opportunity for microbiologists to step beyond the confines of previous methodologies in their attempts to understand the eco-physiology of microbes in their natural habitats. These markers added the capability for direct screening through *LacZ* expression by inserting this reporter gene in beneficial soil bacteria such as phosphate solubilisers and nitrogen fixers and study their multiplication and colonization in fly ash amended soils after their introduction in order to assess the optimum dose of fly ash required for their survival and to determine its possible negative impact on soil microbes.

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

1.3 Objectives

- Monitoring of *lacZ* tagged beneficial soil microbes in wheat nursery soil treated with fly ash.
- To study the effect of fly ash on percent wheat germination.
- To monitor the effect of fly ash and co-inoculated beneficial soil microbes on biomass production.

Present study is focused on monitoring the behaviour of inoculated transformants, effect of fly ash and effect of co-inoculation of two beneficial soil micro-organism on growth of wheat nursery in a pot culture experiment with pure soil and soil amended with fly ash at 0, 5, 10% (w/w basis) under sterile and non-sterile conditions over a period of five months. *Pseudomonas* and *Azotobacter* were tagged with *lacZ* marker so as to enable their detection as distinct from the indigenous populations, to monitor its behaviour in soil amended with fly ash and finally to assess their fate.

Chapter-2

Review of Literature

2.1 Effect of co-inoculation of soil beneficial micro-organisms

Early work on free-living bacteria in soil indicated that certain strains, when applied to seeds or roots, may benefit crops by stimulating plant growth or by reducing the damage from soil-borne plant pathogens. Free-living bacteria may also influence the symbiosis between microorganisms and plants and thereby stimulate plant growth indirectly. Plant growth promoting rhizobacteria (as biofertilizers) increases plant growth through many modes, these modes of action include fixing N_2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant–microbe symbioses (Vessey, 2003). A range of diazotrophic plant growth-promoting rhizobacteria participates in interactions with C_3 and C_4 crop plants (e.g. rice, wheat, maize, sugarcane and cotton), significantly increasing their vegetative growth and grain yield (Kennedy *et al.,* 2004). Plant growth promoting rhizobacteria increases plant growth either indirectly or directly, indirectly by preventing the deleterious effects of phytopathogens while directly by providing plants with a compound synthesized by bacterium or facilitating the uptake of certain nutrient from environment (Glick, 1995).

Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan (Khan *et al.,* 2004). To assess this hypothesis, local isolates of *Azotobacter* and *Pseudomonas sp*. were screened for their intrinsic ability to produce IAA in the presence of varying amounts of L-tryptophan and their effect on root elongation of germinating seeds of test plants. It was found that inoculation of wheat seedlings with *Azospirillum brazilense* increased the number and length of lateral roots. Inoculation of canola seeds with *Pseudomonas putida* GR12-2, which produces low levels of IAA, resulted in 2 - or - 3 fold increase in the length of seedling roots.

Synergistic effects of the inoculation with plant growth-promoting Rhizobacteria and an Arbuscular Mycorrhizal Fungus on the performance of wheat (Khan and Zaidi, 2007) were observed. Numerous studies have shown a substantial increase in plant growth and seed yield following inoculation with PGPR strains including PSMs and N_2 fixers on legumes (Perveen *et al.,* 2002). However, where P is scarce, it was found that plants inoculated with arbuscular mycorrhizal (AM) fungi either alone or in combination with PSMs increased the P uptake in wheat (Raja *et al*., 2002) or maize (*Zea mays L*.) (Evans and Miller, 1990). Zaidi and Khan (2007) have suggested a synergistic relationship between PSMs (*Pseudomonas striata* and *Penicillium*) and *A. chroococcum*, allowing a better use of poorly soluble P sources and increased dry matter accumulation, grain yield, and P uptake of wheat plants. During interaction, the PSMs increase the availability of P and efficiency of N2 fixation by phosphate solubilizing activity and releasing the plant growth promoting substances (Kucey *et al.,* 1989). Similarly, a significant increase in the dry matter yield of wheat plants with co-inoculation of rock-phosphate solubilising fungi *Aspergillus niger* and *Penicillium citrinum*, and *G. constrictum* was reported (Omar, 1998).

Effect of *Azotobacter chroococcum* (PGPR) on the growth of Bamboo (*Bambusa bamboo*) and Maize (*Zea mays*) plants was studied and was found to be beneficial for bamboo plantation as they enhance growth and induced IAA production and Phosphorus solubilization (Dhamangaonkar Sachin N, 2009). Yield of rice (Yanni and El- Fattah 1999), cotton (Anjum *et al.,* 2007), and wheat (Hegazi *et al*., 1998; Barassi *et al.,* 2000) increased with the application of *Azotobacter.*

Phosphorus (P) is one of the major essential macronutrients for plant growth and development (Ehrlich 1990). The concentration of soluble P which is accessible to plant in soil is usually very low. To convert insoluble phosphates compounds in a form accessible to the plant is an important trait for a PGPR in increasing plant yields. Bacterial strains belonging to genera *Pseudomonas, Bacillus, Rhizobium* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate (Rodríguez. *et al,*. 2006). Strains from genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers, while tricalcium phosphate and hydroxyl apatite seem to be more degradable substrates than rock phosphate (Banerjee. *et al*., 2006). The production of organic acids especially gluconic acid seems to be the most frequent agent of mineral phosphate solubilization by bacteria such as *Pseudomonas sp.* (Rodríguez and Fraga, 1999).

2.2 Fly ash

Fly ash is an amorphous mixture of ferroaluminosilicate minerals which is generated from combustion of ground or powdered coal at temperatures ranging from 400-1500^{o}C with 2% excess air (Mattigod *et al*., 1990). It 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. In India fly ash is produced more than 112 mt per annum of which nearly 4 mt is released into the atmosphere. Kalra *et al.* (1997) have reported that FA production in India will exceed 140 million tons by 2020. The disposal of such a huge amount of FA is one of the major problems in developing countries. Fly ash particles are small enough to escape emission control devices and easily get suspended in air. Repeated exposure to fly ash can cause irritation in eyes, skin, nose, throat and respiratory tract and result in arsenic poisoning (Finkelman *et al.,* 2000). Therefore, disposal and utilization of fly ash needs careful assessment. Approximately 13 million tonnes of fly ash produced by power stations in Australia is currently being explored for its ameliorating properties in soils with inherent structural and nutritional limitations and for biomass production of plants such as clover (*Trifolium subterraneum*) and turfgrass (*Cynodon dactylon*). Apart from acting as source of nutrients fly ash has been observed to have a positive effect on water holding capacity, hydraulic conductivity and pH (Yunusa *et al.,* 2005).

Fly ash is chemically heterogenous in nature on account of being composed of large number of trace and heavy metals in variable proportions. Although fly-ash contains traces of toxic elements and heavy metals but due to its physical characteristics and presence of some macro and micro nutrients in it, fly ash can be used as soil amendments and soil conditioner and enhance plant growth. It was observed by Kumar and Chauhan (2008) that the toxic effect of fly ash is insignificant and concentration of toxic elements was found to be within permissible limit.

Fly ash addition generally has shown positive impact on plant biomass production and nutrient uptake (Ciravolo and Adriano, 1979; Elfving *et al.,* 1981). It can be used for wasteland reclamation, agriculture and forestry as it plays a vital role in altering physical, chemical and microbiological status of soil. Judicious application of fly ash can bring about favourable alteration in the nutrient status of soil, provided all aspects are constantly monitored for overall benefit. Field and greenhouse studies (Sharma *et al.,* 1990) indicate that on account heterogenous nature of fly ash can improve agronomic properties of soil and benefit plant growth. 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).

2.2.1 Physico-chemical properties of fly ash

The mineralogical, physical and chemical properties of fly ash (Page *et al.*, 1979; Adriano *et al.,* 1980; Carlson and Adriano, 1993) depends on the nature of parent coal, conditions of combustion, type of emission control devices and storage and handling methods. Fly ash is composed predominantly of small, glassy, hollow particles with low to medium bulk density ranging from 2.1 to 2.6 g cm-3 (Adriano *et al.,* 1980) and with moisture retention ranges from 6.1% at 15 bar to 13.4% at 1/3 bar with an average diameter of <10 mm, high surface area and light texture which are aggregated into micron and sub-micron spherical particles of sizes ranging from 0.01 to 100 mm (Davison *et al.,* 1974), with smaller particles entrapped within large spheres (Fischer *et al.,* 1976). Approximately 90-99% of fly ash consists of Si, Al, Fe, Ca, Mg, Na and K. Major matrix elements in fly ash are Si and Al together with significant percentage of K, Fe, Ca and Mg. Al in fly ash is mostly bound in insoluble aluminosilicate structures, which considerably limits its biological toxicity (Page *et al*., 1979). Fly ash contains all naturally-occurring elements and is substantially rich in trace elements like lanthanum, terbium, mercury, cobalt, chromium (Adriano *et al.,* 1980). Maiti *et al.,* 1990 observed Ca to be the dominant cation in ESP ash and fly ash collected from dump sites, followed by Mg, Na and K. Minerals like quartz, mullite, haematite, magnetite, calcite and borax were also identified in fly ash (Hogdson and Holliday, 1966). The authors opined that oxidation of C and N during combustion drastically reduces their quantities in ash. The pH of fly ash varies from 4.5-12.0 depending largely on the sulphur content of the parent coal (Plank and Martens, 1974) and the type of coal used for combustion affects the sulphur content of fly ash (Page et al., 1979). Eastern U.S. coals that include anthracite are generally high is S and produce acidic ash, while western U.S. coals, which includes lignites, tends to be lower in S and higher in Ca and produce alkaline ash (Natusch *et al.,* 1975; Page *et al.,* 1979). The coal produced in India is poor in S but high in ash content (40%) whereas the coal produced in U.S. is rich in S (2%) and contains only 5-10% ash.

Table 1: Major and trace elements in ESP fly ash and soil

Elements	Soil Lit. value*	Fly Ash (ESP) Lit. value*
Major elements (%)		
Al	4-30	0.1-17.3
Ca	0.7-50	0.11-22.2
Fe	0.7-55	1-29
Mg	0.06-0.6	0.04-7.6
Na	0.04-3.0	0.07-2.03
K	0.04-3.0	0.15-3.5
S	0.01-2.0	0.1-1.5
P	0.005-0.2	0.04-0.8
N	0.01-1.0	-
Trace elements (mg/kg)		
As	0.1-40	2.3-6300
B	2-100	10-618
Cd	0.01-7.0	0.7-130
Co	1-40	7-520
Cr	5-3000	10-1000
Cu	2-100	14-2800
Hg	-	0.02-1.0
Mn	100-4000	58-3000
Mo (2-40)	0.2-5.0	7-160
Ni (50)	10-1000	6.3-4300
Pb (100)	2-100	3.1-5000
Se (5-10)	0.1-2.0	0.2-134
Zn (300)	10-300	10-3500

(*Page et al. 1979; Values in parentheses indicate critical levels in soil; Pendias and Pendias, 1984)

(Jala, S., Goyal, D., 2006. Fly ash as a soil ameliorant for improving crop production-a review. *Bioresour. Technol.* 97, 1136-1147)

2.3 Fly ash as a soil-ameliorating agent

2.3.1 Fly ash for improving soil fertility

Fly ash has immense potential as a soil-ameliorating agent in agriculture, forestry and wasteland reclamation because of its heterogenous nature. Previous work (Reynolds *et al.,* 1999) to determine the feasibility of converting waste disposal problem into a soil benefaction strategy has proven true. Fly ash has also been reported to improve the nutritional status of soils *via* increase in cation exchange capacity (CEC) and by provision of some essential nutrients. It has been studied as a useful soil-amending agent with agronomic and environmental benefits (Zhang *et al.,* 2004). Studies have been carried out to report the efficacy of fluidized bed combustion (FBC) and flue gas desulfurization (FGD) byproducts, when amended with dairy, swine and broiler litter manures, in reducing phosphorus (P) solubility and potential impact on water quality (Zhang *et al.,* 2004). Pilot scale studies have been conducted on use of fly ash at rates of 100 to 650 tonnes per hectare of land as soil modifier and microfertilizer under field conditions for vegetable crops in Orissa, Madhya Pradesh and Uttar Pradesh, with a positive influence reported on the soil nutrient status as well as on plant growth (Saxena *et al.,* 2005). Fly ash upon amelioration at 10, 20, 30 and 40% (w/w) in clay, sandy-clay loam, sandy and sandy loam soil has been reported to increase its pH, electrical conductivity and modify water retention capacity (Kalra *et al.,* 2000). Fly ash is predominantly composed of silt-size particles so its addition in soils very high in either sand or clay can improve soil texture (Chang *et al.,* 1977) by reducing soil bulk density and increasing aeration. Fly ash addition at 70 t/ha was reported to alter the texture of sandy and clay soils to loamy soils (Capp, 1978) and improve the water-holding capacity of sandy soils (Chang *et al.,* 1977; Sharma *et al.,* 1990). The water holding capacity of sandy/loamy soils increased by 8% due to fly ash amendment (Chang *et al.,* 1977) and the accompanied increase in hydraulic conductivity helped in reducing surface encrustation. Fly ash has been found to improve soil porosity and workability (Page *et al.,* 1979).

Fly ash, which can be acidic or alkaline depending on the source, can be used to buffer the soil pH (Elseewi *et al.,* 1978 a, b; Phung *et al.,* 1978). Fly ash decreased bulk density, which in turn improved soil porosity and increased water retention capacity (Page *et al.,* 1979). 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

(Adriano *et al.,* 1980; El-Mogazi *et al.,* 1988) and improving the nutrient status of soil (Wallace *et al.,* 1980; Elseewi and Page, 1984).

A number of studies have shown that addition of alkaline ash can increase the pH of acidic soils (Elseewi and Page, 1984; Petruzzelli *et al.,* 1987). Mulford and Martens (1971) indicated that application of about 5% fly ash to Tatum silt loam soil increased the EC of the soil saturation extract from about one to about four mmhos cm^{-1}.

2.3.2 Effect of fly ash on soil microbes

Addition of unweathered fly ash to sandy soils severely inhibited microbial respiration, enzyme activity and soil nitrogen cycling processes such as nitrification and N mineralization. Boron toxicity is the major limiting factor; boron induces inhibition of microbial respiration which can be prevented by the co application of a readily oxidizable organic substrate (Page *et al.,* 1979). Lal *et al.* (1996) recorded higher microbial activity in soil amended with up to 8% fly ash. 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). Schutter and Fuhrmann (2001) proposed that fly ash has no detrimental effect to microbial community instead fly ash amendment may benefit fungi and gram-negative bacteria relative to other components of soil microbial community. This can be explained due to enhanced crop growth and soil texture, pH and nutrient content as a result of fly ash amendment. Fly ash-sludge mixtures containing 10% ash had positive effect on soil microorganisms in terms of enzyme activity for urease, dehydrogenase and phosphatase, N and P cycling and reduction in the availability of heavy metals (Lai *et al*., 1999). Fly ash composted with wheat straw and 2% rock phosphate (w/w) for 90 days had an encouraging effect on chemical and microbiological parameters of the compost and fly ash up to 40-60% did not exert any detrimental effect on either C:N ratio or microbial population (Gaind and Gaur, 2003).

2.3.3 Effect of fly ash in Agriculture

Field and greenhouse studies both indicated that many chemical constituents of fly-ash benefit plant growth and can improve the agronomic properties of the soils (Chang *et al.,* 1977). Fly ash addition generally results in consistently favourable impact on plant growth and nutrient uptake (Aitken *et al.,* 1984). Addition of unweathered fly ash up to

8% to calcareous or acidic soils resulted in higher crop yield due to increased availability of S from fly ash (Page et al., 1979). Fly ash up to 50t/ha was used to study the effect on the soil properties and yield of wheat (*Triticum aestivum L.*), mustard (*Brassica juncea L.*), rice (*Oryza sativa L.*) and maize (*Zea mays, L.*). The yield of wheat increased for 20t/ha fly ash while paddy and mustard were observed to survive well in soil amended with 10t/ha of fly ash, all three crop plants showed improved growth over control (Kalra *et al.,* 1998, 2003). A lower application of fly-ash (5–10%) in soils enhances seed germination as well as seedling growth, although higher application (20–30%) either delays or drastically inhibits plant growth, development and other specific parameters (Singh et al. 1997). Tomato cultivars grown on fly ash amended soils had higher tolerance to wilt fungus *Fusarium oxysporum* (Khan and Singh, 2001). 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 (Goyal *et al.,* 2002). Fly ash can be used in problematic soil as an amended material and also it acts as source of plant nutrition for crop production (Mittra and Ghosh *et al.,* 2003). Mittra *et al.* (2003) developed an integrated plant nutrition system utilizing fly ash, paper factory sludge, farmyard manure, crop residues and chemical fertilizers for rice-peanut cropping system. In his work fly ash applied at 10 t ha^{-1} in combination with organic sources was found to increase the grain yield of rice, pod yield of peanut and equivalent yield of both crops by 31, 24 and 26% respectively as compared to chemical fertilizers alone. Studies were conducted by Rautaray *et al.* (2003) on sandy loam acid lateritic soils to observe direct effect of fly ash, organic wastes and chemical fertilizers on rice (*Oryza sativa*) and their residual effects on mustard (*Brassica napus* var *glauca*) grown in sequence. The integrated use of all the three amendments was observed to show an increase in rice-mustard yield by 14%, compared to use with fertilizers 10% and fly ash alone at 3%, respectively.

Lignite fly ash (LFA), being alkaline and endowed with excellent pozzolanic properties, a silt loam texture, and plant nutrients, has the potential to improve soil quality and productivity (Jha and Sinha. 2007). Fly ash does not seem to be a good source of phosphorus as it was found inferior to monocalcium phosphate (Martens, 1971). It, however, accelerated Ca^{2+} and Mg^{2+} uptake by legumes (Adriano *et al.,* 1978; Page *et al.,* 1979). Although, higher B availability limits the use of fly ash in crop production (Page *et al.,* 1979), the problem can be overcome by proper weathering of the fly ash, which reduces the availability to below toxic level (Townsend and Gilham, 1975). Increased selenium accumulation in plant tissues with increased fly ash dose warrants close monitoring and use of appropriate quantity of weathered fly ash depending upon the end use of the produced biomass (Straughan *et al.,* 1978).

The large scale use of fly ash in agriculture and wasteland development holds a potential to increase on an average 15% yield of grains, oil seeds, sugarcane, cotton and about 25–30% of vegetables resulting in another green revolution (Kumar et al., 2005). It was evident from the results that the addition of fly ash (10 - 200 ton per ha) increased the yield of different crops from 10-40%. Thus the use of fly ash in agriculture has proved to be economically rewarding.

In addition to increase in the yield of produce, significant increase in biomass yield has also been found. Farmers have also reported that the size of grains and their luster get improved with application of fly ash, resulting in better quality marketing assessment inter-alia better realization. The significant improvement has been reported by farmers regarding pest control especially in case of rice & sugarcane, due to fly ash application. A number of farmers reported that there was no soil born pest attack due to fly ash application. The presence of calcium, magnesium, sulphur, iron and other nutrients in most of the fly ash samples was found to improve the quality of crop produce in respect of protein and oil content.

There is no adverse effect of addition of fly ash, on the contrary there is an advantage; the protein, iron & calcium contents are higher in the produce of fly ash treated plots and trace/ heavy metal concentrations in the produce is not at all affected by fly ash addition. This is desirable as it is good for human being. Fly-ash based *Azotobacter* and *Azospirillum* formulation alone and in combination with chemical fertilizer was evaluated for bio-efficacy on wheat. Population of *Azotobacter* and *Azospirillum* was also evaluated in treated soil. The results of the studies showed that, seed treatment with *Azotobacter* and *Azospirillum* and soil treated with chemical fertilizer alone and in combination significantly enhanced the seed germination, plant height, plant biomass and crop yield compared to control (Kumar *et al.,* 2010). Use of fly-ash as carrier in these formulations is an effective way of utilization of problematic fly-ash waste in a useful manner.

2.4 Monitoring of tagged microorganisms

To monitor microbes in nature, various methods have been developed and the most straightforward way is to track the phenotype of the marker gene. Antibiotic resistant genes such as the *nptII* gene encoding resistance to kanamycin, was the first to be employed as marker. Genes encoding metabolic enzymes have also been used as non-selective markers. These include *lacZY* (encoding b-galactosidase and lactose permease) and *gusA* (encoding β-glucuronidase (GUS). The enzymes encoded by *lacZ* and *gusA* cleave the uncolored substrates X-gal or X-glc, respectively, to produce blue colored products. The difference with this method, compared to that above, is that it does not rely on incorporation of an inhibitory compound to the agar medium. Increasingly, it is becoming apparent that the best solution for tracking a microbe is to use more than one marker simultaneously. Molecular tagging of inoculant strains (such as *Rhizobium, Bradyrhizobium, Azotobacter, Azospirillum, Bacillus polymyxa and Pseudomonas striata*) is essential for ecological monitoring and to assess their performance in the field. The *lac*Z has been used to study nodule infection by *Rhizobium* (Boivin *et al*. 1990) and root colonization by *Azospirrilum* (Katipitiya *et al*. 1995). We have isolated nitrogen fixing and phosphate-solublizing bacteria from fly ash affected soils. In this proposal we intend to tag these bacteria and study their performance *in situ*.

Chapter-3

Materials and Methods

3.1 Instruments used

Instruments used during the course of this work were Autoclave (Equitron, Mumbai); Atomic absorption spectrophotometer (*GBC 932AA, Australia*); Centrifuge (Sigma); Electrophoresis assembly (Tarson, Mumbai); Kjeldahl Assembly; Orbital Shaker, Scigenics Biotech, (orbitek); Spectrophotometer (Hitachi); pH meter (Thermo Orion Model 290) ; EC meter (Orion Model 125)

3.2 Chemical and Reagents

Chemicals and microbiological media were procured from Ranbaxy Laboratories and SD fine-chem. Ltd., SRL and Hi Media, Mumbai. Nutrient agar, Jensens media and Pikovskaya media were used for bacterial enumeration. Chemicals used were chloramphenicol, Xgal, IPTG etc. purchased from SD fine-chem. Ltd and Hi Media, Mumbai

3.3 Collection and processing of fly ash and soil

Fly ash from the electrostatic precipitator (ESP fly ash) was collected from GGS Superthermal Power Plant, Ropar, Punjab was directly analyzed for physicochemical properties after air-drying. Soil from agricultural field, Thapar Technology Campus, Patiala, Punjab was collected from a 0-30 cm depth and processed and analyzed for physical, chemical characteristics. Bulk density and water holding capacity was determined using keen boxes (Black *et al.,* 1965). pH and electrical conductivity (Jackson, 1967) was determined by soil suspension method with a soil to water ratio of 1:2. Organic carbon content was determined as per the protocol (Walkley and Black, 1934); Available N, P, S (Jackson, 1967). Analysis of total K and heavy metals was done in $HNO_3/HClO_4$ digested samples (Page *et al.,* 1982) and analyzed by Atomic Absorption Spectrophotometer (GBC 932). Among the microbiological parameters, enumeration of bacteria, *Azotobacter* and *Phosphorus solubilizer* was done on Nutrient Agar, Jensen's media and Pikovskaya's media respectively.

3.4 Nursery trial (November, 2010 – April, 2011)

ESP fly ash from GGSTP (Guru Gobind Singh Super Thermal Power Plant), Ropar was mixed with alkaline soil of Patiala taken from topmost soil profile 0-30 cm**,** on a v/v basis at concentrations of 0%, 5%, 10%. The different fly ash-soil mixtures were placed in 36 pots. Nursery trial with wheat variety HD 2687 was carried out in triplicate with twelve different treatments. All the treatments were in sterile and non- sterile system. Details of the treatments are as follows:

Soil (Control)

Soil + MA

Soil + 5% fly ash (w/w)

Soil + 5% fly ash + MA

Soil + 10% fly ash (w/w)

Soil + 10% fly ash + MA

*MA: Microbial Amendment is a consortium of *Azotobacter* CBD15 *(lacZ*$^{+}$*)* and *Pseudomonas striata (lacZ*$^{+}$*)*

The seeds sown in treatments amended with MA were given seed treatment and were coated with the above said consortium. A portion of the soil samples were drawn from top 0-10 cm layer for physical, chemical and microbiological analysis prior to beginning of trial**.** The soil after collection was divided into two portions, one of which was immediately refrigerated for later microbiological analysis. The remaining part was gently broken up for clods and macro aggregates using pestle and mortar followed by removal of gravel and other debris. The soil was taken in large petri-plates, air-dried and sieved through a 2 mm mesh sieve and used for physical analysis. The 2 mm-sieved soil was crushed to pass through 0.2 mm sieve for chemical analysis. The same sampling was done after 60 days of treatment and at the time of harvesting of wheat (in April) and processed for chemical and microbiological analysis.

3.5 Soil analysis

3.5.1 Chemical analysis

3.5.1.1 pH

pH was determined as per the method given by Jackson (1967)

1. Soil/fly ash (10 g) was placed in a 100 ml beaker and 20 ml of distilled water was added making a soil-water/fly ash-water suspension of 1:2 ratio.
2. The soil was stirred well for five minutes and kept undisturbed for some time followed by stirring again.
3. pH was measured using a Thermo Orion Model 290 pH meter after calibration with buffers of pH 4.0, 7.0 and 9.2.

3.5.1.2 Electrical conductivity

Electrical conductivity was measured in µS cm^{-1} as per the method given by Jackson (1967).

1. Soil/fly ash (10 g) was placed in a 100 ml beaker and 20 ml distilled water was added.
2. The soil-water mixture was allowed to stand undisturbed until the soil settled completely.
3. EC was measured using a conductivity meter (Orion Model 125) after calibrated with 0.01 M potassium chloride (1413 µS cm^{-1}).

3.5.1.3 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%) and sodium fluoride (NaF).

Procedure

1. Soil/fly ash (10 g) 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. H_2SO_4 (20 ml) was added and the flask was allowed to stand undisturbed for 30 minutes after which 200 ml of distilled water was added.
3. To the mixture, 10 ml of Orthophosphoric acid, 0.5 g of NaF and 1 ml diphenylamine indicator was added.
4. The contents were ultimately titrated with freshly prepared 0.5 N ferrous ammonium sulphate till the end-point is observed from blue-violet to green. A blank was also run without soil.

Calculation

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

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

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

3.5.1.4 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 2l with distilled water.
3. Reagent B (freshly prepared): 1.058g of ascorbic acid in 200 ml of reagent A and mixed.
4. Sulphuric acid (2.5 M): 140 ml of concentrated H_2SO_4 diluted to 1l.
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 1l.

Procedure

1. Soil/fly ash (0.5g) was placed in a 100 ml Erlenmeyer flask followed by the addition of 10 ml extracting solution.
2. The solution was kept on a shaker for 30 minutes and filtered through Whatman No.42 filter paper.
3. Aliquot (2ml) of the filtrate was transferred to a test tube followed by addition of 0.2 ml of 2.5 M H_2SO_4, 3.1 ml of distilled water, 1.6 ml of Reagent B and another 3.1 ml of distilled water.
4. A blank was prepared as above. For the standard curve: 0, 0.5, 1, 1.5, and 2 ml of standard solution were placed in test tubes separately. 2 ml of extracting solution, 0.2 ml of 2.5 M H_2SO_4, 1.6 ml Reagent B was added and the final volume was made upto 10 ml. The P concentrations of these solutions were 0, 2.5, 5, 7.5, and 100 ppm respectively. After 10 minutes, the P concentration was read at 882 nm.

Calculation

P in soil (ppm) = P in extract (ppm) x 100 (the standard soil to solution ratio).

3.5.1.5 Available nitrogen

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

Reagents

1. Potassium per manganate (0.32%): 3.2 g of $KMnO_4$ dissolved in water and final volume made up to 1l.
2. NaOH (2.5%): 25 g of sodium hydroxide pellets dissolved in water and volume made up to 1l.
3. Boric acid (2%): 20 g of boric acid powder dissolved in warm H_2O by stirring and diluted to 1l.
4. Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green dissolved in 100 ml of ethyl alcohol. 20 ml of the mixed indicator added to each litre of 2% boric acid solution and final pH adjusted to 4.5 with dilute HCl or dilute NaOH.
5. Potassium hydrogen phthalate (0.1N): Dissolve 20.422 g of the salt in water and dilute to 1l.
6. NaOH (0.1 N): 4g of NaOH dissolved in water and diluted to 1l, standardized against 0.1 N potassium hydrogen phthalate solution.

7. **7.** H_2SO_4 (0.02 N): 0.1 N H_2SO_4 prepared by adding 2.8 ml of concentrated H_2SO_4 to about 990 ml of distilled water. From this 0.02 N H_2SO_4 made by diluting a suitable volume five times with distilled water and standardized against 0.1 N NaOH solution.

Procedure

1. Soil/fly ash (1g) was weighed and placed in a 800 ml Kjeldahl flask.
2. The soil was moistened with 2 ml distilled water and any adhering soil on the neck was washed down followed by addition of 20 ml of 0.32% $KMnO_4$. Glass beads measuring 0.4 mm were added to prevent bumping.
3. 2% boric acid (20 ml) 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. 2.5 % NaOH (20 ml) 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. The conical flask containing distillate was removed before switching off the heater to avoid back suction.
7. 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 soil in ppm = (X) x 0.00028 x 100/5 where X stands for the titre value of 0.02 N H_2SO_4 consumed.

3.5.1.6 Available sulphur

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

Reagents

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

Procedure

1. 0.15 % calcium chloride (10 ml) was added to 1 g soil sample taken in a 150 ml conical flask.
2. The sample was agitated at 130 r.p.m. for 30 minutes followed by filtration through Whatman No. 42 filter paper.
3. Filtrate (2 ml) was taken in a test tube and 0.1 g of 30-60 mesh barium chloride was added, swirled followed by 0.1 ml of gum acacia. The volume was made upto 2.5 ml with distilled water and absorbance read at 420 nm.

   Standard curve: Different volumes 0, 5, 10, 15 and 20 ml of 100 ppm standard K_2SO_4 solution were taken in 25 ml volumetric flasks.1 ml of 0.15% calcium chloride, 0.1 g barium chloride, 0.1 ml gum acacia were added and volume made up with distilled water. The absorbance was read at 420 nm.

3.5.1.7 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 1l after adjusting the pH to 7.0.
3. Whatman No.42 filter paper.

Equipment

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

Procedure

1. Air-dried soil (10 g) 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).

3.5.1.8 Heavy metal analysis

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. Soil/fly ash (1g) 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, Co, Cr, Pb, Cd, Cu and Zn in soil samples using an atomic absorption spectrophotometer (GBC 932AA).

3.5.2 Physical analysis

3.5.2.1 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 is fitted with a brass lid.

Procedure

1. A filter paper strip of the size of the base of the keen box was cut.
2. The filter disc was weighed and placed in a petridish containing water for measuring the moisture absorbed by the filter paper.

3. The disc was placed at the bottom of the keen box and weighed followed by filling of the box with soil/fly ash. Each time the box was tapped to make a uniform soil/fly ash column.
4. The box containing soil/fly ash was weighed and kept in a petridish containing water for overnight saturation.
5. The box was removed the next day, wiped and weighed followed by overnight drying at 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

3.5.2.2 Bulk density

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

Apparatus

Specific gravity bottle.

Procedure

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

Calculation

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}

3.5.3 Microbiological analysis

3.5.3.1 Enumeration of bacteria

3.5.3.1a 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})

Table 2: Nutrient agar composition (gl^{-1})

Constituents	Amount
Peptone	5.0g
Sodium chloride	5.0g
Beef extract	1.5g
Yeast extract	1.5g
pH	7.0

Procedure

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

3.5.3.1b Enumeration on free-living nitrogen fixing bacteria

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

- Jensens Medium (g l^{-1})

Table 3: Jensens Medium composition (gl^{-1})

Constituents	Amount
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	15.0g

Procedure

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

3.5.3.1c Enumeration of phosphate solubilizing bacteria

The phosphate-solubilizing bacteria were enumerated on Pikovskya media (Pikovskya, 1948).

- Pikovskya medium (g l^{-1})

Table 4. Pikovskya Medium composition (gl^{-1})

Constituents	Amount
Glucose	10.0g
Tri calcium phosphate	5.0g
$(NH_4)_2SO_4$	0.5g
NaCl	0.2g
$MgSO_4$.7H2O	0.1g
KCl	0.2g
Yeast extract	0.5g
$MnSO_4$	0.025g
$FeSO_4$.7H2O	0.020g
Agar	15g
pH	7±0.2

Procedure

1. Soil (1g) was added to 10 ml water blank and shaken well.
2. 1 ml from this was added to a test tube containing 9 ml water making a dilution corresponding to 10^{-1}.
3. Further dilutions were prepared in a similar way up to 10^{-4}.
4. Inoculum (100µl) was taken from dilution 10^{-3} and $10^{-4}$and added to pikovskya media 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.

3.5.3.2 Enumeration and Monitoring of *lacZ*$^+$ marked *Pseudomonas* and *Azotobacter* by selective plating method. (Cappuccino, 1987)

The phosphate solubilizer and N_2 fixing bacteria have been tagged with plasmid pMMB277 carrying lac-cat marker gene which is *lacZ*$^+$ and chloramphenicol resistant, which gives blue coloured colonies on media spreaded with X-gal + IPTG. Bacterial counts of these *lacZ*$^+$ marked *Pseudomonas* and *Azotobacter* in soil with and without fly ash was carried out on selective media plates on X-gal + IPTG spreaded PKV + chl$^+$ and JM + chl$^+$ media plates respectively.

Requirements: Chloramphenicol containing PKV and JM media, (v/v) fly ash amended soil (0, 5 & 10%), IPTG and X-gal stock solution.

Preparation of stock solutions

IPTG (Isopropyl-b-D-thiogalactopyranoside):

100mg ml^{-1} in sterile distilled water

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

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

Chloramphenicol

Stock solution: 10 mg ml^{-1} dissolved in 0.2 ml ethyl alcohol and 0.8 ml of distilled water.

Working solution: 10µg ml^{-1}

Procedure

1. A cocktail of above solutions was prepared as follow:

Table 5: Cocktail composition for 10 plates

Constituent	Amount
X-gal	300µl
IPTG	80µl
Sterile DW	450µl

2. From the cocktail prepared above, 830µl was taken and spread on Chloramphenicol containing media (both PKV and JM) plates and left for 15 minutes.
3. Soil (1g) was added to 10 ml water blank and shaken well.
4. 1 ml from this was added to a test tube containing 9 ml water making a dilution corresponding to 10^{-1}.
5. Further dilutions were prepared in a similar way upto 10^{-4}.
6. Inoculum (100µl) was taken from dilution 10^{-3} and 10^{-4}and added to X-gal + IPTG plates prepared as above and the inoculum was spread with the help of a spreader.
7. The plates containing inoculum were incubated at 30ºC for 24 hours and observations recorded.

3.6 Biometric data collection

Nursery trial

At the end of the nursery trial i.e. at the time of harvesting after 120 days of sowing (in April, 2011), wheat plants were cut just 1 inch above the soil surface and various biometric and growth parameters were recorded. Biometric parameters included no. of plants/pot, no. of spikes, length of plant, length of spike, no. of grains per spike, weight of grains and shoot biomass of above soil portion.

3.7 Effect of fly ash on wheat germination

To study the effect of fly ash on wheat germination an experiment was carried out in petri-plates (of 200mm diameter). Fly ash was amended at different proportion (i.e. 0, 5 and 10%, w/w basis) in soil and its effect on two parameters was monitored, (1) percentage wheat germination and (2) radical/plumule ratio. Three treatments were studied in triplicates (control soil, soil + 5% fly ash and soil + 10% fly ash) for which nine petri-plates were taken. 350 g of control soil (moisture content: 4.66% ±0.15) was taken in triplicate in 3 petri-plates such that each petri-plate was half filled with the soil. Soil was mixed with 5% fly ash on w/w basis i.e. 332.5 g of soil mixed with 17.5 g of fly ash and was filled in another 3 petri-plates. Similarly soil + 10% fly ash mixture was prepared by mixing 315 g of soil with 35 g of fly ash and was filled in 3 petri-plates. A filter paper disc cut at the size of petri-plate was kept over the soil in each petri-plate. Each petri-plate was watered properly. 20 wheat (variety- HD 2687) seeds were placed (each seed at a distance of approx 1.5 cm) over filter paper in each petri-plate and these petri-plates were kept in dark. They were continuously watered and monitored for seed germination. After two days no. of seed germinated in each petri-plate was recorded and percentage seed germination was calculated. When radical and plumule of germinated seed attained certain length, they were uprooted and monitored for radical and plumule length and radical/plumule ratio was calculated.

Similarly pot experiment was conducted to evaluate the effect of fly ash incorporation in soil on percentage germination of wheat. Effect of fly ash amendment in soil at different proportion was also studied on *Mung bean* in same way carried out in 9 petri-plates and percentage seed germination was recorded.

3.8 Soil DNA isolation and total DNA estimation

3.8.1 Soil DNA isolation

Total soil DNA was isolated from each treatment of nursery soil by using HiPurATM Soil DNA kit.

1. **Lysis**

   To the HiBead Tube provided, 750 µl of Soil Lysis Solution (SL) was added followed by addition of 500 mg of soil sample. The tubes were mixed several times by inverting them.

2. HiBead tubes were secured horizontally on flat-bed vortex pad using tape and were vortexed at maximum speed for 10 minutes.
3. The tubes were centrifuged at 14,000 rpm for 1 minute at room temperature.
4. The supernatant obtained in above step was transferred to a new 2.0 ml collecting tube.
5. Inhibitor Removal Solution (IRSH) (250µl) was added to the collected supernatant, after that was vortexed for 5 seconds and was incubated at 4 □ C for 5 minutes.
6. The tube was centrifuged at 12,000 rpm for 1 minute at room temperature.
7. **Binding**

   The supernatant was transferred to a clean 2.0 ml collecting tube and 1.2 ml of Binding Solution (SB) was added to it followed by vortexing for 5 seconds.

8. **Loading onto HiElute Miniprep Spin Column**

   Lysate (650µl) was loaded on the HiElute Miniprep Spin Column and centrifuged at 12,000 rpm for 1 minute at room temperature. The flow-through so obtained was discarded. Another 650 µl of lysate was loaded onto the column and repeated the centrifugation. The flow-through was discarded and the remaining lysate was loaded onto the column and centrifuged as before. The flow-through was discarded.

9. **Wash**

   Wash Solution concentrate was prepared as indicated in instruction by adding 2.4 ml of was solution concentrate (WSP) provided in kit to 5.7 ml of ethanol. 500 µl of this diluted Wash Solution was added to the column and was centrifuged at 12,000 rpm at room temperature for 1 minute.

10. The flow-through was discarded and this wash step was repeated again. Discarded the flow-through and centrifuged the column at 12,000 rpm at room temperature for 1 minute to remove any residual ethanol.

11. **DNA Elution**

    The column was transferred to a new 2.0 ml collecting tube and 100 µl of Elution Buffer (ET) was added directly onto the center of the column membrane. The tube was centrifuged at 12,000 rpm for 1 minute at room temperature.

3.8.2 Determination of purity and yield of DNA

The concentration of the DNA in the sample was measured by determining absorbance at 260 nm, and was calculated based on the value of 1.0 A(260nm) unit = 50 µg/ml of DNA, taking into account the dilution factor of the sample. Purity of the DNA was determined by taking absorbance reading at 260 and 280 nm. A260/A280 ratios were calculated to evaluate levels impurities.

1. Isolated DNA (10µl) was taken and was added in 3ml of sterile distilled water.
2. Spectrophotometer was set for measurement of absorbance at two wavelengths i.e. 260 and 280 nm.
3. Absorbance at 260 and 280 nm of each DNA sample was noted and A260/A280 was calculated.

3.8.3 Analysis of DNA samples on agarose Gel Electrophoresis

Solutions prepared: a) 5x TBE buffer (per litre) – 54 g Tris base, 27.5 g boric acid, 20 ml of 0.5 M EDTA (pH 8.0)

b) 0.7% agarose gel- 0.7g of agarose dissolved in 100 ml of TBE buffer

1. Prepare 0.7% agarose gel in 0.5x TBE buffer containing 0.5-1 mg/ml ethidiumbromide (EtBr staining may be carried out after gel electrophoresis).
2. Slowly load the DNA samples mixed with gel-loading buffer into the slots of the submersed agarose gel using a micropipette (also load DNA molecular wt. markers like 1 kb DNA ladder / l DNA + *Hind* III digest).
3. Apply a voltage of 1-5 V/cm (measured as the distance between the electrodes). Run the gel until the tracking dye bromophenol blue has migrated the appropriate distance through the gel.
4. Turn off the electric current, examine the gel by ultraviolet light and photograph the gel. Compare the size of DNA band(s) with molecular weight markers

Results

4.1 Physico-chemical characterization of soil and fly ash

The physical and chemical properties of fly ash depends on the nature of parent coal and condition of combustion (Carlson and Adriano, 1993) thus the characterization of fly ash provides useful information for its economic utilization. Both soil and fly ash was physico-chemically characterized (Table 6). It was found that pH of soil was alkaline (8.75) whereas fly ash had acidic pH of 6.09. Fly ash had high EC (0.16 mS/cm) as compared to that of soil (0.10 mS/cm). Fly ash had high available phosphorus and sulphur content as compared to soil, whereas, organic carbon content was more in soil. The heavy metals in fly ash were in order of Fe>Zn>Mn>Pb>Co>Cr>Cu>Cd. Fly ash had WHC of 65.5% which was higher than that of soil. Values of K, Pb, Fe, Zn, Co, Ni, Cu, Mn, Cr and Cd are mentioned in Table 6.

Table 6: Physico-chemical characterization of soil and fly ash

Parameters	Soil	Fly ash*
pH	8.75 ± 0.24	6.09 ± 0.07
EC(mS/cm)	0.10 ± 0.002	0.16 ± 0.0
Ava. Phosphorus (ppm)	1.95 ± 0.04	17.36 ± 0.81
Ava. Sulphur (ppm)	48.39 ± 3.70	288.71 ± 3.52
Ava. Nitrogen (%)	0.006 ± 0.000	0.004 ± 0.0
Organic carbon (%)	0.10 ± 0.01	0.05 ± 0.01
Water holding capacity (%)	34.68	65.5
K (ppm)	1490.33 ± 139.49	209.0 ± 12.29
Pb (ppm)	13.15 ± 0.84	15.67 ± 0.30
Fe (ppm)	11894.33 ± 53.76	2227.33 ± 86.04
Zn (ppm)	38.0 ± 3.51	59.67 ± 0.88
Co (ppm)	5.23 ± 0.08	5.98 ± 0.24
Cu (ppm)	6.02 ± 0.31	1.53 ± 0.07
Mn (ppm)	125.38 ± 4.69	21.05 ± 0.81
Cr (ppm)	6.92 ± 0.28	5.42 ± 0.06
Cd (ppm)	2.65 ± 0.06	1.12 ± 0.10

***Source: GGS Super thermal Plant, Ropar (Punjab)**

4.2 Physico-chemical characterization of nursery soil treated with fly ash at different interval of wheat growth

Physico-chemical properties like pH, EC, WHC, bulk density, total organic carbon, available nitrogen, phosphorus and sulphur are sensitive indicators of soil quality and sustainability in understanding the complexities of the nutrient profile in soils and therefore were examined to study the effect of fly ash on soil fertility and productivity at different interval such as one at the start of trial (i.e. 0 day), after 60 days of sowing and at the time of harvesting (i.e. after 120 days).

4.2.1 Chemical properties

- **pH**

Effect of fly ash amendment on pH of wheat nursery soil was studied (Table 7). It was seen that there was no drastic increase or decrease in pH of soil with either fly ash or microbial amendment. Soil pH remained in alkaline range over different time interval. pH of nursery soil at day 60 and day 120 showed slight increase with increase in percentage amendment of fly ash. There was no significant variation in pH of sterile v/s non-sterile soil system. Soil amended with both fly ash and microbes had slightly higher pH as compared to fly ash alone as shown in Table 7.

Table 7: pH of wheat nursery soil at different time intervals

S.No.	Treatment		pH of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	8.75 ± 0.24	7.9 ± 0.05	8.34 ± 0.03
		(NS)	8.75 ± 0.24	8.04 ± 0.02	8.32 ± 0.01
2	Control Soil +MA	(S)	8.66 ± 0.16	7.98 ± 0.03	8.31 ± 0.03
		(NS)	8.66 ± 0.16	7.83 ± 0.06	8.23 ± 0.04
3	Soil+5%FA	(S)	8.43 ± 0.07	8.09 ± 0.02	8.34 ± 0.01
		(NS)	8.43 ± 0.07	8.11 ± 0.05	8.36 ± 0.01
4	Soil+5%FA+MA	(S)	8.55 ± 0.06	8.14 ± 0.02	8.28 ± 0.03
		(NS)	8.55 ± 0.06	8.09 ± 0.02	8.32 ± 0.01
5	Soil+10%FA	(S)	8.50 ± 0.05	7.93 ± 0.08	8.42 ± 0.01
		(NS)	8.50 ± 0.05	8.13 ± 0.04	8.41 ± 0.02
6	Soil+10%FA+MA	(S)	8.60 ± 0.04	8.05 ± 0.02	8.44 ± 0.03
		(NS)	8.60 ± 0.04	8.06 ± 0.02	8.41 ± 0.02

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

- **EC**

EC of control soil at 0 day was measured to be 100µS/cm which increased with increase in fly ash percentage in soil. Increase in electrical conductivity of soil by any factor, increases the availability of soluble salts (Pitchel and Hayes, 1990). At the start of the trial EC of soil having 5% amendment of fly ash was 150µS/cm whereas in soil having 10% fly ash 162µS/cm EC was recorded (Table 8). Electrical conductivity ranged from 91.93µS/cm to 191.57µS/cm after 60 days of trial in control soil and in 10% fly ash treated soil and after 120 days EC was in the range of 105.03µS/cm to 187.57µS/cm. There was a marked increase in EC with increase in fly ash percentage, which can be attributed to increase in the concentration of soluble salts. There was marginal variation in EC in non-sterile systems as compared to sterile system and in soil amended with FA and MA both as compared to fly ash alone.

Table 8: EC of wheat nursery soil at different time intervals

S.No.	Treatment		EC (µS/cm) of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	100 ± 0.01	98.17 ± 06.81	105.03 ± 3.76
		(NS)	100 ± 0.01	91.93 ± 08.00	115.93 ± 2.58
2	Control Soil +MA	(S)	110 ± 0.01	99.10 ± 06.63	116.09 ± 1.70
		(NS)	110 ± 0.01	95.20 ± 01.22	141.01 ± 1.65
3	Soil+5%FA	(S)	158 ± 0.01	162.63 ± 18.07	165.27 ± 1.53
		(NS)	158 ± 0.01	168.27 ± 14.65	169.01 ± 4.05
4	Soil+5%FA+MA	(S)	132 ± 0.01	165.00 ± 11.55	172.93 ± 6.37
		(NS)	132 ± 0.01	171.13 ± 11.35	174.03 ± 0.81
5	Soil+10%FA	(S)	162 ± 0.02	185.17 ± 18.34	178.43 ± 3.44
		(NS)	162 ± 0.02	191.57 ± 08.51	187.57 ± 4.49
6	Soil+10%FA+MA	(S)	135 ± 0.01	186.00 ± 03.45	175.13 ± 1.65
		(NS)	135 ± 0.01	141.97 ± 23.29	177.07 ± 5.78

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

- **Organic Carbon**

Percent total organic carbon (TOC) increased in nursery soil with increase in fly ash percentage. Quantification of soil organic carbon (SOC) has fascinated the attention of many researchers as being an important indicator of soil quality and productivity (Krishnan *et al.,* 2009). Organic carbon content increased in the pot soil over the period of nursery trial. In control soil it increased from 0.034% to 0.26%, the increase was same in soil amended with fly ash. At the time of harvest in April 2011, the organic carbon content was maximum in soil amended with 5% fly ash and microbial amendment (Table 9).

Table 9: Organic Carbon in wheat nursery soil at different time intervals

S.No.	Treatment		Organic Carbon (%) of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	0.034 ± 0.02	0.20 ± 0.02	0.26 ± 0.03
		(NS)	0.034 ± 0.02	0.18 ± 0.01	0.31 ± 0.01
2	Control Soil +MA	(S)	0.039 ± 0.03	0.32 ± 0.05	0.32 ± 0.01
		(NS)	0.039 ± 0.03	0.23 ± 0.01	0.28 ± 0.01
3	Soil+5%FA	(S)	0.205 ± 0.01	0.35 ± 0.01	0.29 ± 0.003
		(NS)	0.205 ± 0.01	0.31 ± 0.03	0.28 ± 0.02
4	Soil+5%FA+MA	(S)	0.179 ± 0.02	0.37 ± 0.03	0.29 ± 0.01
		(NS)	0.179 ± 0.02	0.33 ± 0.01	0.33 ± 0.003
5	Soil+10%FA	(S)	0.182 ± 0.02	0.22 ± 0.01	0.24 ± 0.003
		(NS)	0.182 ± 0.02	0.23 ± 0.03	0.27 ± 0.02
6	Soil+10%FA+MA	(S)	0.163 ± 0.04	0.28 ± 0.03	0.28 ± 0.01
		(NS)	0.163 ± 0.04	0.25 ± 0.01	0.28 ± 0.02

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

- **Available nitrogen**

Available nitrogen in nursery soil was increased from 0.006% to 0.03% in control soil. It was higher in all treatments at the time of harvest. Highest value of available nitrogen was found after harvesting in soil amended with 5% FA and microbial amendment, which was 0.054% as shown in Table 10. An increase in available nitrogen percentage was seen in soil amended with microbial consortium. No significant pattern of variation was seen in sterile and non-sterile system (Fig. 1).

Table 10: Available Nitrogen in wheat nursery soil at different time intervals

S.No.	Treatment		Available Nitrogen (%) of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	0.006 ± 0.001	0.03 ± 0.001	0.03 ± 0.0001
		(NS)	0.006 ± 0.001	0.04 ± 0.00	0.043 ± 0.0004
2	Control Soil +MA	(S)	0.008 ± 0.001	0.04 ± 0.00	0.05 ± 0.0005
		(NS)	0.008 ± 0.001	0.03 ± 0.00	0.041 ± 0.0003
3	Soil+5%FA	(S)	0.045 ± 0.001	0.05 ± 0.00	0.048 ± 0.00
		(NS)	0.045 ± 0.001	0.04 ± 0.001	0.042 ± 0.00
4	Soil+5%FA+MA	(S)	0.034 ± 0.001	0.05 ± 0.00	0.052 ± 0.00
		(NS)	0.034 ± 0.001	0.043 ± 0.00	0.054 ± 0.00
5	Soil+10%FA	(S)	0.049 ± 0.001	0.03 ± 0.00	0.04 ± 0.00
		(NS)	0.049 ± 0.001	0.03 ± 0.00	0.044 ± 0.0002
6	Soil+10%FA+MA	(S)	0.034 ± 0.001	0.04 ± 0.001	0.042 ± 0.0001
		(NS)	0.034 ± 0.001	0.04 ± 0.00	0.051 ± 0.0002

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

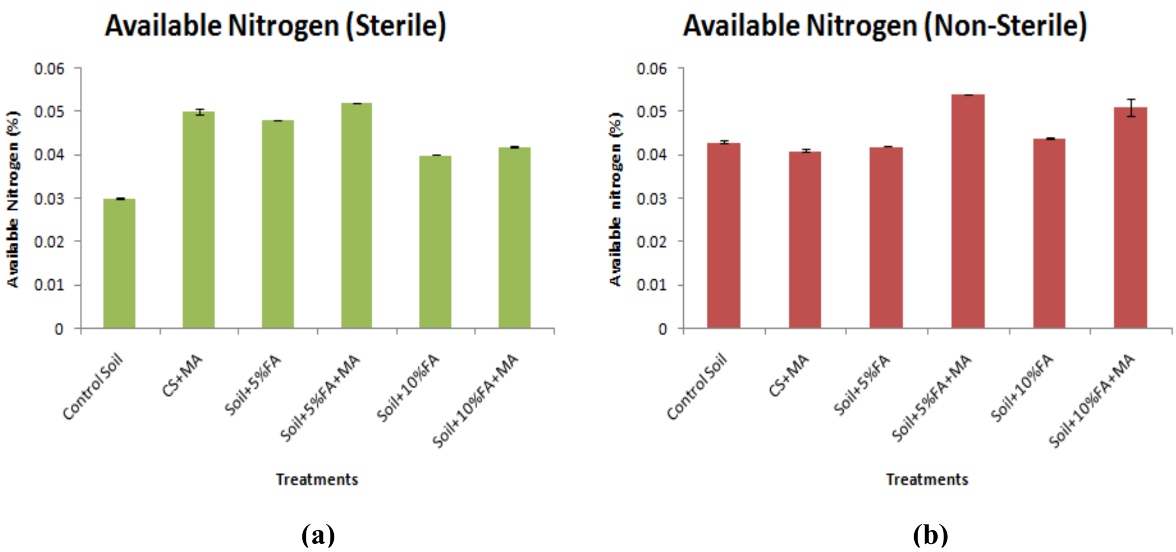


Fig 1: Available Nitrogen in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil systems with 0, 5 and 10 % fly ash.

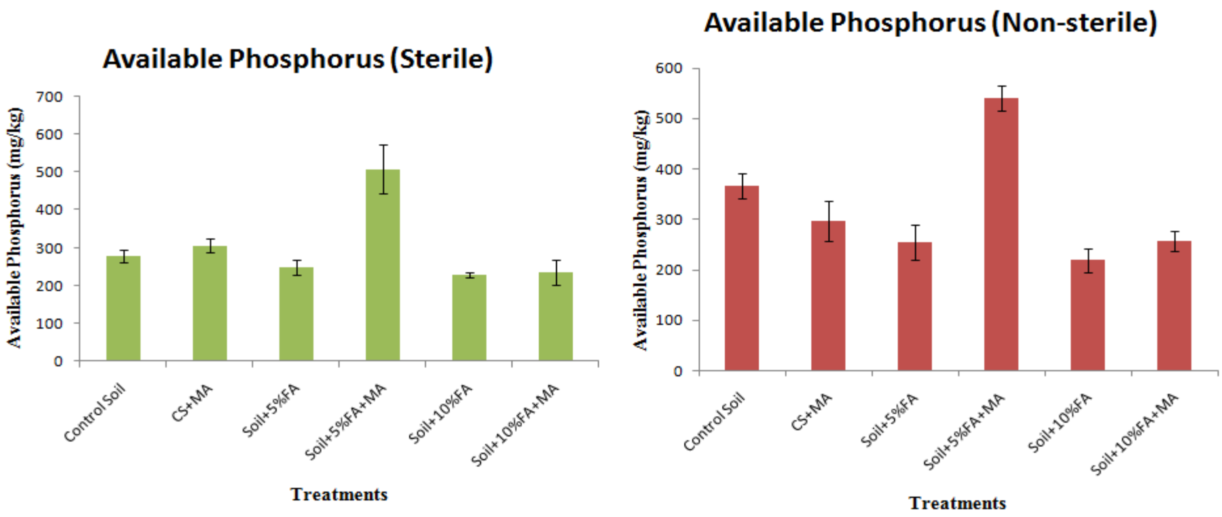
- **Available Phosphorus**

Available phosphorus increased in the soil during the course of nursery trial of wheat crop. The increase was more pronounced in treatments amended with microbes. It was 248.35 mg/ kg and 227.91 mg/kg in soil amended with 5% and 10% fly ash respectively but it was more in soil amended with both fly ash and PSB, 509.01 mg/kg and 235.30 mg/kg respectively (Table 11). This shows that the available phosphorus was highest in soil amended with 5% fly ash and MA. Also increase in available phosphorus content was higher in non-sterile system as compared to sterile system (Fig. 2).

Table 11: Available Phosphorus in wheat nursery soil at different time intervals

S.No.	Treatment		Available Phosphorus (mg/kg) of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	1.95 ± 0.04	374.55 ± 63.22	279.68 ± 16.60
		(NS)	1.95 ± 0.04	343.16 ± 34.14	365.85 ± 25.24
2	Control Soil +MA	(S)	2.88 ± 0.44	397.18 ± 20.07	305.79 ± 17.86
		(NS)	2.88 ± 0.44	372.98 ± 15.24	297.09 ± 40.15
3	Soil+5%FA	(S)	203.88 ± 2.14	370.28 ± 73.86	248.35 ± 20.24
		(NS)	203.88 ± 2.14	386.19 ± 55.04	254.45 ± 33.98
4	Soil+5%FA+MA	(S)	69.47 ± 1.87	226.59 ± 12.18	509.01 ± 64.80
		(NS)	69.47 ± 1.87	237.91 ± 25.99	540.29 ± 25.62
5	Soil+10%FA	(S)	202.45 ± 2.71	192.65 ± 33.72	227.91 ± 06.96
		(NS)	202.45 ± 2.71	235.30 ± 39.78	218.76 ± 24.42
6	Soil+10%FA+MA	(S)	65.72 ± 0.70	170.02 ± 25.24	235.30 ± 33.92
		(NS)	65.72 ± 0.70	147.39 ± 13.57	256.19 ± 20.07

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment



(a) **(b)**

Fig 2: Available Phosphorus in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil systems with 0, 5 and 10 % fly ash.

- **Available Sulphur**

During the nursery trial available sulphur was increased in control soil from a range of 13.21-14.82 mg/kg to 21.08-54.62 mg/kg. Similarly there was an increase in all the treatments with time. Available sulphur also increased with increase in fly ash percentage (Table 12). It was seen that there was slight reduction in available sulphur in soil amended with both FA and MA as compared to FA alone.

Table 12: Available Sulphur in wheat nursery soil at different time intervals

S.No.	Treatments		Available Sulphur (mg/kg) of wheat nursery soil		
			At 0 day	At 60 day	At 120 day
1	Control Soil	(S)	14.82 ± 1.21	29.00 ± 1.46	40.03 ± 1.82
		(NS)	14.82 ± 1.21	56.23 ± 3.93	54.62 ± 1.50
2	Control Soil +MA	(S)	13.21 ± 0.22	24.62 ± 8.66	33.37 ± 5.35
		(NS)	13.21 ± 0.22	24.20 ± 2.95	21.08 ± 3.67
3	Soil+5%FA	(S)	79.02 ± 0.78	25.87 ± 4.03	25.04 ± 1.85
		(NS)	79.02 ± 0.78	73.95 ± 32.12	69.83 ± 17.93
4	Soil+5%FA+MA	(S)	65.00 ± 1.01	65.87 ± 8.74	68.99 ± 21.48
		(NS)	65.00 ± 1.01	41.49 ± 7.43	46.07 ± 8.41
5	Soil+10%FA	(S)	29.83 ± 2.29	86.49 ± 9.78	70.25 ± 4.11
		(NS)	29.83 ± 2.29	77.70 ± 11.79	65.04 ± 4.18
6	Soil+10%FA+MA	(S)	23.29 ± 0.89	85.66 ± 14.59	57.37 ± 2.60
		(NS)	23.29 ± 0.89	44.33 ± 8.88	46.09 ± 2.60

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

4.2.2 Physical properties

- **Water holding capacity**

WHC increased with increase in fly ash percentage and over the period of nursery trial. Effect of MA was not so pronounced on WHC of soil. WHC capacity in control soil increases from 34.68 % to 35.74% after 120 days of trial and the increase was same in soil amended with fly ash. It increased to 47.33% and 45.42% in soil amended with 5% and 10% fly ash respectively (Table 13). Nearly similar results were obtained for non sterile system. Water holding capacity reveals the relationship between the soil and its water and important for the plant growth. The water holding capacity of soils also depends to their particle size distribution and texture (Ahuja, 2008).

- **Bulk Density**

Fly ash incorporation decreases the bulk density of soil. Bulk density of soil was reported to be 1.19 gm/cm^3. With 5% amendment the bulk density of soil becomes 1.12 gm/cm^3. But it was seen that there was increase in bulk density in soil having 10% amendment i.e. 1.41 gm/cm^3. Microbial amendment lead to decrease in the bulk density of soil which was 1.05 gm/cm^3 and 1.06 gm/cm^3 in 5% fly ash and 10% fly ash amendment respectively as shown in Table 13.

Table 13: Physical analysis of nursery soil at 0 day

Treatments		WHC (%)		Bulk Density (gm/cm^3)
		At 0 day	At 120 day	At 0 day
Control soil	(S)	34.68	35.40	1.19
Control soil	(NS)	34.68	35.74	1.19
(Soil + MA)	(S)	37.42	37.43	1.15
(Soil + MA)	(NS)	37.42	35.53	1.15
(Soil + 5% FA)	(S)	45.11	47.33	1.12
(Soil + 5% FA)	(NS)	45.11	44.14	1.12
(Soil + 5% FA + MA)	(S)	44.55	45.68	1.05
(Soil + 5% FA + MA)	(NS)	44.55	44.88	1.05
(Soil + 10% FA)	(S)	45.71	48.10	1.41
(Soil + 10% FA)	(NS)	45.71	46.41	1.41
(Soil + 10% FA + MA)	(S)	44.03	45.42	1.06
(Soil + 10% FA + MA)	(NS)	44.03	44.61	1.06

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

4.3 Microbiological analysis of various treatments in nursery soil at different interval of wheat growth

Enumeration of bacteria and genetic monitoring of inoculated beneficial soil microbes in wheat nursery soil

Enumeration was carried out with two dilutions 10^{-3} and 10^{-4} on nutrient agar, PKV and JM plates. Total bacterial count showed increase in soil during wheat growth in all treatments including control but was higher than control (Table 14, 15). Addition of microbial inoculants led to further rise in the same, whereas, in fly ash treated soil microbial inoculants had minimized the inhibitory effect of fly ash. Phosphate solubilizing bacteria showed increase in soil during wheat growth in all treatments including control but was higher in soil amended with both fly ash and microbes. The number of phosphate solubilizing bacteria was more in Pikovskaya's media as compared to that of media supplemented with chloramphenicol, IPTG and X-gal. The population of transformed nitrogen fixing bacteria was also higher in inoculated soils indicating that there is good establishment of inoculated bacteria alongwith fly ash.

Ecological monitoring of tagged *Pseudomonas* and *Azotobacter* was done on PKV and JM respectively containing chloramphenicol (10µg ml^{-1}), IPTG and X-gal and the bacteria were identified as blue colonies (Plate 11, 12). The present study examined the feasibility of using chloramphenicol resistance (Cmr) with the added capability of direct screening by lacZ expression. Further this reporter gene was inserted in beneficial soil bacteria such as *Pseudomonas sp.* and *Azotobacter sp.* and the bacteria were monitored in terms of multiplication and colonization in fly ash amended soil after their introduction in order to assess the optimum dose of fly ash required for their survival and growth. These tagged bacteria were inoculated in soil amended with 0 to 10% fly ash (v/v) under sterile and non-sterile conditions. The enumeration of inoculated bacteria on selective media showed that with an increase in fly ash concentration there was a positive effect on soil microbial population and an optimum concentration of 10% is tolerable for microbes.

Table 14: Total bacterial count on NA, PKV, JM plates with dilution 10^{-3}

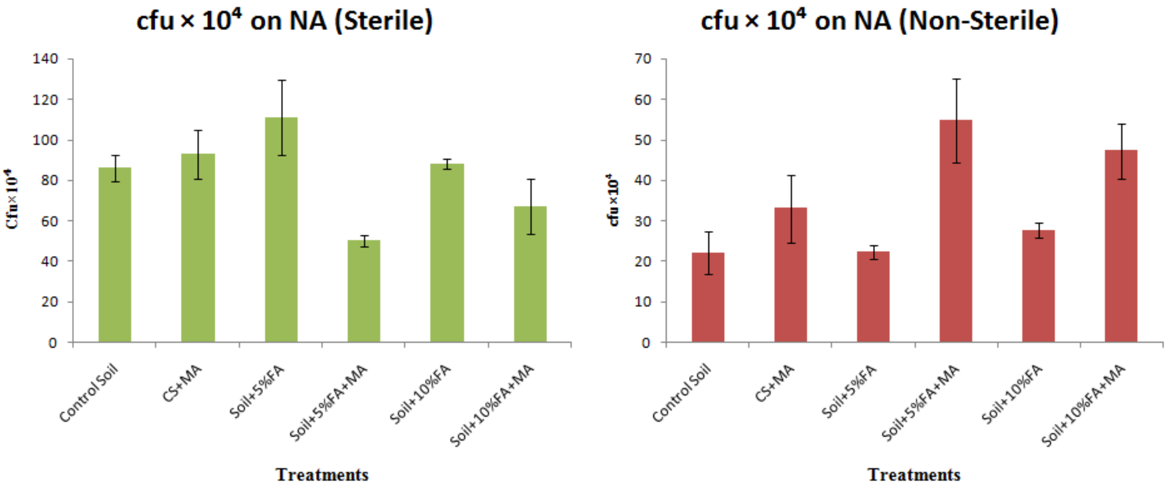
Treatments		NA cfu × 10^4		PKV cfu × 10^4		PKV + chl+ +X-gal + IPTG cfu × 10^4		JM cfu × 10^4		JM + chl+ + X-gal + IPTG cfu × 10^4	
		At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day
Soil (control)	**(S)**	66 ± 10.0	86 ± 06.7	53.3 ± 15.0	45.3 ± 11.5	-	-	82.7 ± 8.2	29.0 ± 1.3	-	-
	(NS)	17.3 ± 1.8	22 ± 05.3	14.7 ± 2.9	15.7 ± 05.8	3.0 ± 0.6	1.0 ± 0.0	9.3 ± 3.2	07.3 ± 1.8	1.7 ± 00.3	1.0 ± 0.0
Soil + MA	**(S)**	79.3 ± 10.4	92.8 ± 11.9	67.0 ± 14.6	94.0 ± 08.3	-	03.3 ± 1.8	79.0 ± 19.5	82.7 ± 3.9	-	4.3 ± 1.2
	(NS)	33.3 ± 12.0	33.0 ± 08.3	65.7 ± 20.3	34.0 ± 2.2	1.3 ± 0.3	1.7 ± 0.3	11.0 ± 5.6	18.7 ± 9.7	4.7 ± 1.2	2.7 ± 0.3
Soil+5%FA	**(S)**	76.3 ± 22.1	111 ± 18.5	31.3 ± 6.3	43.0 ± 15.9	1.7 ± 0.3	-	48.3 ± 21.7	59.7 ± 5.5	5.0 ± 0.0	-
	(NS)	9.3 ± 3.2	22.3 ± 01.8	36.3 ± 1.9	23.0 ± 2.1	01.0 ± 0.0	2.0 ± 0.6	28.8 ± 9.5	38.7 ± 7.3	2.0 ± 0.5	1.3 ± 0.3
Soil+5%FA+MA	**(S)**	22.3 ± 7.4	50.0 ± 02.9	48.0 ± 1.5	62.7 ± 04.3	2.3 ± 0.8	3.0 ± 1.0	106.0 ± 3.2	71.0 ± 9.1	1.0 ± 0.0	2.7 ± 0.9
	(NS)	23 ± 4.2	54.7 ± 10.3	40.7 ± 5.2	11.7 ± 01.5	1.0 ± 0.0	1.3 ± 0.3	42.0 ± 9.1	27.3 ± 1.4	3.0 ± 2.0	1.3 ± 0.3
Soil+10%FA	**(S)**	70.7 ± 24.4	88.7 ± 02.3	16.7 ± 10.2	79.0 ± 02.1	-	-	43.0 ± 25.2	71.3 ± 2.3	-	-
	(NS)	15 ± 3.2	27.7 ± 01.8	10.0 ± 2.7	35.7 ± 01.2	1.0± 0.0	4.7 ± 1.5	11.3 ± 1.4	32.3 ± 5.4	-	1.0 ± 0.0
Soil+10%FA+MA	**(S)**	67.3 ± 13.5	67.3 ± 13.5	33.0 ± 10.0	60.3 ± 0.8	05.0 ± 0.6	3.7 ± 1.2	105.7 ± 3.4	36.3 ± 2.9	1.3 ± 0.3	2.3 ± 0.7
	(NS)	16.7 ± 1.9	47.3 ± 06.8	6.00 ± 3.6	50.0 ± 07.6	01.3 ± 0.3	1.3 ± 0.3	98.3 ± 4.4	55.0 ± 14.4	2.3 ± 0.3	1.0 ± 0.0

NA: Nutrient agar media: Chl+: Chloramphenicol; - : no results

Table 14: Total bacterial count on NA, PKV, JM plates with dilution 10^{-4}

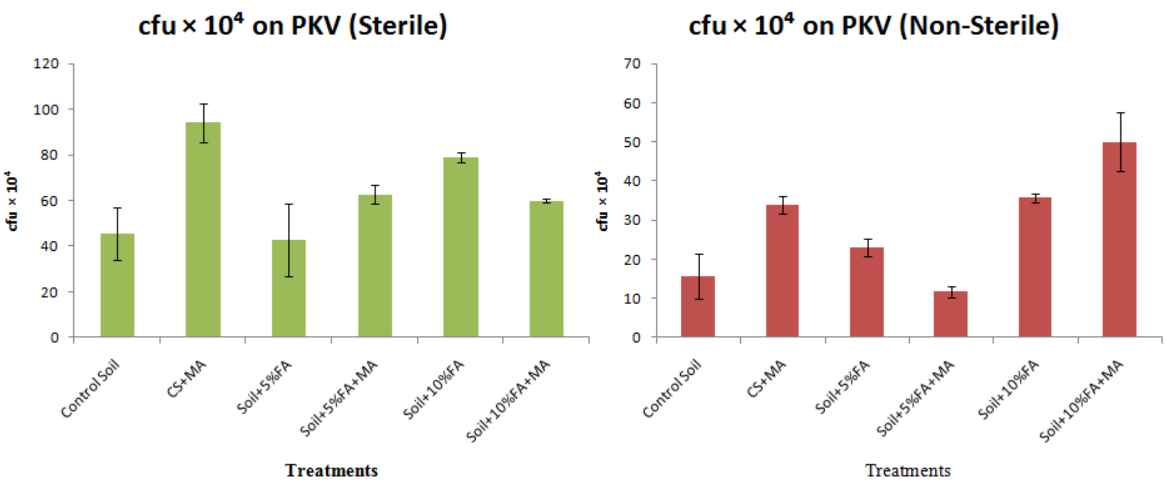
Treatment		NA (cfu × 10^5)		PKV (cfu × 10^5)		PKV + chl+ +X-gal + IPTG (cfu × 10^5)		JM (cfu × 10^5)		JM + chl+ + X-gal + IPTG (cfu × 10^5)	
		At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day	At 60 day	At 120 day
Soil (control)	**(S)**	19 ± 5.7	18.0 ± 2.6	16.0 ± 7.2	6.0 ± 2.3	-	-	8.3 ± 2.9	5.0 ± 2.5	-	-
	(NS)	6.0 ± 2.0	4.0 ± 0.5	4.7 ± 0.8	4.3 ± 2.3	1.0 ± 0.0	-	2.3 ± 0.3	2.3 ± 0.8	1.0 ± 0.0	-
Soil + MA	**(S)**	22.0 ± 15.6	24.0 ± 7.3	13.3 ± 1.2	17 ± 3.1	-	2.0 ± 0.5	12.7 ± 2.7	9.3 ± 1.4	-	1.0 ± 0.0
	(NS)	15.0 ± 7.0	8.0 ± 02.5	26.7 ± 25.1	2.7 ± 1.6	1.0 ± 0.0	4.3 ± 3.3	2.67 ± 0.3	4.7 ± 1.4	1.0 ± 0.0	3.7 ± 0.0
Soil+5%FA	**(S)**	16.7 ± 17.7	16.3 ± 2.4	9.0 ± 4.3	6.7 ± 3.2	-	-	51.0 ± 5.8	7.7 ± 0.3	2.0 ± 0.5	4.0 ± 0.3
	(NS)	6.3 ± 2.3	06.7 ± 0.8	8.7 ± 1.8	7.7 ± 0.3	-	1.0 ± 0.0	12.3 ± 8.9	5.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.0
Soil+5%FA+MA	**(S)**	25.7 ± 0.8	24.7 ± 1.4	4.0 ± 1.7	7.7 ± 0.6	02. ± 0.5	1.0 ± 0.0	7.0 ± 3.2	16.3 ± 0.8	2.0 ± 0.5	8.0 ± 0.0
	(NS)	5.0 ± 0.5	17.0 ± 3.2	6.3 ± 1.2	5.3 ± 0.8	-	1.0 ± 0.0	13.7 ± 2.4	10.0 ± 1.1	1.0 ± 0.0	-
Soil+10%FA	**(S)**	19.7 ± 14.1	34.7 ± 2.4	3.0 ± 1.1	25.7 ± 4.1	-	-	17.3 ± 2.7	23.7 ± 2.4	-	-
	(NS)	2.00 ± 0.0	10.0 ± 1.1	2.3 ± 0.8	11.0 ± 1.1	-	1.3 ± 0.3	4.7± 1.2	8.7 ± 1.7	-	-
Soil+10%FA+MA	**(S)**	12.3 ± 3.8	12.3 ± 3.8	3.0 ± 1.5	26.7 ± 2.0	1.7 ± 0.3	1.0 ± 0.0	32.3 ±10.3	8.3 ± 2.0	2.0 ± 1.0	2.3 ± 0.0
	(NS)	2.7 ± 0.7	21.7 ± 04.4	1.7 ± 0.6	19.0 ± 4.5	1.0 ± 0.0	-	9.0 ± 1.7	13.3 ± 1.4	1.3 ± 0.3	1.0 ± 0.0

NA: Nutrient agar media: Chl+: Chloramphenicol; - : no results



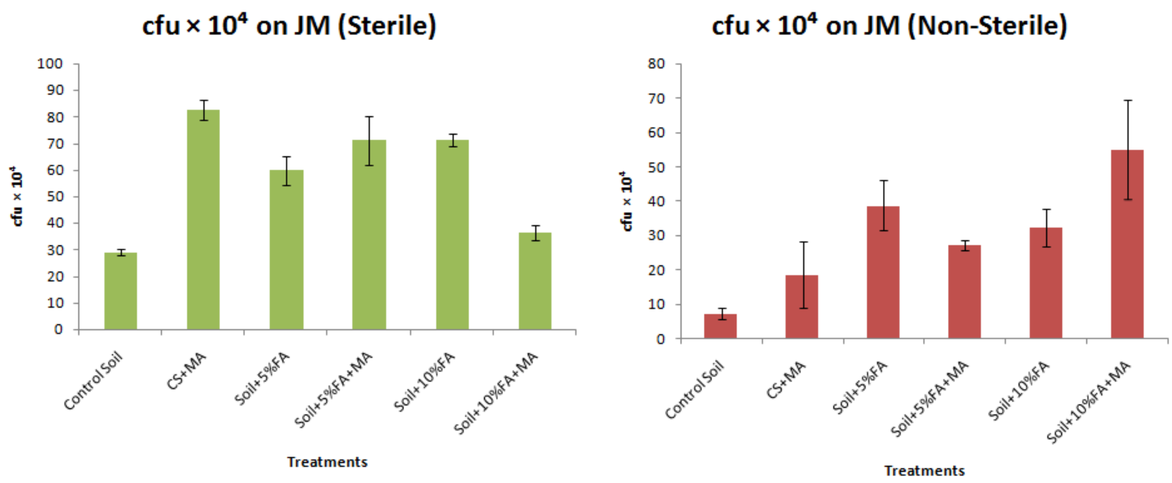
(a) **(b)**

Fig 3: Bacterial count on Nutrient Agar in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil systems soil systems with 0, 5 and 10 % fly ash.



(a) **(b)**

Fig 4: Bacterial count on Pikovskaya media in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil systems soil systems with 0, 5 and 10 % fly ash.



(a) **(b)**

Fig5: Bacterial count on Jensens medium in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil system soil systems with 0, 5 and 10 % fly ash.

4.4 Biometric parameter analysis

At the time of maturity in soil amended with 5% fly ash and microbial amendment of non-sterile system, there was maximum number of plants and maximum number of spikes per pot (Table 16 and 17; Plate 1 and 2). Nine plants per pot were selected for determination of length of plant, length of spike, number of grains, weight of grains and shoot biomass (Table 18). Length of plant was found to be maximum in nursery soil treated with 10% FA in non-sterile system (Plate 1 and 2). Length of spike did not show any drastic variation among various treatments. Range of number of seeds produced in control soil was 8.1 to 15.7 whereas in soil treated with 5% FA it was 10.2 to 17.2 and in soil treated with 10% FA it was 10.6 to 13.1 (Plate 7, 8, 9, 10). The maximum number of seeds were produced in soil treated with fly ash and MA in sterile system. Similarly, weight of grains was maximum (i.e. 5.06 g) in soil treated with 5% FA and microbial amendment in sterile soil system. Addition of 5% fly ash along with microbial inoculants led to increase in biomass production. In terms of weight of grains there was increase as compared to control. The fly ash along with microbial inoculants had a positive effect on wheat growth and grain yield.

Table 16: Number of wheat plants and number of spikes per pot in nursery trial (in sterile system) at harvesting

Treatment	No. of plants/pot				No. of spikes /pot			
	R1	R2	R3	Mean ± S. E.	R1	R2	R3	Mean ± S. E.
Soil (control)	20	18	12	16.67 ± 2.40	20	18	12	16.67 ± 2.40
Soil+MA	18	19	9	15.33 ± 3.18	18	19	9	15.33 ± 3.18
Soil+5% FA	18	15	18	17.00 ± 1.00	18	15	18	17.00 ± 1.00
Soil+5% FA+MA	19	18	16	17.67 ± 0.88	19	18	16	17.67 ± 0.88
Soil+10% FA	19	20	9	16.00 ± 3.51	19	20	9	16.00 ± 3.51
Soil+10% FA+MA	18	18	18	18.00 ± 0.00	18	18	18	18.00 ± 0.00

FA: Fly ash, MA: microbial amendment

Table 17: Number of wheat plants and number of spikes per pot in nursery trial (in non-sterile system) at harvesting

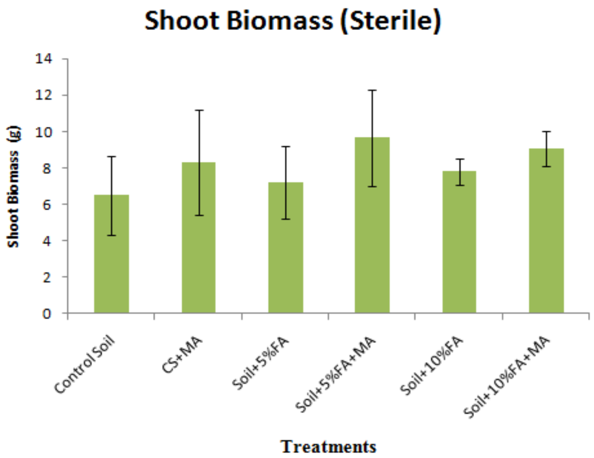
Treatment	No. of plants/pot				No. of spikes /pot			
	R1	R2	R3	Mean ± S. E.	R1	R2	R3	Mean ± S. E.
Soil (control)	18	16	16	16.67 ± 0.67	18	16	16	16.67 ± 0.67
Soil+MA	19	16	16	17.00 ± 1.00	19	16	16	17.00 ± 1.00
Soil+5% FA	17	18	18	17.67 ± 0.33	16	17	17	14.67 ± 1.86
Soil+5% FA+MA	18	17	20	18.33 ± 0.88	18	12	20	16.67 ± 2.40
Soil+10% FA	19	15	19	17.67 ± 1.33	19	15	19	17.67 ± 1.33
Soil+10% FA+MA	19	17	16	17.33 ± 0.88	19	17	16	17.33 ± 0.88

FA: Fly ash, MA: microbial amendment

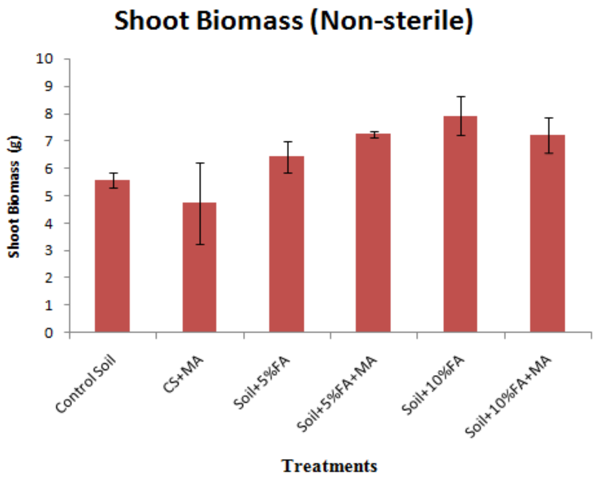
Table 18: Biometric parameters of wheat crop in nursery trial after 120 days at harvesting

Treatment		Length of plant (cm)	Length of spike (cm)	No. of Grains/ spike	Wt. of grains (gm)	Shoot Biomass (gm)
Soil (control)	(S)	43.26 ± 7.99	10.47 ± 0.77	08.7 ± 00.11	1.91 ± 0.06	6.49 ± 2.14
	(NS)	48.29 ± 0.27	9.39 ± 0.11	08.1 ± 00.07	2.33 ± 0.04	5.58 ± 0.27
Soil + MA	(S)	43.74 ± 11.40	10.19 ±0.78	15.7 ± 00.20	4.52 ± 0.05	8.31 ± 2.90
	(NS)	51.87 ± 2.57	9.77 ± 0.35	07.7 ± 00.04	1.69 ± 0.05	4.74 ± 1.48
Soil+5%FA	(S)	45.90 ± 7.11	10.68 ± 0.82	10.2 ± 00.50	2.81 ± 0.24	7.23 ± 1.99
	(NS)	47.72 ± 5.48	9.92 ±0.33	10.44 ± 00.33	2.74 ± 0.21	6.43 ± 0.58
Soil+5%FA+MA	(S)	45.89 ± 9.29	10.37 ± 0.43	17.2 ± 00.09	5.06 ± 0.06	9.65 ± 2.65
	(NS)	42.77 ± 7.32	9.38 ± 0.19	11.6 ± 00.16	3.7 ± 0.03	7.25 ± 0.11
Soil+10%FA	(S)	45.77 ± 8.73	10.99 ± 0.18	11.7 ± 00.07	1.17 ± 0.02	7.81 ± 0.73
	(NS)	52.78 ± 2.65	10.56 ± 0.22	10.6 ± 00.10	2.68 ± 0.04	7.82 ± 0.72
Soil+10%FA+MA	(S)	51.42 ± 1.37	10.95 ± 0.18	13.1 ± 00.94	3.07 ± 0.67	9.08 ± 0.96
	(NS)	52.44 ± 1.37	9.89 ± 0.35	11.9 ± 00.48	2.72 ± 0.67	7.20 ± 0.64

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment



(a)



(b)

Fig 6: Shoot Biomass in wheat nursery soil at the time of harvesting in (a) sterile and (b) non-sterile soil system with 0, 5 and 10 % fly ash.

4.5 Percentage wheat germination

Wheat germination was studied in petri-plates and was monitored, after 2 days of sowing. Percentage germination was highest in soil amended with 5% fly ash as compared to control soil and the soil amended 10% with fly ash (Table 19; Plate 4 and 5). Similar results were obtained for percentage seed germination in *Mung bean* (Table 20; Plate 6). In wheat germination experiment done in pots, percentage wheat germination was nearly same in both control soil and soil amended with 5% fly ash but was low in soil amended with 10% fly ash (Table 21; Plate 3). 5% fly ash amendment in soil had positive role in increasing percent seed germination. After 5 days of sowing wheat sowed in petri-plates was evaluated for radical/plumule ratio. It was seen that this ratio was nearly same in both control soil and soil amended with 5% FA. This ratio was very less in case soil amended with 10% FA (Table 22).

Table 19: Percentage wheat germination in petri-plates

S.No.	Percentage seed germination (%)				
	Treatments	R1	R2	R3	Mean ± S.E.
1	Control soil	70	80	75	75.00 ± 2.89
2	Soil+5% Fly ash	90	80	85	85.00 ± 2.89
3	Soil+10% Fly ash	30	42	55	42.33 ± 7.23

Table 20: Percent *Mung bean* germination in petri-plates

S.No.	Percentage seed germination (%)				
	Treatments	R1	R2	R3	Mean ± S.E.
1	Control soil	8	9	7	8.00 ± 0.58
2	Soil+5% Fly ash	7	10	8	8.33 ± 0.88
3	Soil+10% Fly ash	8	7	6	7.00 ± 0.58

Table 21: Percent wheat germination in pot experiment

S.No.	Percentage seed germination in pot (%)				
	Treatments	R1	R2	R3	Mean ± S.E.
1	Control soil	9	10	9	9.33 ± 0.33
2	Soil+5% Flyash	10	9	8	9.00 ± 0.58
3	Soil+10% Flyash	4	7	1	4.00 ± 1.73

Table 22: Radical/Plumule ratio of wheat grown in petri-plates

Radical/Plumule Ratio					
S.No.	Treatments	R1	R2	R3	Mean ± S.E.
1	Control soil	2.34	1.66	1.76	1.89 ± 0.21
2	Soil+5% Flyash	1.87	1.64	2	1.84 ± 0.10
3	Soil+10% Flyash	1.6	1.59	1.86	1.68 ± 0.09

4.6 Determination of purity and yield of soil DNA

During the wheat nursery trials, there was an increase in soil DNA concentration with time. There was no drastic reduction in soil DNA with fly ash amendment, in fact there was increase in soil DNA concentration with time. This shows that there is no negative effect of fly ash on soil microbial population. Soil DNA concentration in control soil on day 60 was 0.75 µg/ml which increased to 1.58 µg/ml at the time of harvest. Similar increase was found in all other treatments as shown in Table 23. Soil DNA isolated from all the samples by using HiPurATM Soil DNA kit was separated on 0.7% agarose as shown in Plate 13.

Table 23: Total DNA estimation in wheat nursery soil at different time intervals

		Total soil DNA estimation of wheat nursery trial			
		On day 60		On day 120	
S.No.	Treatment	A260/280	DNA (µg/ml)	A260/280	DNA (µg/ml)
1	Control Soil (S)	1.67	0.75	1.16	1.58
2	Control Soil (NS)	1.5	1.35	1.18	1.65
3	Control Soil +MA (S)	1.22	1.2	1.5	1.35
4	Control Soil +MA (NS)	1.38	1.8	1.6	1.2
5	Soil+5% FA (S)	1.5	0.45	1.18	5.33
6	Soil+5% FA (NS)	1.25	0.75	1.18	2.48
7	Soil+5% FA+MA (S)	1.18	1.35	1.23	1.5
8	Soil+5% FA+MA (NS)	1.34	1.4	1.31	2.1
9	Soil+10% FA (S)	1.5	0.9	1.22	2.63
10	Soil+10% FA (NS)	1.6	1.05	1.2	2.25
11	Soil+10% FA+MA (S)	1.1	2.25	1.8	1.35
12	Soil+10% FA+MA (NS)	1.15	2.65	1.67	0.75

S: sterile, NS: non-sterile, FA: Fly ash, MA: microbial amendment

Chapter 5

Discussion

5.1 Physico-chemical characterisation of fly ash and soil

Fly ash collected from GGS Thermal Power Plant, Ropar, Punjab was analyzed for physic-chemical characterisation after air drying and alkaline soil from Thapar Technology Campus, Patiala (Punjab) collected from the topmost (0-30 cm) soil, was processed and subjected to physicochemical characterization.

Characterization of soil and fly ash revealed that pH of soil was alkaline and on the other hand pH of fly ash was acidic (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 followed by formation of insoluble hydroxides and oxides.

The fly ash used in the experiment had more EC than the soil, which shows that the fly ash contained more amounts of soluble salts. Essential elements were measured in order K>P>N while in soil was K>N>P. Fly ash contained more phosphorus as compared to soil, but had less nitrogen and organic carbon percentage than soil. It had been opined that the oxidation of C and N during combustion drastically reduces their qualities in ash. Fly ash used was rich in sulphur as compared to soil which implied that the type of coal used for combustion was rich in S and produces acidic fly ash as type of coal used for combustion affects the S content of fly ash (Page *et al*., 1979). The micronutrients varied as Fe>Zn>Mn>Cu in fly ash while in soil it varied as Fe>Mn>Zn>Cu. It implies that fly ash was rich in Fe and Zn. Also cobalt was higher in fly ash as compared to soil. The presence of micronutrients in fly ash can thus be beneficial for maintaining nutrient balance in soil from the fertility angle; however its dosage of application has to be properly determined (Wallace et al., 1980, Aitken and Bell, 1984; Sikka and Kansal, 1994). With regard to physical properties, WHC of fly ash was much higher as compared to soil this may be certainly due to dominance of silt-sized particles in fly ash.

5.2 Physico-chemical characterization of nursery soil treated with fly ash at different interval of wheat growth

Chemical properties

pH:

The native soil was alkaline with pH 8.75 and its amelioration with acidic fly ash resulted in only a slight decrease, which ranged from pH 8.43 to 8.50. Also with time pH slightly decreased in all the treatments but at the time of harvesting it increased. There was no significant variation in pH of nursery soil i.e. amendment with fly ash was not able to shift the pH of nursery soil towards acidity. The role played by the inherent buffering capacity of the soil might have helped in resisting pH changes resulting in pH range upto 7.93-8.42.

Electrical Conductivity:

There was an increase in electrical conductivity with increase in fly ash percentage. Electrical conductivity increased due to enrichment with fly ash, which is composed of various essential and non-essential cations and anions (Wong and Wong, 1989). Increase in electrical conductivity of soil by any factor, thereby increases the availability of soluble salts (Pitchel and Hayes, 1990).

Organic Carbon:

Organic carbon (TOC) increases in nursery soil with increase in fly ash percentage and it also increased in soil over the period of nursery trial. This was in accordance with Fang *et al.* (1998) according to whom organic carbon in fly ash amended soil increased with addition of fly ash up to 10%. Organic carbon content showed no distinct variation in both sterile and nonsterile soil. The marginal increase in OC content may be due to the fact that the soil initially had a very low OC content and it had not been supplemented with organic manure. Organic carbon percentage was found to be highest in soil with FA and microbial amendment this is because microbes add organic matter to soil.

Available Nitrogen

Nitrogen content in the fly ash amended soil showed a significant increase ranging from 0.006 to 0.054 %. The native soil of Patiala is rich in nitrogen. Highest value of available nitrogen percentage was found in soil amended with both fly ash and microbial consortium. The increased in soil nitrogen can be attributed due to fixation of nitrogen by added *Azotobacter* consortium and it can also be due to decomposition of leaf litter resulting in enzyme-aided nutrient mineralization carried out by the microbial amendment (Klose et al., 2004).

Available Phosphorus:

Phosphorous increased in soil as a result of fly ash amendment and was found to be highest in soil amended with both FA and microbial consortium as microbial consortium includes PSB (*Pseudomonas* sp.). This increase in phosphorus content may be attributed because of phosphate-solubilizers and partly due to fly ash itself. Available phosphorous decreased under sterile and non-sterile conditions in zero day nursery soil amended with both fly ash and microbes which could be due to consumption of the phosphorous present in soil and fly ash by microbes for growth and metabolism (Gaind and Gaur, 1991) Fly ash had inherent phosphorous which in due course of time might have been utilized by the microorganisms and converted into available form. In this way microorganisms increased the availability of phosphorous by utilizing the phosphorous present in soil and fly ash (Gaind and Gaur, 1991). In addition to this nutrient mineralization from plant litter and soil organic matter might have contributed to increased adaptability of the phosphate solubilizers in soil micro-environment (Klose et al., 2004). Over all available phosphorous were higher in non-sterile soil which might be due to presence of native microorganisms.

Available Sulphur:

Available sulphur increased with increase in fly ash percentage as the fly ash used had an acidic pH and was rich in S which implied that the type of coal used for combustion was rich in S (Page *et al*., 1979) due to which its amendment in nursery soil increases the available sulphur concentration. Thus, increase in sulphur can be attributed to acidic pH of fly ash and presence of sulphur predominantly in SO^4 form as a result of which SO_4^-S increases in proportion to fly ash added (Plank and Martens, 1974; Page et al., 1979; Natusch et al., 1975).

Physical properties

Water Holding Capacity:

Water holding capacity of the soil ranged from 34.68 % to 35.74% and increased with increase in fly ash percentage, which could be due to dominance of silt-sized particles in fly ash and cenospheric nature of fly ash (Chang et al., 1977; Aitken et al., 1984; Sharma et al., 1990).

Bulk Density:

Bulk density in fly ash-amended soil ranged from 1.15 to 1.19 g $cm^{-3}$and decreased with 5% fly ash addition as reported in other studies (Chang et al., 1977).

5.3 Microbiological analysis: Enumeration of bacteria and genetic monitoring of inoculated beneficial soil microbes in wheat nursery soil.

Population increase of microbes was studied in soil amended with 0 to 10% fly ash under sterile and non-sterile conditions over a period of 120 days period. Bacterial counts in sterile soil were lower as compared to non-sterile soil. Addition of microbial inoculants led to further increase in microbial population which was in accordance with the study on application of fly ash at 40 t/ ha in conjunction with phosphate solubilizer, *Pseudomonas striata* improved the bean yield and fly ash did not exert any detrimental effect on the population of *P. striata* in soil (Gaind and Gaur, 2002).

Reporter gene systems have become indispensable tools for understanding gene regulation in prokaryotes and eukaryotes (Loper and Lindow, 1997). Reporter genes like lac Z are most popular on account of their easy detectability, sensitivity, specific activity and rapidity (Bronstein et al., 1994). Due to the ease and sensitivity of its detection, lac Z is the most commonly used reporter gene in microbial ecology (Slauch and Silhavy, 1991). The ecological monitoring in terms of population buildup was studied by enumeration on chloramphenicol, IPTG and X-gal containing nutrient agar plates. The enumeration of inoculated bacteria on selective media indicated that with an increase in fly ash concentration there was a positive effect on soil microbial population and an optimum concentration of 10% is tolerable for microbes indicating that its application provides micronutrients for growth. This was in accordance to the study that fly ash-sludge mixture containing 10% fly ash had positive effect on soil micro-organisms (Lai *et al.,* 1999).

5.4 Biometric parameter analysis

Various biometric parameters, viz. no. of plants/pot, no. of spikes, length of plant, length of spike, no. of grains, weight of grains and shoot biomass per pot were studied to determine the impact of FA either singly or in combination with microbial consortium of *P.striata* and *Azotobacter sp.* on growth of wheat with the addition of fly ash alone the shoot biomass was increased to 7.23 g and in fact, the combination of 5% fly ash with MA led to further increase in total biomass to 9.65 g in sterile system, plant length to 47.72 cm, no. of grains produced to 17, and weight of grains to 5.06. In almost all the parameters soil amended with 5% FA and with microbes showed more pronounced effect as compared to other treatments. Soil amended with 5% fly ash in non-sterile systems increased the grain yield by 18% and shoot biomass by 15% as compared to control soil and in treatment comprising of 5% fly ash and microbial consortium grain yield and shoot biomass increased by 59% and 30% respectively as compared to control soil. Soil amended with 10% fly ash showed nearly same results as control soil. Therefore, it's addition is not inhibitory for wheat growth. These results were in accordance with the study that the application of fly-ash based *Azotobacter* and *Azospirillum* formulation alone and in combination with chemical fertilizers significantly enhanced the seed germination, plant height, plant biomass and crop yield compared to control (Kumar *et al.,* 2010). Also application of a treatment comprising of 10% fly ash, 20% farmyard manure and microbial consortium promoted growth in *Populus deltoides* and significantly increased plant height, collar diameter and total dry biomass (Aggarwal and Goyal, 2008).

5.5 Percentage wheat germination

Percentage germination was maximum in soil amended with 5% fly ash as compared to control soil and the soil amended 10% with fly ash, which was in accordance with lower dose (5-10%) of application of fly-ash in soils enhances seed germination (Singh *et al.* 1997). Wong and Wong (1989) reported an increase in seed germination, shoot length and cotyledon length of *Brassica parachinensis* and *B. chinensis* on 6% fly ash amendment to sandy loam soil while those in 12 and 30% treated sandy soil and 30% treated sandy loam soil showed a significant reduction. It was seen that root and shoot ratio was nearly same in both control soil and soil amended with 5% FA. This ratio was reduced in case of soil amended with 10% FA. Decrease in total root length and cotyledon length of seedlings of desert annuals has been reported with increased ash amendment up to 50% (Vollmer *et al.,* 1982).

5.6 Determination of purity and yield of soil DNA

There was no drastic reduction in soil DNA with fly ash amendment, in fact there was increase in soil DNA concentration with time. This shows that there is no negative effect of fly ash on soil microbial population. These all findings were in accordance with the application of fly ash at 40t/ha in conjucation 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.straita* in soil (Gaind and Gaur 2002).

Fly ash contains basic mineral elements, which make it similar to the earth's crust. Its addition to soil results in agronomic benefits enabling raising a green cover and even growing of crops on nutrient-deficient soils, and can be successfully used to correct soil pH. Presence of high concentrations of elements like K, Mg, Fe, Zn and Ca in readily available ionic form increases their uptake by plants. Soils prone to wind or water erosion can be stabilized through fly ash amendment. The fine nature of fly ash is effective in increasing water holding capacity of sandy soils. These improvements in physical and chemical nature coupled with a positive effect on microbial denizens make the problem soils productive.

Based on the above findings, fly ash can be recommended as a potential nutrient supplement for agriculture thereby solving the solid waste disposal problem to some extent. However, bioaccumulation of toxic heavy metals in plants part and their critical levels for human health and soil need to be investigated. Usually the properties of fly ash vary depending upon the properties of coal, combustion condition, storage as well as other factors. Therefore, it will also be necessary to investigate the potential of fly ash generated from a particular industry in agriculture on a long-term basis in order to determine an optimum concentration for soil amendments. An ultimate goal would be to utilize fly ash in agriculture to such an extent so as to achieve enhanced yield without affecting soil quality and minimizing the accumulation of toxic metals in plants below critical levels for human health.

Conclusion

1. 5% fly ash and microbial consortium of *Pseudomonas* sp. *and Azotobacter* sp. as soil amendment had a positive effect on growth of wheat crop as judged by various biometric parameters of plant growth.

2. It showed significant increase in biomass production as compared to other treatments. It also showed increase of various plant nutrients in soil like available phosphorus and available nitrogen but was more pronounced in soil treated with both fly ash and beneficial soil microbes.

3. Combined addition of fly ash and microbial inoculants can be used as a good potting mixture for improving survival rates and plant growth, which would also result in meaningful utilization of fly ash.

4. There was an enhanced effect of 5% fly ash amendment on percentage seed germination as compared to control soil and soil treated with 10% fly ash.

5. Fly ash addition improved soil properties such as it increased water holding capacity, EC and reduced bulk density.

6. That total bacterial count showed increase in soil during wheat growth in all treatments. There was no negative effect of fly ash on soil microbial population.

7. Total soil DNA was not affected by the addition of 5-10% fly ash in soil.

References

1. Adriano, D.C., Page, A.L., Elseewi, A.A., Chang, A.C., Straughan, I., 1980. Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review. *J. Environ. Qual*. 9, 333-344.
2. Adriano, D.C., Woodford, T.A., Ciravolo, T.G., 1978. Growth and elemental composition of corn and bean seedlings as influenced by soil application of coal ash. *J. Environ. Qual*. 7, 416-421.
3. Aggarwal, H., Goyal, D., 2009. Impact of addition of soil amendments and microbial inoculants on nursery growth of *Populus deltoides* and *Toona ciliata. Agroforst Syst*. 75, 167-173.
4. Ahuja, L. R., Nachabe, M. H., and Rockiki, R., 2008. Soils: Field Capacity, Encyclopedia of Water Science, Second Edition
5. Aitken, R.L., Campbell, D.J., Bell, L.C., 1984. Properties of Australian fly ash relevant to their agronomic utilization. *Aust. J. Soil Res*. 22, 443-453.
6. Anjum, MA., Sajjad, MR., Akhtar, N., Qureshi, MA., Iqbal, A., Jami, AR., Hassan, M., 2007. Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen. *J Agric Res* 45(2), 135–143
7. Banerjee, MR., Yesmin, L., Vessey, JK., 2006. Plant growth promoting rhizobacteria as biofertilizers and biopesticides. In: Rai MK (ed) Handbook of microbial biofertilizers. Haworth Press, New York.
8. Barassi, CA., Creus, CM., Casanovas, EM., Sueldo, RJ., 2000. Could Azospirillum mitigate abiotic stress effects in plants? Auburn University.
9. Black, C.A. (Editor-in-Chief), D.D.Evans, J.L.White, L.E.Ensmingher and F.E.Clarke, Associate Editors. R.C.Dinauer, Managing Editor, 1965. Methods of Soil Analysis. Part 1 Physical and Mineralogical Properties Including Statistics of Measurement and Sampling, 9 Series Agronomy. *American Society of Agronomy, Inc*., Madison, Wisconsin, USA.
10. Boivin, C., Camut, S., Malpica, CA., Truchet G., Rosenberg, C, 1990. *Rhizobium meliloti* genes encoding catabolism of trigonelline are induced under symbiotic conditions. *The Plant Cell*. 2, 1157-1170.

11. Bronstein, I., Fortin, J., Stanley, P.E., Stewart, G.S.A.B., Kricka, L.J., 1994. Chemiluminescent and bioluminescent reporter gene assays. *Anal. Biochem*. 219, 169-181.
12. Capp, J.P., 1978. Power plant fly ash utilization for land reclamation in the eastern United States. In F.W Schaller and P. Sutton (ed.) in Reclamation of drastically disturbed lands, ASA, Madison, Wisconsin, pp. 339-353.
13. Cappuccino, J.G., Sherman, N., 1987. Microbiology. A laboratory manual. Benjamin Cummings Publications.
14. Carlson, C.L., Adriano, D.C., 1993. Environmental impacts of coal combustion residues. *J. Environ. Qual.* 22, 227-247.
15. Chang, A.C., Lund, L.J., Page, A.L., Warneke, J.E., 1977. Physical properties of fly ash amended soils. *J. Environ. Qual*. 6 (3), 267-270.
16. Chesnin, L., Yien, C.H., 1950. Turbidimetric determination of available sulphates. Proc. Soil Sci. Soc. Am. 15, 149-151.
17. Ciravolo, T.G., Adriano, D.C., 1979. Utilization of coal ash by crops under greenhouse conditions. In M.K.Wali (ed.) Ecology and Coal Resource Development.Vol 2. Permagon press, New York, pp. 958-966.
18. Davison, R.L., Natusch, D.F.S., Wallace, J.R., Evans, C.A., Jr. 1974. Trace elements in fly ash: Dependence of concentration on particle size. *Environ. Sci. Technol.* 8, 1107-1113.
19. Dhamangaonkar, S.N., and Misra, P., 2009. Effect of Azotobacter chroococcum (PGPR) on the Growth of Bamboo (*Bambusa bamboo*) and Maize (*Zea mays*) Plants. *Biofrontier.* 1, 24-31
20. Ehrlich HL., 1990. Geomicrobiology, 2nd edn. Dekker, New York, p 646
21. Elfving, D.C., Bache, C.A., Gutenmann, W.H., Lisk, D.J., 1981. Analysis of crops grown on waste amended soils. *Biocycle.* 12, 44-47.
22. El-Mogazi, D., Lisk D.J., Weinstein, L.H., 1988. A review of physical, chemical and biological properties of fly ash and effects on agricultural ecosystems. *Sci. Tot. Environ.* 74, 1-37.
23. Elseewi, A.A., Page, A.L., 1984. Molybdenum enrichment of plants grown on fly ash treated soils. *J. Environ. Qual.*13, 394-398.
24. Elseewi, A.A., Bingham, F.T., Page A.L., 1978 a. Availability of sulfur in fly ash to plants. *J. Environ. Qual.* 7, 69-73.

25. Evans, A.C., Miller, M.H., 1990. The role of external mycelia network in the effect of soil disturbance upon vesicular arbuscular mycorrhizal colonization in maize. New Phytologist 114, 65-71.
26. Fang, M., Wong, J.W.C., Li, G.X., Wong, M.H., 1998. Changes in biological parameters during co-composting of sewage sludge and coal ash residues. *Bioresour. Technol*. 64 (1), 55-61.
27. Finkelman, R.B., Belkin, H.E., Zhang, B.S., Centeno, J.A., 2000. Arsenic Poisoning caused by residential coal combustion. In Proc.of the 31st *Intl. Geol. Cong.* Guizhou Province, China.
28. Fischer, G.L., Chang D.P.Y., Brummer, M., 1976. Fly ash collected from electrostatic precipitator. Microcrystalline structures and the mystery of the spheres. *Science*. 19, 553.
29. Gaind, S., Gaur, A.C., 1991. Thermotolerant phosphate solubilizing bacteria and their interaction with mung bean. *Plant Soil*. 133, 141-149.
30. Gaind, S., Gaur, A.C., 2002. Impact of fly ash and phosphate solubilising bacteria on soybean productivity. *Bioresour. Technol*. 85, 313-315.
31. Gaind, S., Gaur, A.C., 2003a. Evaluation of fly ash as a carrier for diazotrophs and phosphobacteria. *Bioresour. Technol*. 95, 187-190.
32. Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41, 109-117.
33. Goyal, D., Kaur, K., Garg, R., Vijayan, V., Nanda, S.K., Nioding, A., Khanna, S and Ramamurthy, V., 2002. Industrial fly ash as a soil amendment agent for raising forestry plantations. In 2002 EPD Congress and Fundamental of Advanced Materials for Energy Conversion. Ed. by Patrick R Taylor; TMS Publication. Warrendale, Pennsylvenia, pp. 251-260.
34. Hegazi, NA., Faye, M., Amin, G., Hamza, MA., Abbas, M., Youssef, H., Monib, M., 1998. Diazotrophs assoiciated with non-legumes grown in sandy soil. In: Malik KA, Mirza MS, Ladha JK (eds) Nitrogen fixation with non-legumes. Kulwer, Dordrecht, pp 209–222
35. Hodgson, D.R., Holliday, R., 1966. The agronomic properties of pulverized fuel ash. *Chem. Ind*. 20, 785-790.
36. Jackson, M.L., 1967. Soil Chemical Analysis. Asia Publishing House. Bombay.
37. Jala, S., Goyal, D., 2006. Fly ash as a soil ameliorant for improving crop production-a review. *Bioresour. Technol*. 97, 1136-1147.

38. Jensen, H.L., 1954. The Azotobacteriaceae. *Bact. Rev*. 18, 195-214.
39. Jha. SK., Sinha. AK., *et al.,* 2007. Management of lignite fly ash for improving soil fertilitynand crop productivity. *Environ Manage*. 40 (3), 438-52.
40. Kalra, N., Harit, R.C., Sharma, S.K., 2000. Effect of fly ash incorporation on soil properties of texturally variant soils. *Bioresour. Technol.* 75 (1), 91-93.
41. Kalra, N., Jain, M.C., Joshi, H.C., Choudhary, R., Harit, R.C., Vatsa, B.K., Sharma, S.K., Kumar, V., 1998. Fly ash as a soil conditioner and fertilizer. *Bioresour. Technol.* 64 (3), 163-167.
42. Kalra, N., Jain, M.C., Joshi, H.C., Choudhary, R., Kumar, S., Pathak, H., Sharma, S.K., Kumar, V., Kumar, R., Harit, R.C., Khan, S.A., Hussain, M.Z., 2003. Soil properties and crop productivity as influenced by fly ash incorporation in soil. *Environ. Monit. Assess*. 87 (1), 93-109.
43. Kalra, N., Joshi, H.C., Chaudhary, A., Chaudhary, R., Sharma, S.K., 1997. Impact of fly ash incorporation in soil on germination of crops. *Bioresour. Technol*. 61, 39-41.
44. Katipitiya, S., New, PB., Elmerich, C., Kennedy, IR., 1995. Improved N2 fixation in 2,4 D treated wheat roots associated with *A lipoferum*: studies of colonization using reporter genes. *Soil Biol. Biochem*. 27, 447-452.
45. Kennedy, IR., Choudhury AIMA, KecSkes, ML., 2004. Non-Symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Boil Biochem.* 36(8), 1229-1244
46. Khan, MS., Ahmad, F., Ahmad, I., 2004. Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan. *Turk J Biol.* 29, 29-34
47. Khan, M.R., Singh W.N., 2001. Effects of soil application of fly ash on the fusarial wilt of tomato cultivars. *Intl. J. Pest Mng*. 47 (4), 293-297.
48. Klose, S., Wernecke, K.D., Makeschin, F., 2004. Microbial activities in forest soils exposed to chronic depositions from a lignite power plant. *Soil Biol. Biochem*.36 (12), 1913-1923.
49. Krishan, G., Srivastav, S. K., Kumar, S., Saha, S. K., Dadhwal, V. K., 2009, Quantifying the underestimation of soil organic carbon by the Walkley and Black technique – examples from Himalayan and Central Indian soils, Current Science, pp. 1133-1136
50. Kucey, R.M.N., Janzen, H.H., Leggett, M.E., 1989. Microbiology mediated increases in plant available phosphorous. Advanced Agron. 42, 199-228.

51. Kumar, V., Chandra, A., Singh, G., 2010. Efficacy of fly ash based biofertilizers vs perfected chemical fertilizers in wheat (*Triticum aestivum*). *Ijest*. 2 (7), 31-35
52. Kumar, V., Chauhan, U. K., 2008. Evaluation of fly-ash as a carrier for *Beauveria bassiana* formulation, *An International J. The Ecoscan.* 2 (1), 107-109.
53. Kumar, V., Singh, G., Rai, R., 2005. Fly ash: A Material for another green revolution. *Fly Ash Utililization Programme (FAUP),* TIFAC, DST.
54. Lai, K.M., Ye, D.Y., Wong, J.W.C., 1999. Enzyme activities in a sandy soil amended with sewage sludge and coal fly ash. *Water, Air and Soil Pollut*. 113, 261- 272.
55. Lal, J.K., B. Mishra and A.K. Sarkar, 1996. Effect of fly ash on soil microbial and enzymatic activity. *J. Ind. Soc. Soil Sci*. 44, 77-80.
56. Loper, J.E., Lindow, S.E., 1997. Manual of Environmental Microbiology. C.J.Hurst (Editor-in-chief). G.R. Koudsen, M.J. McInerney, L.D.Stetzenbach, M.V.Walter (Eds.). ASM Press Washington, D.C. pp 482-492.
57. Maiti, S.S., Mukhopadhyay, M., Gupta, S.K., Banerjee, S.K. 1990. Evaluation of fly ash as a useful material in agriculture. *J. Ind. Soc. Soil. Sci*. 38, 342-344.
58. Martens, D.C., 1971. Availability of plant nutrients in fly ash. *Compost Sci.* 12, 15-19.
59. Mattigod, S.V., Rai, D., Eary, L.E., Ainsworth C.C. 1990. Geochemical factors controlling the mobilization of inorganic constituents from fossil fuel combustion residues: I. Review of the major elements. *J. Environ. Qual.* 19, 188-201.
60. Merwin, H.D., Peech, M., 1951. Exchangeability of soil potassium in sand, silt and clay fractions, as influenced by the nature of the complimentary exchangeable cations. *Soil Sci. Soc. Am. Proc*. 15, 125-158.
61. Mittra, B.N., Karmakar, S., Swain, D.K., Ghosh, B.C., 2003. Fly ash- a potential source of soil amendment and a component of integrated plant nutrient supply system.
62. Natusch, D.S.F., Bauer, C.F., Matusiewicz, H., Evans., C.A., Baker, J., Loh, A., Linton, R.W., Hopke, P.K., 1975. Characterization of trace elements in fly ash. 1975 Proc. Int. Conf. On Heavy Metals in Environment. Toronto, Ontario, Canada, 2 (II), 553-575.
63. Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorous by extraction with sodium bicarbonate. USDA Circ., 939**.**
64. Omar, S.A., 1998. The role of rock-phosphate-solubilizing fungi and vascular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14, 211-218.

65. Page, A.L., 1982. Methods of Soil Analysis Part II. Soil Sci. Society of America, Madison, Wisconsin.

66. Page, A.L., Elseewi, A.A., Straughan I.R., 1979. Physical and Chemical properties of fly ash from coal-fired power plants with special reference to environmental impacts. *Residue Rev.* 71, 83-120.

67. Perveen, S., M.S. Khan., Zaidi, A., 2002. Effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiatus*). Ind. *J. Agric. Sci*. 72, 421-423.

68. Petruzzelli, G., Lubrano, L., Cervelli, S., 1987. Heavy metal uptake by wheat seedlings grown in fly ash amended soils. *Water, Air Soil Pollut*. 32, 389-395.

69. Phung, H.T., Lam, H.V., Lund L.J., Page, A.L., 1979. The practice of leaching Boron and salts from fly ash amended soils. *Water, Air and Soil Pollut*. 12, 247-254.

70. Phung, H.T., Lund L.J., Page, A.L., 1978. Potential use of fly ash as a liming material, In D.C. Adriano and I.L. Brisbin (ed.) Environmental chemistry and cycling processes. CONF-760429, U.S. Department of Commerce, *Springfield, VA*. pp. 504-515.

71. Pikovskya, R.I., 1948. Mobilization of phosphorous in soil in connection with the vital activity of some microbial species. *Mikrobiologiya*. 17, 362-370.

72. Pichtel, J.R., Hayes, J.M., 1990. Influence of fly ash on soil microbial activity and populations. *J. Environ. Qual*. 19, 593-597.

73. Plank, C.O., Martens D.C., 1974. Boron availability as influenced by application of fly ash to soil. *Soil Sci. Soc. Am. Proc*. 38, 974-977.

74. Raja, A.R., Shah, K.H., Aslam, M., Memon, M.Y., 2002. Response of phosphobacterial and mycorrhizal inoculation in wheat. *Asian J. Plant Sci*. 1, 322-323.

75. Rautaray, S.K., Ghosh, B.C., Mittra, B.N., 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. *Bioresour. Technol*. 90 (93), 275-283.

76. Reynolds, K.A., Kruger, R.A., Rethman, N.F.G., 1999. The manufacture and evaluation of an artificial soil prepared from fly ash and sewage sludge. Proc. Intl. Ash Utiliz. Sympos. Lexington, Kentucky, U.S.A. pp. 378-385.

77. Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv.* 17, 319–339

78. Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, T., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil.* 287,15–21

79. Saxena, M., Asokan, P., Murali, S., 2005. Pilot scale demonstration study on impact of fly ash on soil fertility and crop yield. International Conference on Energy Environment and Disasters- INCEED2005, Charlotte, NC, U.S.A, July 24 to 30.
80. Schutter, M.E., Fuhrmann, J.J., 2001. Soil microbial community responses to fly ash amendment as revealed by analyses of whole soils and bacterial isolates. *Soil Biol. Biochem.* 33, 1947-1958.
81. Sharma, B.M., Aggarwal, R.K., Kumar, P., 1990. Water retention and nutrient availability in a fly ash amended desert sandy soil: A study in vitro. *Arid Soil Res.* Rehab. 4, 53-58.
82. Sikka, R., Kansal, B.D., 1994. Characterization of thermal power-plant fly ash for agronomic purposes and to identify pollution hazards. *Bioresour. Technol*. 50, 269-273.
83. Singh, S.N., Kulshreshtha, K., Ahmad, K.J., 1997. Impact of fly ash soil amendment on seed germination, seedling growth and metal composition of *Vicia faba* L. *Ecol. Engg*. 9, 203-208.
84. Slauch, J.M., Silhavy, T.J., 1991. Genetic fusions as experimental tools. Methods Enzymol. 204, 213-248.
85. Straughan, I., Elseewi A.A., Page, A.L., 1978. Mobilization of selected trace elements in residues from coal combustion with special reference to fly ash. In Trace Substances in Environmental Health- XII. A Symposium, (ed.) D.D. Hemphill. University of Missouri, Columbia, pp. 389-402.
86. Subbiah, B.V., Asija, G.L., 1956. A rapid procedure for the determination of available nitrogen in soils. *Curr. Sci.* 25, 259-260.
87. Townsend, W.N., Gillham, E.W.F., 1975. Pulverized fuel ash as a medium for plant growth. In M.J. Chadwick and G.T.Goodman (ed.). The ecology of resource degradation and renewal. Blackwell Scientific, Oxford, pp. 287-304.
88. Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant soil*, 255, 571-586.
89. Vollmer, A.T., Turner, F.B., Straughan, I.R., Lyons, C.L., 1982. Effects of coal precipitator ash on germination and early growth of desert annuals. *Environ. Exp. Bot.* 22, 409-413.
90. Walkley, A., Black, C.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29-38.

91. Wallace, A., Alexander, G.V., Soufi, S.M., Mueller, R.T., 1980. Micronutrient supplying power of pyrite and fly ash. *J. Plant Nutr*. 2, 147-153.
92. Wong, M.H., Wong, J.W.C., 1989. Germination and seedling growth of vegetable crops in fly ash amended soils. *Agric. Ecosyst. Env*. 26, 23-25.
93. Yanni, YG., El-Fattah, FKA., 1999. Towards integrated biofertilization management with free living and associative dinitrogen fixers for enhancing rice performance in the Nile delta. *Symbiosis* 27, 319–331
94. Yunusa I.A.M, Eamus, D., De Silva, D.L., Murray, B.R., Burchett, M.D., Skilbeck, G.C and Heidrich, C., 2005. Prospects for Coal-ash in the management of Australian Soils. World of Coal Ash: Sci., Applications & Sustainability, Lexington, USA, April 11 –15, http://www.Fly ash.info/2005/110yun.pdf.
95. Zaidi, A., Khan, M. S., 2007. Stimulatory effect of dual inoculation with phosphate solubilising microorganisms and arbuscular mycorrhizal fungus on chickpea. *Aust. J. Exp. Agric.* 47, 1016-1022.
96. Zhang, G.Y., Dou, Z., Toth J.D., Ferguson, J., 2004. Use of fly ash as environmental and agronomic amendments. *Environ. Geochem. Health*. 26, 129-134.

Plates

Control Soil

Soil+5% fly ash

Soil +10% fly ash

Control Soil   Soil + MA

Soil + 5% FA   Soil + 5% FA + MA

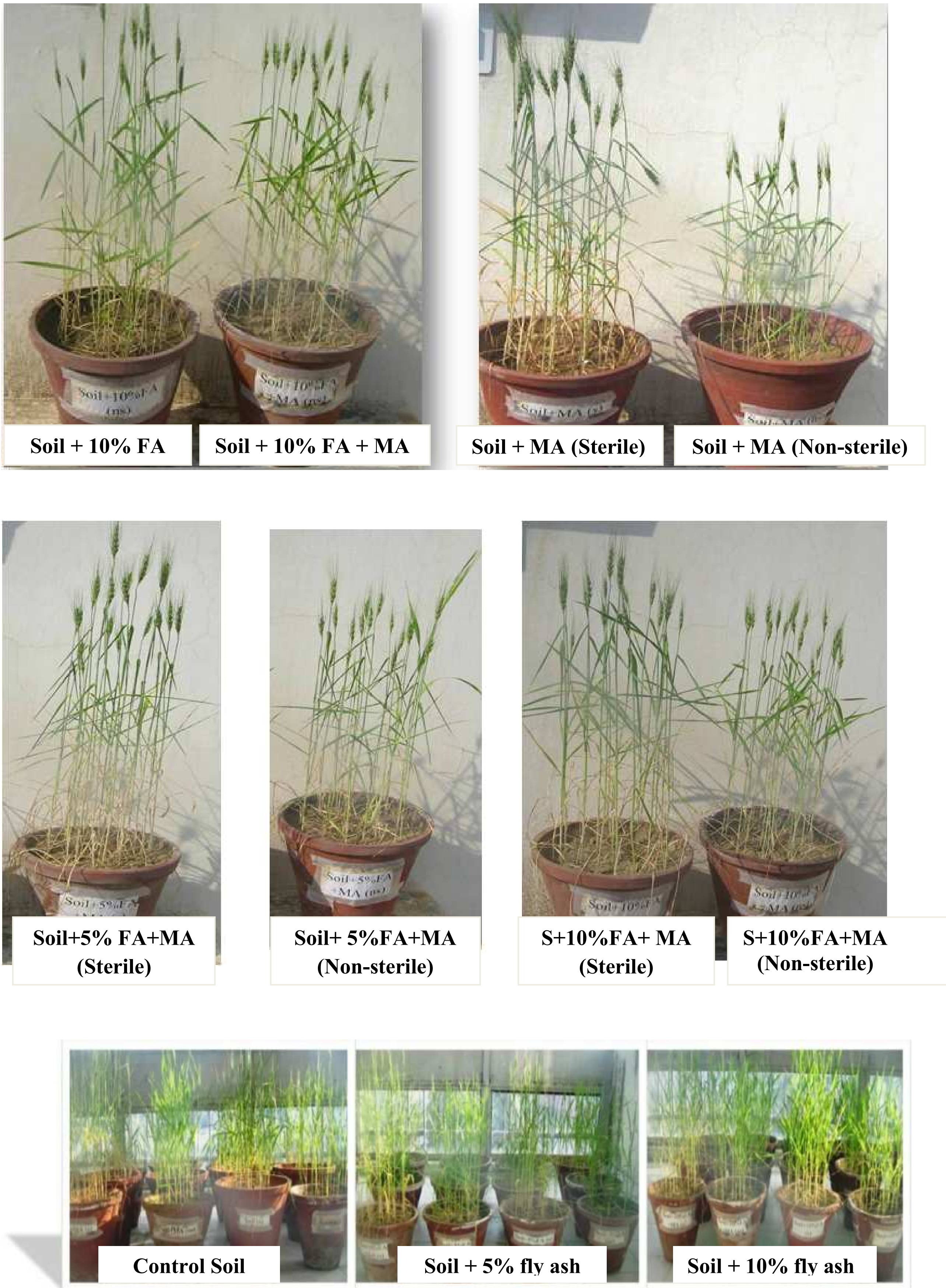


Plate 1: Growth of wheat crops in 0%, 5%, 10% fly ash amended soil after 60 days of sowing


Control Soil


Soil + 5% Fly ash


Soil + 10% fly ash


Control soil Soil + MA


Soil + 5% FA


Soil+5%FA+MA


Soil+10%FA


Soil+10%FA+MA


Soil MA (Sterile) Soil+MA (Non-Sterile)

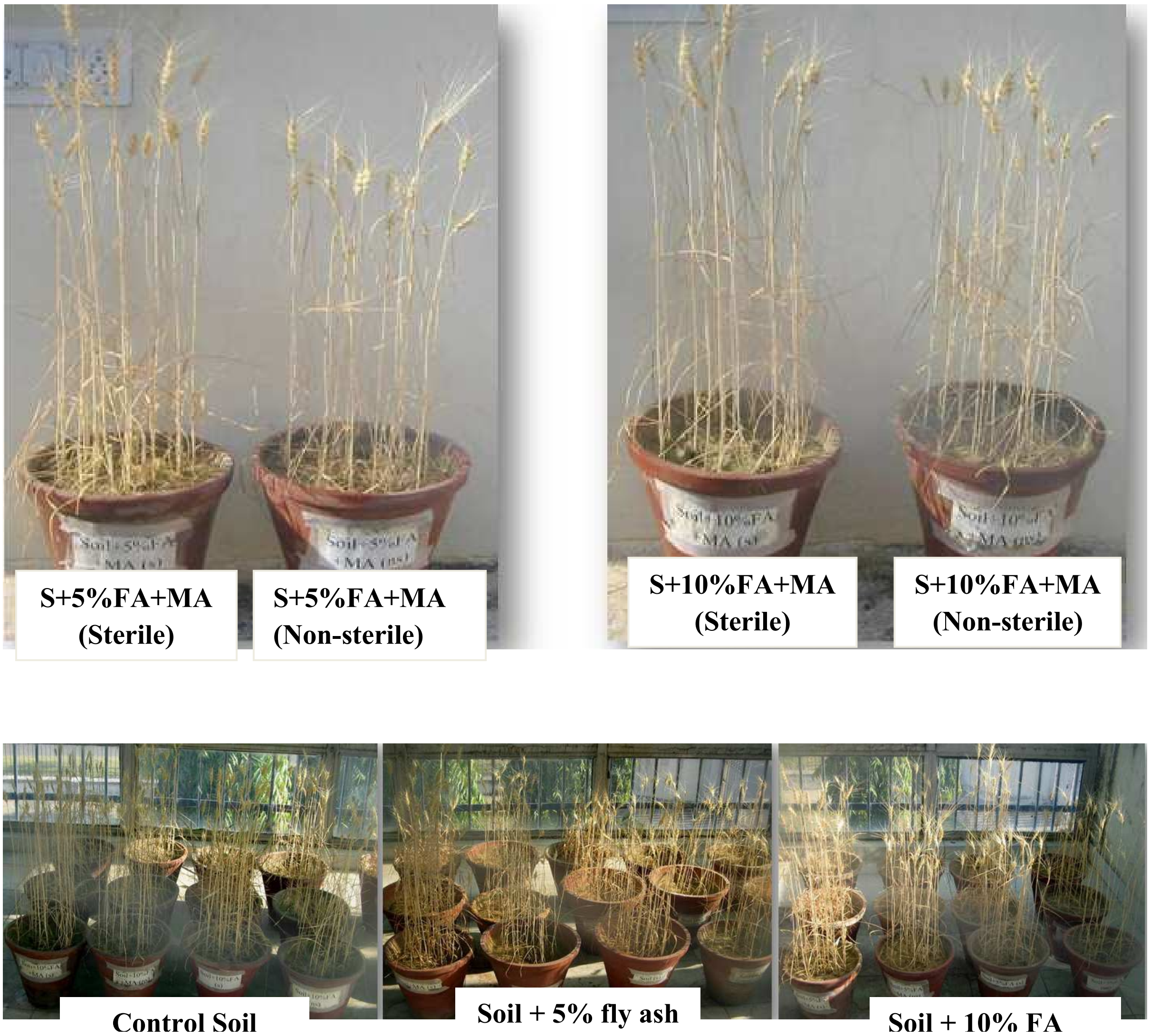


Plate 2: Growth of wheat crops in 0%, 5%, 10% fly ash amended soil at the time of harvesting

Plate 3: Wheat germination in Pot experiment

Plate 4: Wheat germination in petri-plate experiment at the time of sowing

Plates 5: Wheat germination after 2 days of sowing showing radical and plumule

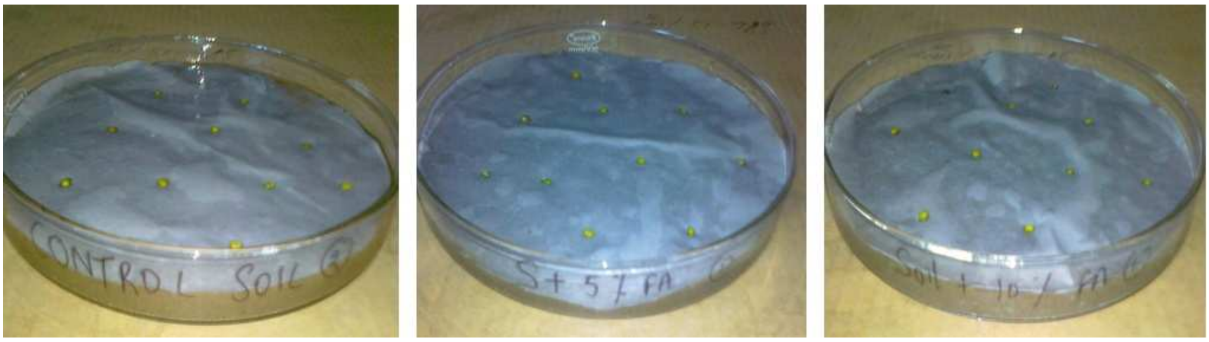


Plate 6: *Mung bean* germination in petri-plate experiment at the time of sowing

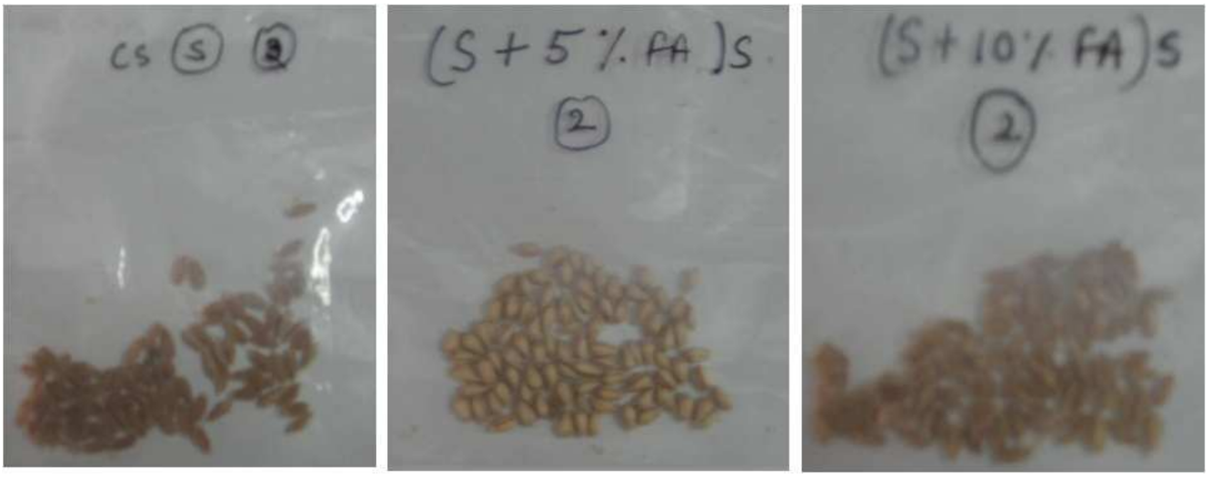


Plate 7: Grain yield in wheat nursery soil amended with 5% and 10% FA in sterile system after harvesting

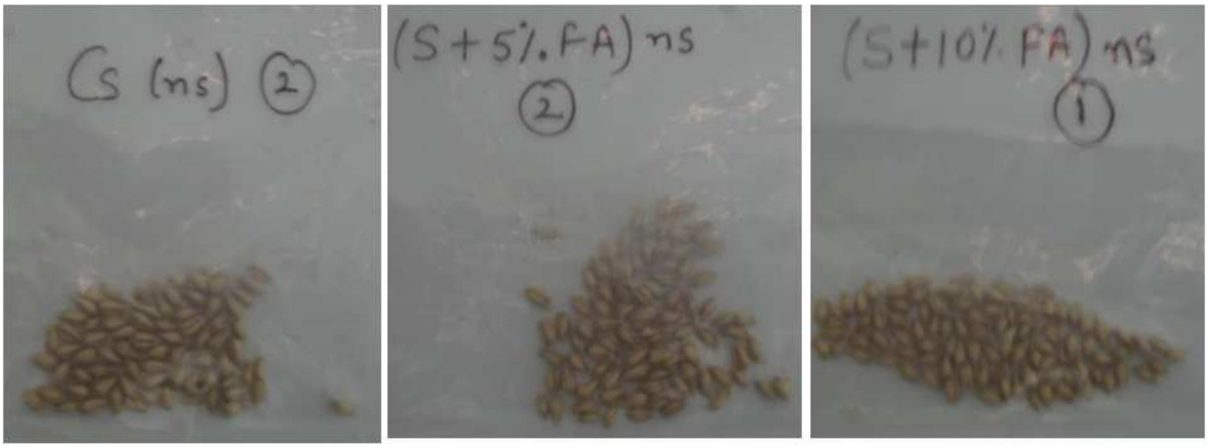


Plate 8: Grain yield in wheat nursery soil amended with 5% and 10% FA in non-sterile system after harvesting

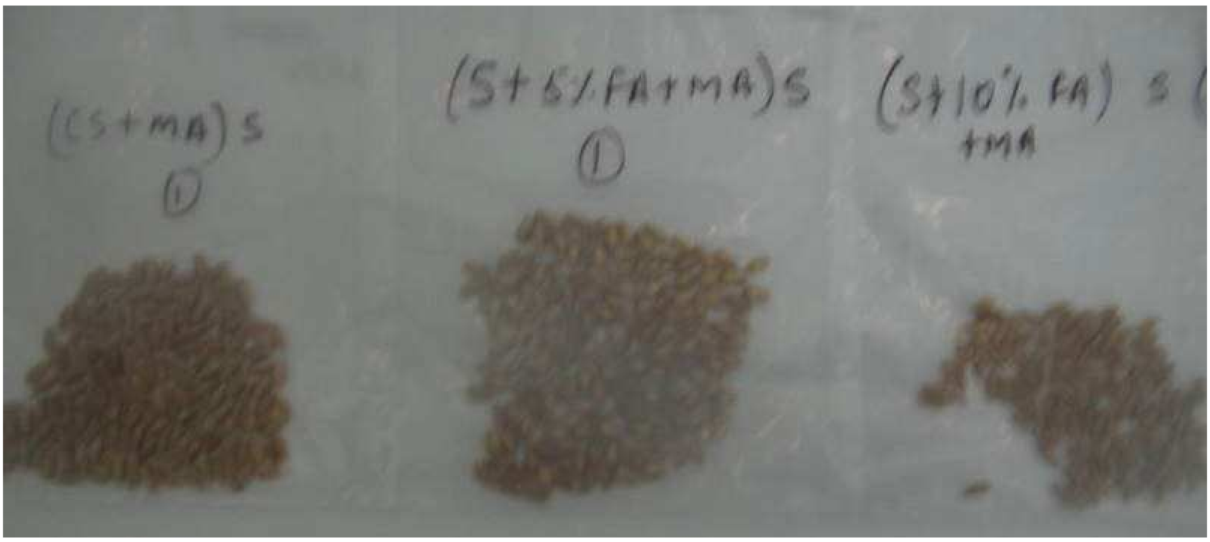


Plate 9: Grain yield in wheat nursery soil amended with 5% and 10% FA and MA in sterile system after harvesting

Plate 10: Grain yield in wheat nursery soil amended with 5% and 10% FA and MA in non-sterile system after harvesting

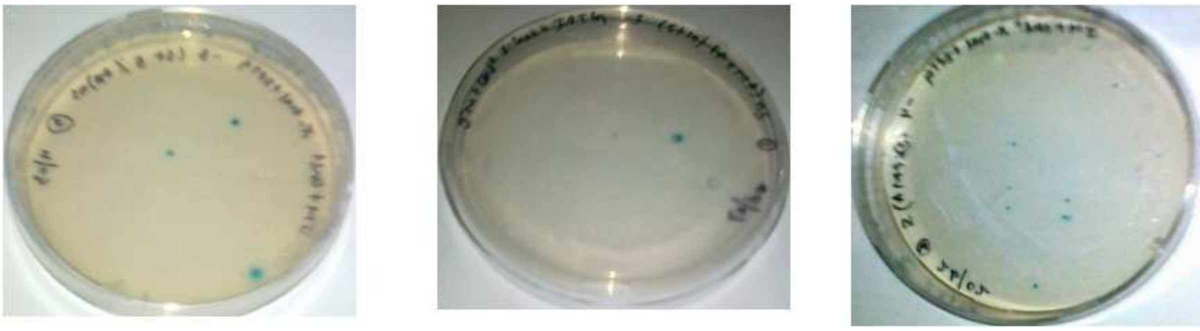


Plate 11: Enumeration of bacteria on Chl^+ + X-gal + IPTG containing Jensens medium in wheat nursery soil at the time of harvesting.

Plate 12: Enumeration of bacteria on Chl^+ + X-gal + IPTG containing Pikovskaya medium in wheat nursery soil at the time of harvesting.

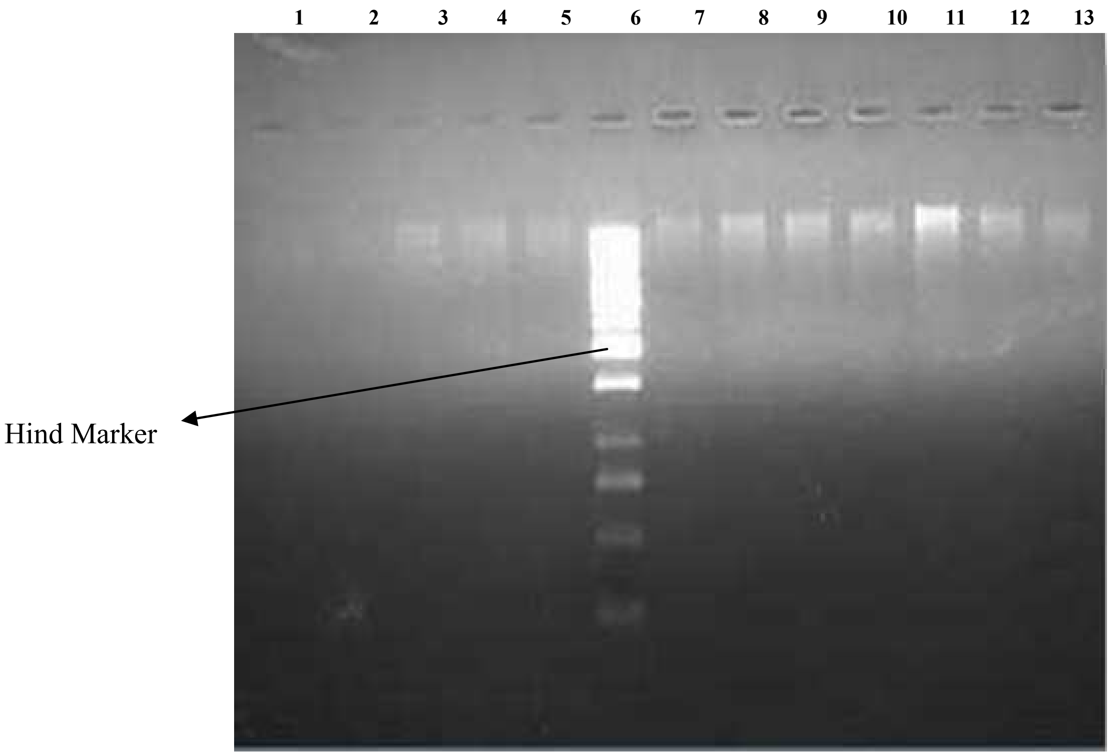


Plate 13: Gel electrophoretogram of total DNA isolation from soil

Lane 1: Control soil (NS) (1)

Lane 2: Control soil (NS) (2)

Lane 3: Soil + MA (NS) (1)

Lane 4: Soil + MA (NS) (2)

Lane 5: Soil + 5% fly ash (NS) (1)

Lane 6: Hind Marker

Lane 7: Soil + 5% fly ash (NS) (2)

Lane 8: Soil + 5% fly ash + MA (NS) (1)

Lane 9: Soil + 5% fly ash + MA (NS) (2)

Lane 10: Soil + 10% fly ash (NS) (1)

Lane 11: Soil + 10% fly ash (NS) (2)

Lane 12: Soil + 10% fly ash + MA (NS) (1)

Lane 13: Soil + 10% fly ash + MA (NS) (2)