# *IMPACT ANALYSIS OF FLY ASH ON GROWTH PERFORMANCE OF SELECTED LEGUMES WITH SPECIAL REFERENCE TO ROOT NODULE BACTERIA*

चयनित् लेग्यूमस् के वृद्धि प्रदर्शन पर फ्लाई ऐश के प्रभावों का मूल ग्रंथियों जीवाणु के विशेष संदर्भ में विश्लेषण

## A THESIS

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by

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# CERTIFICATE

It is certified that the

1. Thesis entitled **" *IMPACT ANALYSIS OF FLY ASH ON GROWTH PERFORMANCE OF SELECTED LEGUMES WITH SPECIAL REFERENCE TO ROOT NODULE BACTERIA"*** submitted by ***Mridula Khandelwal*** is best of my knowledge is an original piece of work carried out by the candidate under my supervision.

2. Literary presentation is satisfactory and the thesis is in a form suitable for publication.

3. Work evinces the capacity of the candidate for critical examination and independent judgment.

4. Candidate has put in at least 200 days of attendance every year.

Date:

Place: **(Dr. Neerja Shrivastava)**

# “A Word of Gratitude”

Though this thesis bears my name the main spirit and dynamic force behind this work is the brilliant genius of my esteemed guide.

Dr. Neerja Shrivastava
Lecturer, Department of Botany,
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I consider myself to be extremely lucky having worked under such a perfect and dynamic teacher. It is only because of her unfailing interest, constant inspiration, simplicity, wisdom, understanding, sympathy, logical approach and stimulating criticism that provided me impetus of this work.

I shall ever remain grateful for the encouragement which my able and efficient guide gave me at every stage of my work. Words are poor medium for expression of thanks to my respected guide.

To her, I express much more than a mere sense of gratitude I shall always gratefully cherish that sweet memory of the tender guard ship for years to come. it is proud privileged to work under such a delightful and pleasant personality

Regards,

(Mridula Khandelwal)
M.Sc., M. Phil.

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*Date:*

*Place: Kota* ***Mrs. Mridula Khandelwal***

# LIST OF ABBREVIATIONS

- BP - Between Paper
- Db - Bulk density
- Dp - Particle density
- EC - Electrical Conductivity
- FA - Flyash
- FYM - Farmyard Manure
- KTPS - Kota Thermal Power Station
- MR - Methyl Red
- MTCC - Microbial Type Culture Collection
- RSSC - Rajasthan State Seed Corporation
- STL - Seed Testing Laboratory
- VAM - Vesicular Arbuscular Mycorrhiza
- VP - Voges Proskauer
- WHC - Water Holding Capacity
- YEMA - Yeast Extract Mannital Agar

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CHAPTER – 1

# INTRODUCTION

# INTRODUCTION

During the process of cultural evolution natural resources have been brutally exploited by man and the environment of this planet is contaminated by rapid growth of urbanization, faster means of transportation, unplanned industrialization and agricultural practices. These activities have compelled living being to face the serious problem of environmental pollution which has come up as dark side of the so called progress putting a big sign of interrogation before the existence of natural ecosystem. In the last decades years increasing industrialization and rapid rate of population growth has caused severe environmental crisis by polluting soil, water and air where soil pollution is confined to a particular area, water pollution can be bound in national and international limits but as air cannot be divisible in any type of boundary.

Soil is an integral part of land resources. It is an irreplaceable and non-renewable component of terrestrial ecosystems. Globally soil and their biota make up a natural asset that needs to be protected. Soil plays a significant role in global environmental changes. Processes in soil often occur very slowly therefore difficult to trace. About 17% of world's soils show clear signs of degradation caused by humans. Country like India has suffered enormous pressures on their land resources. These regions have shown some of the world's highest population increases, and have suffered serious land degradation. The widespread industrialization and increasing consumption have changed the complexion of soil. Thus the soil is getting heavily polluted day by day by toxic materials and dangerous microorganisms which enter the air, water and the food chain. For all this, man is the original and basic pollutant responsible for pollution hazards and toxic effects. The pollutants are directly discharged in soils of surrounding areas and these effluents affect the quality of soil. Contamination of the soil causes to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. In the

race of economic growth soil of Kota city is also pay lot off and the soil quality of Kota city is continuously degraded due to different soil pollutants. These soil pollutants such as flyash (FA) come through different industrial activities which take place in Kota city including thermal power station which is coal based. The other pollutants are automobile waste, pesticides and sewage etc. It is observed that due to rapid growth, the flora of Kota City is also affected and it seems that loss of plant diversity is may be due to loss of fertility of soil, so the health of soil and plants of Kota region are badly affected by flyash. It was also found in primary study that soil pollutant also affects different soil bacteria which play important role in maintaining soil fertility.

For the generation of energy coal is used in Kota Thermal Power Station (KTPS) and by burning of fossil fuels as coal release huge amount of carbon dioxide content in the atmosphere, coal is a very diverse, safe, secure and abundant energy source worldwide, it is a complex heterogeneous material containing both organic and inorganic elements. India is the world's third largest coal producer (after China and United states) and the power generation in the country is predominantly coal based. Thermal power stations spread all over the country. Till now we have added quite a few thermal power plants, super thermal power plants and many more are likely to be commissioned during the coming years. In India about 76% of the coal consumption is for power generation and about 60% of India's installed capacity and 70% of power generation is coal based. The government's endeavour to add more to accomplish its dream of "Power for all" is increase the coal consumption further. Although coal is a competitive fuel for the generation of electricity, it is highly polluting fuel being one of the primary sources of anthropogenic greenhouse gas emissions spewing massive amounts of carbon di-oxide and large amount of Sox, Nox, particulates and other toxins. The low quality indigenous coal further exacerbates the pollution by generation of excessive ash, all of which are hazardous to agricultural crops and human health. Every year Indian Thermal Power Station including KTPS, produce more than 100 million tons of fly ash, which is expected to,

reaches 175 million tons in near future. In India pollution problems are still localized in certain pockets, but exert a potential threat to plants. We can understand that at what extend this flyash affect the macro and micro bacteria of soil. Kota Thermal Power Station (KTPS) in Rajasthan's first major coal based power station. Its capacity is 1045 MW. This was envisaged to create the State's own thermal generating capacity and to meet the growing power demand. Kota thermal power station is located on the left bank of river Chambal in Rajasthan's principal industrial city, Kota. Infrastructural facilities like adequate water availability from Kota Barrage, nearness to broad gauge railway line, load centre and existing transmission system for evacuation of power were some of important factors to select this location.

Kota thermal power plants where coal is used as the primary source of energy generate large number of particulate and gaseous pollutants. Flyash is a by-product from thermal power station causing pollution to the local area of power plant as well as flyash dumping station and transporting tracts, on the other hand air pollutants directly emitted through chimneys of thermal power station due to the combustion of coal also spread in the local environment, emission from thermal power station adversely affect the plant species growing near the source. Thirdly the road transport vehicles like trucks, tankers, trailers involve in transportation of flyash to the industries as cement industry, also contribute in enhancement of pollution level through automobile emission by increasing level of particulate matter and gaseous pollution in the environment. These pollutants affect the local vegetation directly or indirectly. As the cumulative effect of all these factors the biodiversity of that particular area is comparatively less and confined to limited species (except the cultivated plants). At lower dose of pollutants, plant community may not show any symptoms, still there can be substantial production losses.

Air borne fly ash pollutes the atmosphere and causes ailments such as Irritation to skin and cause dermatitis from mechanical abrasion

or alkaline composition. When inhaled irritation to nose, throat and respiratory tract causing coughing and sneezing. In long term exposure repeated inhalation of dust containing crystalline silica can cause bronchitis, silicosis and lung cancer. It may also increase the risk of scleroderma and inflammation of small airways of lungs. Abrasive nature of ash affects structures and exposed metals.

A close look at the process of coal based power generation brings out the fact that a mass transfer is being made from the lithosphere (of coal) in to the biosphere (ash). This mass transfer is making a continuous and irreversible impact on the ecology of biosphere and members of food chain at various levels. The coal used in Indian thermal power plants, have generally high ash content (45-55%) and low fixed carbon content. In addition to the purely organic substance, they also contain large amounts of inorganic compounds and trace elements. These trace elements are generally associated either with organic fraction or inorganic fraction or with both fractions. Flyash contains a high concentration of toxic heavy metals, along with low nitrogen and phosphorous content and pH ranged from 4.5-12.0 depending on the S-content of parental coal. The chemical and physical properties of flyash are determined by several variables such as coal source, degree of pulverization, design of boiler unit, loading and firing conditions, type of emission control devices, handling and storage methods. The physical, geotechnical and chemical parameters to characterize fly ash are the same as those for natural soils, Chemical composition (%/w) of flyash (Source-KTPS, Kota) represent $SiO_2$ (42-62%), $Al_2O_3$(19-28), $Fe_2O_2$( 4-20), CaO(0.6-4.0), $K_2O$(0.5-1.8), $P_2O_5$(Traces), $TiO_2$(Traces), $Na_2O$(0.6), $SO_3$(0.09-0.15), MgO(0.2-2.0), MnO(0.1-0.5). The components of flyash vary considerably, but all types of flyash include substantial amount of silicon dioxide ($SiO_2$) and calcium oxide (CaO), both being endemic ingredients in many coal bearing rock strata.

KTPS a power generation project of such a magnitude and based on coal would lead to a number of impacts on environment via,

atmospheric pollution by gaseous emission, solid waste pollution by way of ash dumping, water pollution by way of waste effluents and ash in the nearby water ways, impacts on agriculture due to ash disposal or sanitation is often the direct cause of on outbreak of water borne disease due to inevitable effluents discharge. The present ash disposal systems which is in use are causing serious disposal and ecological problems on account of its potential for contamination of surface and ground water and surrounding air due to escape in atmosphere causing serious problems to human and plant life. The coal ash pollutes the air as well as water too and requires a huge land area for its disposal. Chemical reaction takes place, when gases like $SO_2$, NOx and hydrocarbons are released into the environment. It degenerates the thriving of flora and fauna in the adjoining area. Major sources of pollution which can occur from ash disposal are -

1- Fugitive dust emission due to wind erosion in dry areas of ash pond.
2- Infiltration of ash leachates from the ponds into ground water.
3- Discharge of fine ash particles from ash pond effluent as suspended solids.

The impact of coal residue i.e. flyash on health and environmental consequences has been reviewed extensively, thus ecologically safer disposal of this huge amount of flyash is of significant concern, certain approaches like utilization of flyash in construction of bricks, in making cement, in road construction, ceramic & refractory products used as mine filler etc. have also been tried but have not yet proved feasible. According to one latest report, nearly 65000 acres of land is presently occupied by ash pond.

Impact of flyash of KTPS on vegetation of Kota shows negative effect according to air pollution but it also shows positive effect in form of ash utilization plan and utilization of flyash in agriculture. It works as a pollutant until it is not present in an appropriate amount. Being fine in size, flyash application improves soil texture of coarse texture soils, increase

soil property, and water holding capacity, available water capacity, water infiltration rate and overall drainage. The agronomic potential as limiting agent to acid soils, alternative micronutrients fertilizers and also a physical conditioner for soils. The application of flyash to soil affects the content of both macro and microelements in the growing crop plants depending upon application rates, stage of weathering and composition of flyash material.

Reclamation of mine spoil, wasteland management and agriculture have also emerged as major bulk utilization area for flyash in country. Flyash can be used for reclaiming the problem soil and enhance the crop productivity depending upon the nature of soil and flyash. It affects physical, chemical and biological properties of soil and has impact on the available macro and micronutrients of plants. Flyash can also be utilized in agriculture, because it helps in neutralizing the problem soil (Acidic/Alkaline) and fly ash application also improving the various physical properties and makes it suitable for cultivation that can increases the yield of crops, others advantages like increase in water holding capacity and lesser pest infection when compared to control was observed. Yield of crops increased to the tune of 20-30% in mine spoil and 40-60% in lateritic soil on application of different proportion of fly ash over the control and no adverse effect in respect of heavy/ toxic metal carry over by succeeding crops was observed due to flyash application even at highest dose were obtained by previous studies.

Kota region has good plant diversity including crop plants. Rajasthan is the one major legumes producing state. In Rajasthan Kota district has major contribution in total production of legumes. The three subfamilies of Fabaceae (Caesalpinioideae, Mimosoideae, and Papilionoideae) contain species that form root nodules. In Mimosoideae, all genera appear to nodulated, but not all species do, Papilionoideae has the highest proportion of nodulating species, and produce all known types of nodules. In Caesalpinioideae, nodulation show by members of all tribes. Along with human diet leguminous plants are also of crucial

importance as animal feed. Alfalfa and Methi are grown over extensive area as forage crops for grazing or as dry hay, and they furnish not only high quality protein but also a variety of biologically active molecules such as vitamins, minerals and other nutrients. Surveys regarding the crops indicate that during 70-90 decades farmers of this area were grown leguminous plants and got good production but now a day's picture is changed. According to official data it is indicate that production of leguminous crop in Kota region is decreases, this may be due to poor soil quality, and flyash is the one of the reason for it. Our aim to contribute in this area by studying impact of flyash on soil, vegetation and on legume crops so we have selected leguminous plants for the present study.

The present study is aimed to find out the resistant species in flyash affected area, tolerant species may be used as pollution indicators and bio-monitor for monitoring the quantitative and qualitative level of pollutants up to level of root nodule bacteria. In the light of above facts present research work taken to study the impact analysis of flyash on growth performance of selected legumes with special reference to root nodule bacteria.

**Present study is broadly divided in to five parts-**

- The first part deals with floristic survey of study site that is Kota Thermal Power Plant (KTPS), collection of flyash sample and soil sample, their physiochemical study and selection of four legumes for further study.
- The second part deals with seed germination test of flyash treated seeds (selected legumes) to find out appropriate concentrations for pot experiments.
- Third includes eco-morphological and biochemical study of selected legumes grown in different soil amended with flyash.
- Fourth part deals with nodulation study in selected plants.

- Fifth part deal with comparative study by isolation purification and culture of *Rhizobium* bacteria from control and flyash amendments (25%) plants, along with biochemical testing to analyze impact of flyash on different *Rhizobium* strains.

Present research is performed to know the impact of flyash on growth performance of following four selected legumes that are well known and highly consumed in this region as food or as fodder. Selected legumes are *Cyamopsis tetragonoloba* L*, Glycine max* L, *Medicago sativa,* L*, Trigonella foenum-graecum* L. Our attempt is to find out how flyash has altered the character of soil and impact of such alters soil character on growth performance of selected plants as well as on root nodule bacteria and ultimately find out the best suitable concentration of flyash as soil amenders for improving the eco-morphological characters and productivity of leguminous plant.

**Detail Introduction of Selected Legume Plants-**

**A.** ***Cyamopsis tetragonoloba*** L. (RMG1002), member of family Fabaceae, also known as Cluster bean occupies very important place among commercially utilized crops in India. It is a rich source of high quality galactomanan gum and protein rich guar meal which is in great demand in the world market. Cluster bean is compatible for nodulation with both fast growing *Rhizobium* and slow growing *Bradyrhizobium* species. It is an annual, bushy, upright herb reaching up to 3 m in height. It has a vigorous root system. The young tender pods can be eaten as vegetables and the seeds are used as livestock feed. The plant is also grown as a green manure or used as fodder. Seed flour is used to improve the strength of paper and stamps or in textile sizing and as a thickener in ice creams and salad dressings.

**B.** ***Glycine max*** L (JS 335) member of family Fabaceae, also known as Soya bean. The bean pods and seeds are a source of oil and protein and are good source of vitamin B. Fermented pods are

used to make soya sauce and other sauces and soya milk. Inoculation with nitrogen-fixing bacteria is desirable, the strain *Rhizobium japonicum* being specific to soya bean. A bushy herbaceous legume reaching a height of 20-180 cm. Oil from the seeds used as wetting and stabilizing agent in food, cosmetics, and pharmaceutical products. The oil is also used in paints, linoleum, oilcloths, printing inks, soaps, insecticides, disinfectants and as a bio-fuel. After oil extraction, the soya meal can be used for manufacturing of fiber, adhesives, and textiles.

**C.** ***Medicago sativa*** L (T9) it belongs to family Fabaceae, also known as alfalfa. It is one of the highest yielding forage legumes. It is grow as a cover crop to reduce erosion. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. It is inoculated with an effective strain of *Rhizobium meliloti*. It is erect, much-branched, perennial plant (30-90 cm) with alternate trifoliate leaves. It is deep-rooted, 2-4 m, or more in well-drained soils. Inflorescences are compact racemes up to 40 mm, borne in axils of upper leaves; purple florets, 8 mm, typically papilionacious. It is mainly grown as a fodder crop. Chlorophyll is extracted from the leaves and the flowers are a source of honey.

**D.** ***Trigonella foenum-graecum*** L (SWATI1) It belongs to family Fabaceae, also known as Fenugreek or methi, It is a useful agro forestry species. The plant and seeds have a characteristic strong odour. An erect, smooth, herbaceous plant that can grow up to 40-80 cm tall. The plant and seeds have a characteristic strong odour and used as condiments and as flavouring agent in artificial maple syrup, cheese and curries. Oil can be extracted from the seeds and used to flavour butterscotch, cheese, licorice, pickles, rum, syrup and vanilla. This oil has potential in the perfume and cosmetic industries. The seeds also contain the drug diospenin, used in the synthesis of hormones. Seed husks are a source of mucilage, oil,

sapogenin and protein. Residue is used as manure and fuel. In India, plants are grown for forage.

Legume plant posses a unique ability to establish symbiosis with nitrogen fixing bacteria of the family Rhizobiaceae. Rhizobia are the name given to the group of genera of alpha-proteobactiria (family Rhizobiaceae) which includes all of the nitrogen fixing species that produce nodules with legumes. This symbiotic relationship reduces the requirements for nitrogenous fertilizers during the growth of leguminous crops and also enriches soil with nitrogen. Rhizobia and their legume host must recognize each other for nodulation to begin. Though legume rhizobium symbiosis has been extensively studied in many crops but no systematic work has been done to exploit the nodulation and nitrogen fixing ability of these legume crops for increasing the production and impact of flyash on germination, ecomorphological and biochemical characters of selected legume plants and also the morphological, cultural and biochemical characterization of rhizobium.

One of the major defining characteristics between the genera of rhizobia is the rate at which they grow (whether they are fast or slow growing). Those that are fast growing synthesize acidic products those that are slow growing synthesize alkaline products. These different nitrogen products are also specific to nodule shape. Nodules on many perennial legumes, such as alfalfa and clover, are finger like in shape. Mature nodules may actually resemble a hand with a centre mass (palm) and protruding portions (fingers), although the entire nodule is generally less then ½ inch in diameter.

Among plant-microbe interactions, legume–rhizobium interactions are unique because they supply 80-90% of total nitrogen requirement of legumes. It involves a complex interaction among host, microbe and environment. As a result, the plant is infected by rhizobium, develop different types of nodules. Root can be infected via the root hairs, damaged epidermal tissues, or intact epidermis. Although symbiotic nitrogen fixation by legumes is generally dominant source of nitrogen

input in soil for imparting fertility but research indicates that the working capacity of nodule are affected by various factors including the pollutant in soil, which pose a severe yield constraint in obtaining plant growth and development.

Pink or red nodules should predominate on a legume in the middle of the growing season. If grey or green nodules predominate, little nitrogen fixation is occurring as a result of an inefficient rhizobium strain it may be due to poor plant nutrition, pod filling or other plant stress. Any stress that reduces plant activity will reduce nitrogen fixation. It indicates that if there is any alteration in character of soil, the root nodules may affected positively or negatively. Factors like temperature and water may not be under the farmer control. But nutrition stress especially phosphorus, potassium, zinc, iron, molybdenum and cobalt can be control by improving soil quality. When a nutritional stress is corrected, the legume responds directly to the nutrient and indirectly to the increased nitrogen nutrition resulting from enhanced nitrogen fixation. Poor nitrogen fixation in the field can easily corrected by inoculation, fertilization, irrigation or other management practices. It is very important for good growth of plant (leguminous) the soil should not be contaminated.

Rhizobium is able to enter into symbiotic relationship with legumes. They fix atmosphere nitrogen and thus not only increase the production of the inoculated crops, but also leave a fair amount of nitrogen in the soil, which benefits the subsequent crop. Following groups of rhizobium have been recognized for inoculating legumes in India. *R leguminosarun, R.melilotti, R.trifoli, R.phaseoli, R.lupinli, R.japonicious* etc.

Rhizobium as a bacteria are a tiny and lower most components of any food chain, but these tiny members have their own importance, without these rhizobium bacteria we can’t imagine legumes and nitrogen cycle in atmosphere. So it is very important especially in area where large amount of soil and air polluted, by various pollutants such as flyash, which is directly dumped in soil. Direct and indirect dumping of flyash may alter

the characters of leguminous plants and bacteria present in nodules of those plants.

The present investigation is an attempt to find out effect of flyash in different concentration on leguminous plants and try to find out the best suitable concentration of flyash in soil amendment for improving the ecomorphological characters and productivity of leguminous plants. Along with morphological aspects, some biochemical analysis (primary metabolites) is also performed, by using 'Petri plate experiment' as well as 'Pot experiments'. Our efforts are to study the impact of flyash on root nodules and study of microbiology and biochemical analysis of selected legume rhizobia by isolating inoculating and culturing the bacteria. Hence in present study, plant species growing wild as well as cultivated in our area were screened for effective rhizobial strain by comparing the data on nodulation, morphological cultural and biochemical characteristic of the strains.

CHAPTER – 2

# STUDY AREA

# STUDY AREA

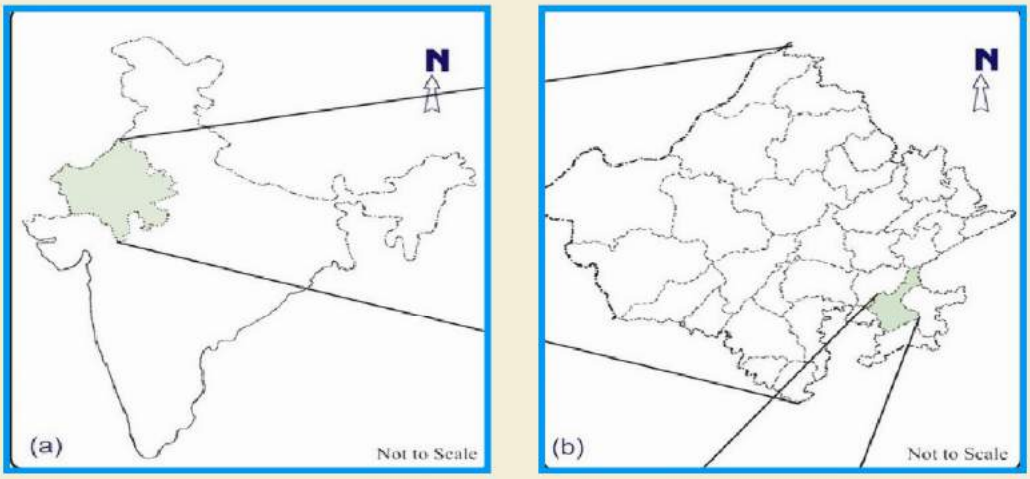
Kota District (South Eastern Rajasthan or Hadoti Plateau) is situated at the edge of Malwa plateau at 23˚45' to 25˚3' North latitudes and 75˚09' to 77˚27' East longitudes in south eastern corner of Rajasthan state (Plate-1). Its total area is 24156.6 square kilometers and from administrative point of view it is known as Kota division, one of the six major administrative units of Rajasthan state. The average surface elevation of land surface is 300 meter above Mean Sea Level (M.S.L), its shape is roughly quadrangular with irregular upper and lower arms some what extending on opposite sides. The district is more or less like a cross in shape, being 144 kilometer in width. It is bounded on the North and North-West by Sawai Madhopur, Tonk and Baran districts, the Chambal River separating these from Kota district and forming the natural boundary. It is well watered, drained by Chambal River and its tributaries flowing in North and North easterly directions.

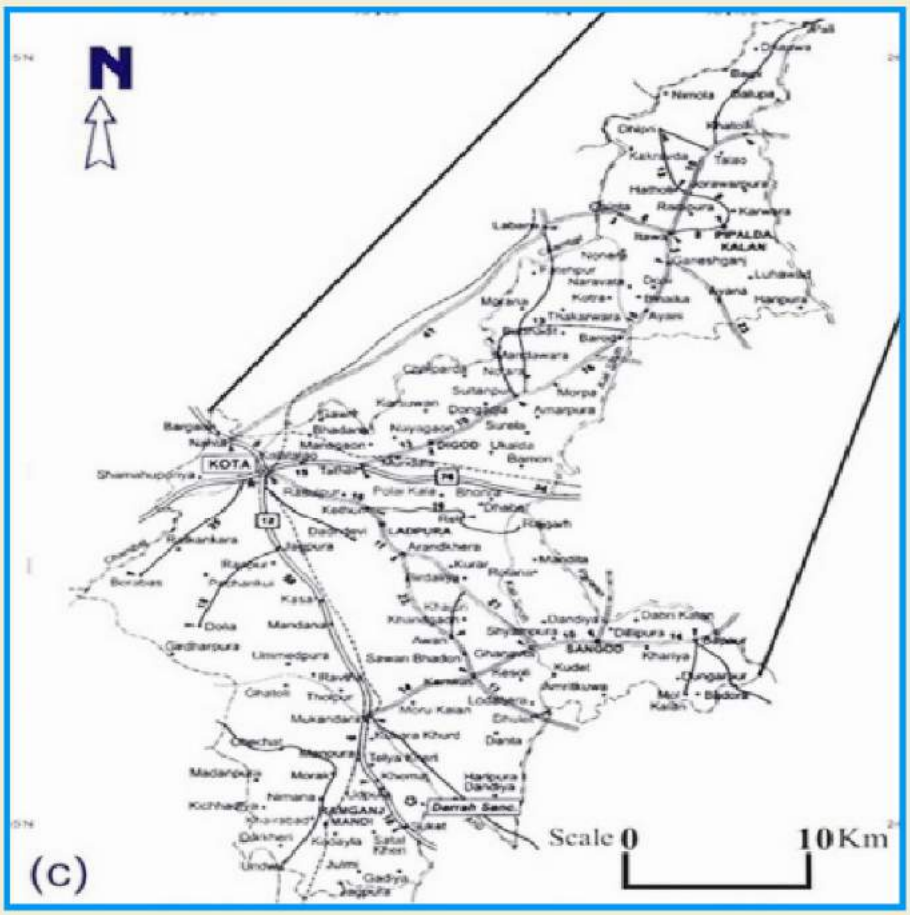
Hadoti plateau is unique due to historical and cultural heritage as well as geographical location and physiography. Hadoti comprising of Kota, Bundi, Jhalawar and Baran district. The name of hadoti is given on the name of Hadas who were the rulers of east while princely state of Kota and Bundi.

## A. PROFILE OF KOTA DISTRICT

Kota region is mainly composed of low hills and discretely distributed plateau area with shallow plains. On the basis of physiography, altitude and topography Kota region can be classified into 5 specific geographical zones-

# PLATE - 1




Source : www.mapsofindia.com

**(a) Map of India Showing State of Rajasthan**
**(b) Rajasthan Showing Kota District (c) Kota District**

1. **Hilly Tracts of Bundi and Mukundra Hills :**

   Bundi and Mukundra hills are extended in the form of series in approximate area of 6022 square kilometer (sq. km.). Numerous small rivulets have got their way through these ranges. Famous Dara pass of Kota district is the distinguishing feature of these ranges.

2. **Riverian Zone of Chambal, Kalisindh and Parvati Rivers :**

   The drainage system of this region is well represented by Chambal, Kalisindh, Parvati and their tributaries but except for Chambal none of these rivers are so much prominent.

3. **Shahabad Upland :**

   This is the far eastern part of state having an area of 2900 sq. km. Its highest point Kasba Thana has got an altitude of 456 meters above MSL. The Most attractive topographic element of this region is, horse shoe shaped hilly tract, in the north-east of village Ramgarh.

4. **Jhalawar Plateau :**

   The region has got an approximate area of 6182 sq. km. and situated in south to Mukundra ranges.

5. **Dug-Gangdhar Upland:**

   It is the smallest physical unit of region having an area of 1429 sq. km. and situated in south west corner.(Plate-1)

## B. CLIMATE OF THE AREA

Climate of Hadoti region is sub humid and this area is included in semiarid and semi humid region. According to longitudinal situation it is placed under subtropical region. Three prominent seasons of area are :

**Winter season** - October - February.

**Summer season** - March - Mid June.

**Rainy season** - Mid June - September.

The whole Hadoti region continues to get heated till the onset of monsoon, by the middle of June, the area gets the monsoon currents from

the Bay of Bengal and Arabians Sea. More than 93% of total rainfall is received from June to September. The average rainfall of the area is 852 mm (34 inches). Average temperature of area 15˚C - 17˚C (Jan.) and 40˚C – 45˚C (during summer).

## C. SOILS OF KOTA DISTRICT

The texture of soil of Kota region comes under loamy to sandy loam type. The western parts of the region possess sandy-loam in preponderance while loamy-sand soil is characteristic of the eastern part of this region. Hadoti region consist of black soil, alluvial soil in river valleys which are rich in potash, calcium, magnesium, alumina and iron but having deficiency of phosphate, nitrogen and organic matter. The soil series dominant in the Kota region and adjoining areas are found to be mainly Chambal soil series and Kota variant soil series.

## D. RELATIVE HUMIDITY

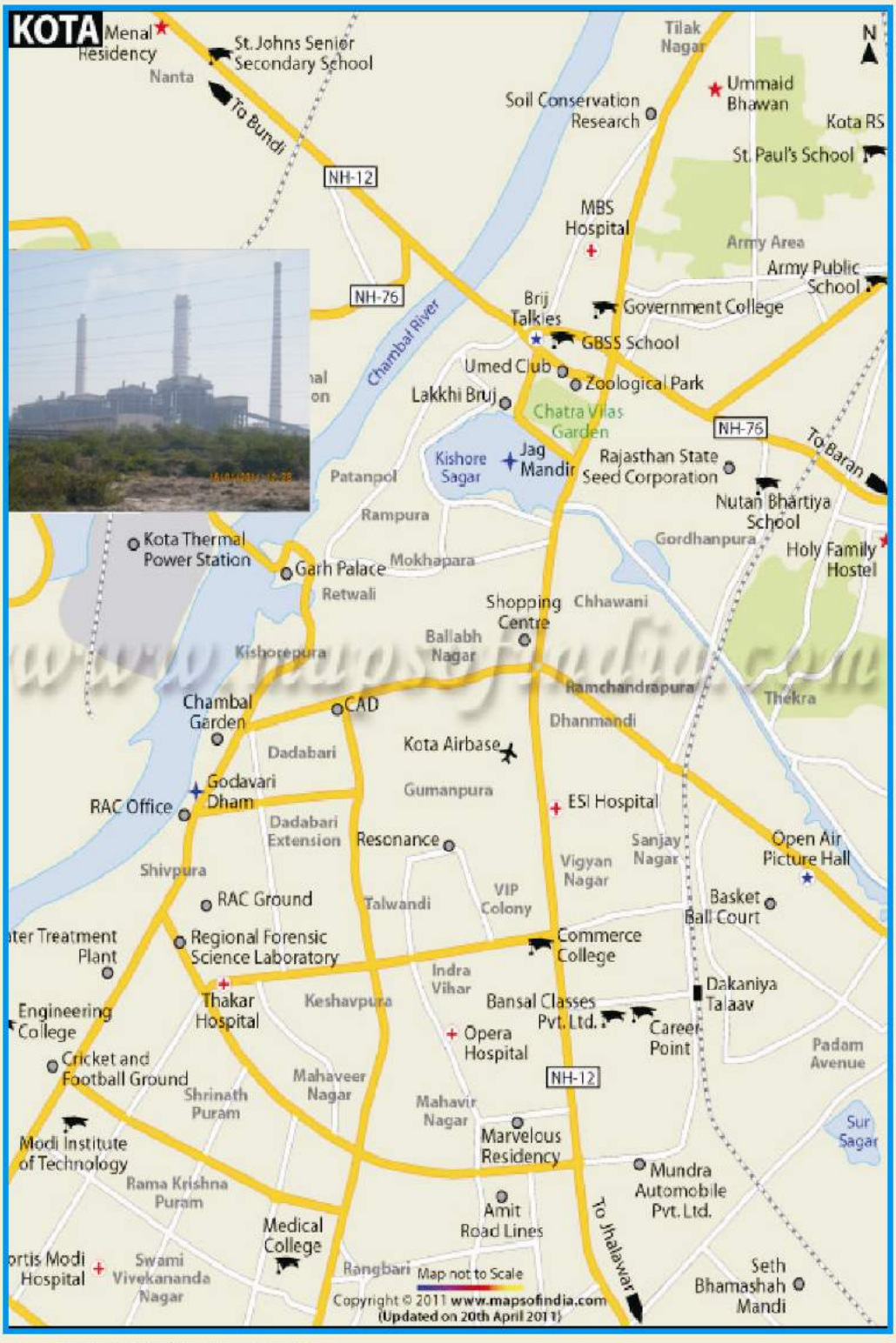
Relative humidity is minimum in summer season, particularly in April ad May when it ranges from 35 to 60% in the morning and 10 to30% in afternoon, Maximum humidity is noted during the rainy season particularly in July to mid Aug.

## E. SELECTED STUDY SITE

### Kota Thermal Power Station (KTPS)

Kota thermal power station is Rajasthan's first major coal based power station operating since 1982 (Plate-2,3). Presently it is operation with installed capacity of 1045 mw. This plant was envisaged to create the State's own thermal generating capacity and to meet the growing power demand. Kota thermal power station is located on the left bank of river Chambal in principal industrial city, Kota. Present study is an attempt to know about the impact of KTPS emission on vegetation present near the KTPS campus, different sites were selected for the survey, screening and collection of plant samples, survey of KTPS Kota, has been done

# PLATE - 2




Source : www.mapsofindia.com

## Map of Kota City Showing Study Sites

# PLATE - 3

(A) Study Site (Kota Thermal Power Plant)

(B) Transporting Tract/Flyash Filling Station

**Showing Different Views of Study Site**

periodically and various plant samples were collected from different sites. And for the purpose of further resent research work of ecomorphological study of selected legumes plants and Rhizobium bacteria present in legume plants growing in flyash amended soil, fly-ash samples were collected from the hopper directly located in KTPS campus. (Plate- 3 A,B).

CHAPTER – 3

# Review of LiteRatuRe

# Review of LiteRatuRe

In present studies, the main objective is to find out the pollutant released from thermal power station especially flyash and its impact on the vegetation in natural habitats by studying ecomophological character and comparative data analysis of vegetation through survey and in artificial conditions by growing representative herb plants that are the legume plants in control / flyash ameliorated conditions and the main emphasis is on the study of root nodule bacteria. The previous researches revealed that there are many contradictory results about flyash emission and amendments so the review includes both the aspects that are beneficial verses hazardous effects of flyash on soil and vegetation, through directly checking of the deposition of flyash on exposed plant organ and indirectly by mixing flyash with soil.

In the last 100 years investigation were conducted on the effects of gaseous pollutants, by many workers. More detailed reviews of the literature of vegetation injury from the pollutant released from industrial and power plant may be found in the publication by Thomas and Hendricks, (1956), Brandt and Heck, (1968), Wentzel, (1956) and Scurfield, (1960), The damaging effects of air pollutant on vegetation have long been recognized by Das Gupta, (1957), Woodwell, (1970), Majernik and Mansfield, (1972), Bleasdale, (1973), Mansfield, (1976), Furukawa, *et al.,* (1980), Kasat and Agrawal, (1981), Koziol and Whatley, (1984), Saxena, (1987), Agrawal, (1985), Singh and Rao,(1983), Gupta, *et al.,* (1988) and Sirohi and Singh, (1989), Several studied with higher plant exposed to air pollution show decrease in seed germination, root and shoot development, foliar injury, chlorophyll contents, economic yield of plants by Pandey and Rao, (1979), Chapekar,(1982), Rabe and Kreeb, (1979), Varshney,*et al.,* (1979), Mishra,(1986), Sharma,(1986), Prasad and Rao, (1985), Dubey *et al.,* (1982), Agrawal and Sharma, (1984). This

decrease has been attributed inhibition due to pollutant's destruction activity.

According to Pandey and Singh, (2010), coal combustion produce $CO_2$, $SO_x$, $NO_x$ and a Varity of product including flyash, flue gas and scrubber sludge, it also consist of minute glass like particles and its deposition on leaves inhibits the normal transpiration and photosynthesis of plant. Collected residues of thermal power plant have adverse effect on the environment and may affect the quality of surface and ground water, soil and vegetation. The volatile gasses contribute in atmospheric pollution while solid waste enhances disposal problems and disturb ecobalance of soil by change in micro flora. In India 75% of electricity is generated by coal based thermal power plants, which produce noxious gasses and nearly 65 million tons of flyash in a year as a byproduct (Sahu, 1998). Flyash consist of minute glass like particles and these very fine particles thus tend to remain airborne for a long period create atmospheric problems. Flyash may affect vegetation directly through deposition on plant surface mostly on leaves and when settle down it accumulate in the soil media and indirectly effect the vegetation. Kalra, *et al.,* (1998), found that fly ash production in India will exceed 140 million tons by 2020. In India Singh and Singh, (1986), Maiti, *et al.,* (1990), Sridhran and Pandian, (1998), Suwalka, (2003), characterized the thermal flyash of various thermal power plants including Kota thermal power plant. Flyash being light in weight easily mixes with air, water and remain for long duration thus pollutes the environment. It may also contaminate underground water with traces of toxic metals. Flyash deposition on land and it corrodes structure, texture and nature affect soil ecobalance.

Disposal in ash pit or pond create land pollution in surrounded areas, affect soil and vegetation of that area and also create harmful effect on public health. 2% of the cost of thermal power plants goes towards flyash disposal system. Annual expenditure on the road transport for dumping flyash is near about Rs 50 crore (Vitekari, *et al.,* 2012). According to one estimate, use of 1 tone of flyash in concrete will avoid 2

tons of carbon di oxide emitted from cement production and reduces green house effect and global warming (Krishnamoorthy, 2000 and Naik and Tyson, 2000).

The flyash contains a high concentration of toxic heavy metals such as Cu, Zn, Cd, Pb, Ni, Cr etc. ( Rautaray, *et al.,* 2003, and Lee, *et al.,* 2006) along with low nitrogen and phosphorus content and pH ranged from 4.5 to 12.0 depending on the S-content of parental coal. Therefore, disposal and utilization of fly ash needs careful assessment to prevent conversion of arable land into landfills and accumulation of toxic metals in soil and use it as an ameliorant for problem soils.

Flyash addition changes the physical properties of soil such as bulk density, water holding capacity, hydraulic conductivity and particle size distribution. major elements present in fly ash (in the order of decrease abundance) are Si, Al, Fe, Ca, C, Mg, K, Na, S, Ti, P and Mn and all exist in their oxidized state (Sharma and Kalra, 2006). Flyash contains considerable amount of macro as well as micro nutrients as plant nutrients (Maiti, *et al.,* 1990 and Suwalka, 2003) mineral such quartz mullite, hematite, magnetite, calcite and borax were also observed in flyash and oxidation of carbon and nitrogen during combustion drastically reduces their quality in flyash (Hodgson and Holliday,1966). Flyash production depends on the quality of the coal, (Singh and Siddiqui, 2003).

It consists of practically all the elements present in soil except organic carbon and nitrogen and can be utilized as a resource material. Because of the dominance of silt size particles in flyash, this material may often be substituted for topsoil in surface mine lands, thereby enchasing physical condition of soil, especially water holding capacity (WHC) (Sharma and Kalra, 2006). Ameliorating effect of flyash incorporation on soil properties has been studied by various workers (Adriano, *et al.,* 1980, Page *et al.,* 1979, Atiken, *et al.,* 1984, Singh and Singh, 1986, Gupta and Agarwal, 1993, Agarwal, 1998, Singh and Yunus, 2000, Sharma, *et al.,* 2002). By use of excessive quantities of flyash to alter pH, it can cause increase soil salinity especially with unweathered flyash (Sharma, *et al.,*

1990). The application of flyash altered the soil texture and increased water holding capacity, pH and electrical conductivity and extractable amount of micro and macronutrients but decreased soil particle density and available soil nitrogen in a study conducted Sharma, *et al.,* (1990). Sharma and Kalra, (2006), studied that because of ability to provide essential macro and micro nutrient for plant nutrition, flyash are being considered for amending agriculture soil to improve both chemical and physical property. Addition of flyash with soil alter characteristics mostly in such a way that it become more fertile i.e. having positive impact on growth and yield, the researches till date shows that it has greater potential in agriculture and related fields. The addition of appropriate quantity of flyash alters the texture of sandy and clayey soil to loamy. The predominantly silt size nature of flyash has been used to improve soil physical properties (Adriano and Weber, 2001). Fly ash addition generally decreased the bulk density of a soil, which in turn improve soil porosity and better ability and enhanced water retention capacity (Page, *et al.,* 1979 and Sharma, *et al.,* 2002). It also increased organic carbon content but did not increase amount of water availability to plants because more water was held in the ash by capillary actions Eisenberg *et al.,* (1986). Chang, *et al.,* (1977) and Page*, et al.,* (1979) also reported modification in bulk density of soil in flyash application experiment with calcareous and acidic soil. It revealed that flyash addition increased the pH of former from 8 to 10.8 and that of the later from 5.4 to 9.9.

According to Page, *et al.,*(1979). The effect of fly ash on chemical properties of soil is influenced by original pH of both ash and soil; applications of alkaline fly ash have invariably been associated with the nutrient status of soil. The lignite fly ash was equivalent to about 20% of reagent grade $CaCo_3$ in reducing soil acidity and supplying Ca needs of plants (due to fly ash as a liming material), According to Phung, *et al.,* (1979), studied that Fresh ash has been found more effective in raising soil pH to levels conducive to maximum plant growth than that of weathered ash in a given soil. A significant increase in EC has also been reported with increase percentage of fly ash by Singh and Singh,(1986),

Chang, *et al.,* (1977), and Adriano and Weber, (2001), observed that an addition of flyash by weight increased the water holding capacity of soil. Improvement in water holding capacity is beneficial to the plants especially under rain fed agriculture. They also observed that the soil hydraulic conductivity improved at lower rate of flyash application but deteriorates when the rate of flyash amendment exceed 20 percent. Soil properties as influenced by fly ash addition in soil have been studied by several researchers as Sharma, *et al.,*(2002), Sikka and Kansai, (1994), Kalra *et al.,* (1997), Sharma, (1998), Deshmukh, (2000), Grewal, *et al.,*( 2001), Garg, *et al* .,(2003).

Non judicial use however may lead to deterioration of soil texture and structure mainly in upper soil layer surface crust formation impending the water intake capacity of soil addition to toxic elements and alteration in physico-chemical properties as pH, EC. These changes in soil can affect the moisture availability, seedling emergence and crop establishment, root and shoot growth and consequent crop yield. (Nowakowa, 1981, Finkelman, *et al.,* 2000). Regardless of with or without application of recommended dose of NPK fertilizers increasing levels of coal flyash resulted in significant increase in pH, total contents of alkaline exchanges cation, Cation exchange capacity and percentage based saturation. This was mainly attributed to the inherent property of flyash (Yeledhalli, *et al.,* 2007).

There is less information available on soil biological properties of flyash. Application of any kind of pollutants such as fly ash is likely to interfere with the microbe mediated processed operating in soil thus misbalancing of the ecobalance. The application of graded levels of flyash resulted in an increase in available nutrient in the soil which modify the physico-chemical properties of soil and affected its biological activity significantly (Yeledhalli *et al.,* 2007). Addition of fly ash to sandy soil and silt loam decease the microbial respiration and nitrification activity in soil. Total bacteria, actinomycetes, fungus, count as well as enzyme activity in the soil decrease with increase ash content attribute to very high pH of fly

ash or presence of toxic elements at potential toxic concentrations. However application of acidic fly ash up to 100 tons/hect in an agricultural soil had no makeable impact on soil heterotrophic microbial activity, by higher level of amendment (400-700 ton/hect) may adversely affect the soil microorganism. In another study 20% flyash decreased bacteria, actinomycetes and fungi by 57, 80, 86% respectively. (Pichtel, 1990, Schutter and Fuhrmann, 2001). Invertase, amaylase, protease, dehydrogenase activity was found to be increased with increasing application of flyash, but decreased with higher levels of flyash application (Sarangi, *et al.,*2001).

Amendments to fly ash with organic matter such as sewage sludge may improve the soil conditions by increasing the cation exchange capacity. This may result in the immobilization of toxic metals and increase the availability of K, Mg and Ca. In addition to this, soil physical properties such as moisture retention and aggregation may also improve, through sludge application increased microbial count as well as enzyme activity but all populations were still lower at the highest ash rates (20% on wt basis) compared to untreated control. Flyash composted with wheat straw and 2% rock phosphate (w/w) or 90 days enhanced the chemical and microbiological properties of the compost. (Gaind and Gaur, 2004). Lal, *et al., (*1996) reported that flyash added to soil at 16% (w/w) increases enzyme activities (urease and cellulase). However, acid phosphates activity was depressed and with flyash application. So, mix application of flyash proved to be beneficial in augmenting proliferation and activity of microorganisms in acid soils. The enzymatic activity of soil is also an important factor for measuring soil biological properties after flyash amendment in soil. The high pH and electrical conductivity of flyash have been suggested to be important elements limiting microbial activity (Elliott,*et al.,*1982). Pati and Sahu, (2004) taken 7 concentrations of flyash amended soil (0%, 2.5%, 5%, 10%, 15%, 25% and 50% ,w/w) for the toxicity test of earthworms. They found little or no inhibition of soil respiration and enzyme activities up to 2.5% flyash amendment. With

further addition of flyash, all the above activities were significantly decreased.

Many researchers added fly ash in the soil to evaluate the long term consequences of flyash on soil environment and crop productivity. Flyash incorporation in the sandy loam soil (up to 40%) modified the soil environment, mainly moisture retention, release and transmission behavior, pH, EC and organic carbon. The texture of the soil-ash admixture remained sandy loam up to 10% ash application, beyond this level the texture turned to loamy soil. Microbial activity got modified favorably up to 10% ash in soil-ash admixture.

Flyash application to sandy soil could permanently alter soil texture, increase micro porosity and improve the water-holding capacity (Ghodrati, *et al.,*1995, Page, *et al.,* 1979, Buck *et al.,* 1990). Flyash addition at 70 t $ha^{-1}$ has been reported to alter the texture of sandy and clayey soil to loamy (Capp, 1978). The particle size range of flyash is similar to silt and changes the bulk density of soil. Chang, *et al.,* (1977) observed that among five soil types, Reyes silty clay showed an increase in bulk density. Application of flyash at 0, 5, 10 and 15% by weight in clay soil significantly reduced the bulk density and improved the soil structure, which in turn improves porosity, work ability, root penetration and moisture-retention capacity of the soil (Kene *et al.,* 1991)). Prabakar, *et al.,* (2004) concluded that addition of flyash up to 46% reduced the dry density of the soil in the order of 15-20% due to the low specific gravity and unit weight of soil. A gradual increase in flyash concentration in the normal field soil (0, 10, 20 up to 100% v/v) was reported to increase the porosity and water-holding capacity (Khan and Khan, 1996). This improvement in water-holding capacity is beneficial for the growth of plants especially under rain fed agriculture. Amendment with flyash up to 40% also increased soil porosity from 43 to 53% and water-holding capacity from 39 to 55% (Singh *et al.,* 1997). Flyash had been shown to increase the amount of plant available water in sandy soils (Taylor and Schumann, 1988). The Ca in flyash readily replaces Na at

clay exchange sites and thereby enhances flocculation of soil clay particles, keeps the soils friable, enhances water penetration and allows roots to penetrate compact soil layers (Jala and Goyal, 2006). Water holding capacities of flyash from different thermal power plants in Eastern India were compared and the effect of size fractionation on the water-holding capacity was determined in an investigation by Sarkar and Rano, (2007).

Lime in flyash readily reacts with acidic components in soil and releases nutrients such as S, B and Mo in the form and amount beneficial to crop plants. Flyash improves the nutrient status of soil (Rautaray *et al.,* 2003). The flyash has been used for correction of sulphur and boron deficiency in acid soils (Chang *et al.,*1977). Application of flyash for increasing the pH of acidic soils (Phung *et al.,* 1979). Most of the flyash produced in India is alkaline in nature, hence, its application to agricultural soils could increase the soil pH and thereby neutralize acidic soils. The hydroxide and carbonate salts give flyash one of its principal beneficial chemical characteristics, the ability to neutralize acidity in soils (Cetin and Pehlivan, 2007). Flyash has been shown to act as a liming material to neutralize soil acidity and provide plant available nutrients (Taylor and Schumann, 1988). Researches have shown that the use of flyash as liming agent in acid soils may improve soil properties and increase crop yield (Matsi and Keramidas, 1999). The electrical conductivity of soil increases with flyash application and so does the metal content. De colorization of effluents by fly ash has been reported earlier by a number of workers (Robinson, *et al.,* 2001) and a mixture of flyash and coal in the ratio of 1:1 can be substituted for activated carbon owing to increase in surface area available for absorption (Gupta *et al.,*1990). Metals like Fe, Zn, Cu, Mn, Ni and Cd have been shown to be available at higher concentrations in DTPA extracts of FA (Gupta *et al.,* 2007). The increased accumulation of essential ions such as Zn, Mn and Cu by the paddy shoot/grain might be due to increased activity of ionic transporters (Hall and Williams, 2003), in turn due to higher essential ion availability in the FA. Sarangi *et al.,(*2001), observed that gradual increases in soil pH,

conductivity, available phosphorus, organic carbon and organic matter with increased application rate of fly ash (Table 5). Flyash is considered to be a rich source of Si and application of FA in Si-deficient soils has been demonstrated to improve the Si content of rice plants as well as their growth (Lee, *et al.,* 2006).

Numerous short-term laboratory incubation studies found that the addition of unweathered flyash to sandy soils severely inhibited microbial respiration, numbers, size, enzyme activity and soil nitrogen cycling processes such as nitrification and N mineralization (Cerevelli, *et al.,* 1986, Wong and Wong, 1986, Pichtel and Hayes,1990). Information regarding the effect of flyash amendment on soil biological properties is very scanty (Schutter and Fuhramann, 2001). These adverse effects were partly due to the presence of excessive levels of soluble salts and trace elements in unweathered flyash. However, the concentration of soluble salts and other trace elements was found to decrease due to weathering of flyash during natural leaching, thereby reducing the detrimental effects over time (Sims, *et al.,* 1995). Moreover, the use of extremely alkaline (pH 11-12) flyash could also be the reason for those adverse effects. The application of lignite flyash reduced the growth of seven soil borne pathogenic microorganisms, whereas the population of *Rhizobium* sp. and P-solubilizing bacteria were increased under the soil amended with either farmyard manure or flyash individually or in combination. Amendment of Class F, bituminous flyash to soil at a rate of 505 Mg $ha^{-1}$ did not cause any negative effect on soil microbial communities and improved the populations of fungi, including arbuscular mycorrhizal fungi and gram negative bacteria as revealed from analysis of community (Schutter and Fuhrmann, 2001). Machulla, *et al.,* (2004), suggested that the microbial communities that developed in 17-20 year old lignite ash deposits in Germany contained specific ash tolerant populations that different significantly from those in surrounding soils. Kumar, *et al.,* (2008), isolated metal tolerant plant growth promoting bacteria from flyash contaminated soils and found that the strain are capable of stimulating plant biomass and enhance phytoextraction of metals (Ni, Zn and Cr) from flyash by

metal accumulating plant i.e., *Brassica juncea* (Indian mustard). Actinomycetes and fungi declined with 5% flyash and all populations declined at the 10 and 20% rate. With 20% flyash bacteria, actinomycetes and fungi decreased by 57, 80 and 86%, respectively (Pichtel, 1990). Garampalli, *et al.,*(2005) revealed on the basis of pot culture experiment that using sterile, phosphorus deficient soil to study the effect of flyash at three different concentrations viz., 10, 20 and 30 g flyash $kg^{-1}$ soil on the infectivity and effectiveness of vesicular arbuscular mycorrhiza (VAM) Glomus aggregatum in pigeonpea (*Cajanus cajan* L). All the concentrations of flyash amendment in soil were found to significantly affect the intensity of VAM colonization inside the plant roots and at higher concentration (30 g flyash $kg^{-1}$ soil), the formation of VAM fungal structure was suppressed completely. The dry weight of the pigeonpea plants under the influence of flyash amendment in VAM fungus infested soils was found to be considerably less (though not significant enough) when compared to the plants grown without flyash that otherwise resulted in significant increase in growth over the plants without *G. aggregatum* inoculation. However, flyash amendment without VAM inoculation was also found to enhance the growth of plants as compared to control plants.

Farmers of Punjab, Haryana and Maharashtra blame thermal power plants and pollution from its far sudden decrease in production and found highly contaminated with heavy metal and not fit for consumption. (Art. India beyond coal. 2013) Flyash particles deposits on standing crops smoke from chimneys pollutes the air that plants use for photosynthesis and no farming technique can avoid contamination from these sources.

The study of thermal power exhausts on plant community and growth by various workers as Agarwal and Gupta, (1993), Dadhich and Kasat, (1988), Khandelwal and Shrivastava, (2013), Physical and chemical characteristics of flyash have been extensively studied by various workers.(Krishna Rani and Sharma, 2010, Khan and Khan, 1996), They conclude that low bulk density increases the potential for dust

formation which create problems in transporting and storage of flyash, Flyash has unusually high surface area and light texture due to the presence of large, porous and carbonaceous particles, Flyash dust under certain conditions of humidity, stick to leaves or fruits and promotes chemical as well as physical injuries, small necrotic dark brown spots appear on the leaves of many vegetables. Its deposition on leaves inhibits the normal transpiration and photosynthesis of plant. According to Kumari, (2009), accumulated flyash on guard cell surface of leaves stimulates the regulation mechanism of stomata opening and closer, thereby affecting normal respiration and photosynthetic rate. Higher amount on foliar deposition of flyash resulted in decreased transpiration rate due to barrier created by thicker layer. Thicker layer of flyash interfere with the light required for photosynthesis and thus reduce the photosynthetic rate. Leaves laden with flyash absorb heat more effectively and consequently the increased leaf temperature results in increased transpiration rates. Dubey, *et al.,* (1982), spread 2-4-6 gram/$m^2$ per day flyash on wheat and gram plants and observed increase in height, in shoot length, in dry weight and pigment concentration.

Agricultural utilization of flyash has been proposed because of its considerable content of K, Ca, Mg, S and P (Kalra, *et al.,* 1997, Singh, *et al.,* 1997). Fly ash addition generally increases plant growth and nutrient uptake (Aitken, *et al.,* 1984). Weinstein, *et al*. (1989), reported that fly ash increased crop yield of alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), Bermuda grass (*Cynodon dactylon*) and white clover (*Trifolium repens*). Addition of unweathered western US flyash up to 8% (w/w) to either calcareous or acidic soils resulted in higher yield of several agronomic crops (Page, *et al.,* 1979) mainly due to increased availability of S to plants. Furr, *et al.,* (1977), demonstrated that alfalfa, sorghum (*Sorghum bicolor*), field corn (*Zea mays*), millet (*Echinochloa crusgalli*), carrots (*Daucas carota*), onion (*Allium cepa*), beans (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), potatoes (*Solanum tuberosum*) and tomatoe (*Lycopersicon esculentum*) could be grown on a slightly acidic soil (pH 6.0) treated with 125 mt $ha^{-1}$ of unweathered flyash. These plants

exhibited higher contents of As, B, Mg and Se. Also winter wheat **(***Triticum aestivum*) grown on a deep bed of flyash produced grains containing higher Se (Stoewsand, *et al.,* 1978). Greenhouse experiments conducted by Sikka and Kansal, (1994) showed that application of 2-4% flyash significantly increased N, S, Ca, Na and Fe content of rice (*Oryza sativa*) plants. The foliar application of flyash also enhances growth and metabolic rates, as well as increasing the photosynthetic pigments of crops like maize and soybean (Mishra and Shukla, 1986). They did not find any residual effect of fly ash application on the following wheat crop except for a slight increase in Fe content of the soil. The post harvest soil samples from rice and wheat also did not show any change in the nutrient content and pH. The iron content of the soil however, increased to 18 from 12%. Khan and Khan, (1996), reported that application at 40% fly ash can increase the yield of tomato by 81% and market value (mean fruit weight). Increased selenium accumulation in plant tissues with increased flyash application warrants close monitoring and use of appropriate quantity of weathered flyash depending upon the end use of the produced bio mass (Straughan, *et al.,* 1978). Application of 5-20% fly ash on w/w basis in the plough layer (0-15 cm) increased both grain and straw yield of pearlmillet (*Pennisetum* sp.) followed by wheat (Grewal, *et al.,* 2001). Lau and Wong, (2001), reported that weathered coal fly ash at 5% resulted in higher seed germination rate and root length of lettuce (*Lactuca sativa*). The amino acid content in soybean (*Glycine max L,*) was found to show an increase when grown in fly ash amended soils in pot cultures (Goyal, *et al.,* 2002). High yield of aromatic grasses particularly palmarosa (*Cymbopogon martini*) and citronella (*Cymbopogon nardus*), in presence of different flyash soil combinations, was attributed to increased availability of major plant nutrients (Neelima, *et al.,* 1995). Fly ash applied at 25% showed higher yield of brinjal (*Solanum melongena*), tomato and cabbage. Oil seed crops such as sunflower (*Helianthus* sp.) and groundnut (*Arachis hypogaea*) also responded favorably to flyash amendment. Medicinal plants such as cornmint (*Mentha arvensis*) and vetiver (*Vetiver zizanoides*) were successfully planted in flyash used in

conjunction with 20% farmyard manure (FYM) and mycorrhiza (Sharma, *et al.,* 2001). The level of 40% fly ash was found to have nematicidal effect and suggested for the management of root knot disease in tomato caused by *Meloidogyne* sp. and providing nutrients (Khan *et al.,* 1997). Tomato cultivars grown on flyash amended soils had higher tolerance to wilt fungus *Fusarium oxysporum* (Khan and Singh, 2001).

This review explores the possibility of using flyash to improve the soil environment and subsequently increase the growth and yield of crops. Various industrial effluents as well as coal residues contains considerable amount of organic matter and plant nutrients, particularly potassium and sulphur, this can be applied to arable land as irrigation water and as an amendment. When applied to crops it may act as a source of plant nutrients (N, K, P, Ca, S, Cu, Mn and Zn) and has been reported to increase the yield of the crop (Nagajyothi, *et al.,* 2009. Druzina, *et al.,* (1983), Tsadilas, *et al.,* (2002), studied the flyash and sewage sludge application on an acid soil and their influence on some soil properties and wheat biomass production.

Chemical fertilizers are very expensive in the open market, which, according to Abubakar, *et al., (*2004), is the main source of fertilizer for about 96% of farmers. Flyash the notorious waste product of coal based thermal power plants, known for its ill effects on agricultural land, may now come as an aid for farming community, because of its great availability and low cost, further possibility of its usage should be investigated. Deepti, S and Mishra, (2014), explore and assess flyash biofertilizers. According to Mehra, *et al.,* (1986), Rai, *et al.,* (2002), Flyash contains almost all essential nutrients but deficient in N and P and so that did not affect the availability of nitrogen (N) in soil. Flyash generally contains sufficient concentrations of the nutrient essential for plant growth. However, C and N are usually present in small amount and it is medium in available K and high in available P. Lignite fly ash had a relatively higher K content than non-lignite coal flyash. These constituents may prove good

for reclaiming saline and alkali soils as well as may enrich the soil in due course of time (Sharma and Kalra, 2006).

Flyash also affects the physiochemical characteristics of soil because it is generally very basic, rich in various essential and non essential elements. Sharma and Kalra, (2006), were reported that flyash improve physical, chemical and biological properties of problem soil and enhance the available macro and macronutrients for plants. Pandy, *et al.,* (2009), reveals that the flyash could be efficiently used in barren or sterile soil for improving quality and enhancing fertility, If it is incorporated at a sufficient rate could exert a beneficial effect on soil water holding capacity in sandy soil, since fine textured substrates can hold more water than coarse textured substrates (Chang, *et al.,* (1977), Campbell, *et al.,* (1983), Aitken, *et al.,* (1984), Gangloff, *et al.,* (2000). Page, *et al.,* (1979), recorded that application of flyash to soil (8% by weight) increases pH of the soil. Fresh ash has been found more effective in raising soil pH to levels conducive to maximum plant growth that of weathered ash in a given soil (Phung, *et al.,* 1979). Flyash amendments to a variety of agricultural soils tended to decrease in the bulk density (Page, *et al.,* 1979; Campbell, *et al.,* 1983).

The hydroxide and carbonate salts give flyash one of its principal beneficial chemicals characteristics, the ability to neutralize acidity in soils (Cetin and Pehlivan, 2007). Yeledhalli, *et al.,* (2007), reported that coal flyash is a modifier of physico-chemical and biological properties of soil.

Addition of flyash in silt loam soil and in sandy soil decreased microbial respiration and nitrification activity in soil (Cerevelli, *et al.,* 1986, Arther *et al.,* 1984, Wong and Wong, 1986). Flyash interfere with the microbe mediated processed operating in soil thus unbalancing the ecobalance (Babich and Stozky, 1974 and Babich, *et al.,* 1983). Singh and Singh, (1986), were found a significant increase in electrical conductivity with increase in percentage of flyash after 25 days of incorporation. Plants grown on soils amended with coal flyashes have the capacity to absorb a range of potentially toxic elements (El-mogazi *et al.,*

1988). Concentration of elements detected in the flyash samples are not significantly higher than those typically found in uncontaminated soil (Alloway, 1990). Sharma, *et al.,* (1990), and Panwar, *et al.,* (1998), reported that flyash improves both the water holding capacity and aeration of soil. Pitchel, *et al.,* (1990), were recorded that increased ash content in soil, decreased the total bacteria, actinomycetes, fungal count as well as enzyme activities such as soil phosphates, sulphatase dehydrogenase and invertase. Lal, *et al.,* (1996), Rajkumar, (2000), were reported an increase in bacterial population due to combined application of flyash and N, P, K and flyash increased the microbial count significant.

Addition of alkaline flyash, which a pH over 9.0 (Cha *et al.,* 1999) can reduce soil acidity to a level, suitable for agriculture (Moliner and Street, 1982), and can increases the availability of trace metals, $So^{-2}_4$ and other nutrients (Wong and Wong, 1989, Ko, 2000). Vallini, *et al.,* (1999), reported an increase in the bacterial count due to application of flyash amendments results in dehydrogenase activity in soil.

Use of flyash to vegetate the landfill areas is an alternative for flyash management, which will serve for both the stabilization and amending the soil quality providing a pleasant landscape (Cheung *et al.,* 2000, Vajpayee *et al.,* 2000, Mishra, *et al.,* 2014). High concentrations of elements (K, Na. Ca, and Mg etc.) in flyash increase yield of agriculture crops. Flyash from coal acts as a good carrier for bio pesticides and fertilizers. It is used as a conditioner to arrest soil erosion, and to induce plant resistance against diseases (Vitekari, *et al.,*2012, Vijaykumar and Narayansamy, 1995). Sharma and Kalra, ( 2006), review that flyash can be used for reclamation the problem of soil and enhance the crop production depending upon the nature of soil and flyash, It may improve physical chemical and biological properties of soil and enhance the available macro and micronutrients for plants the higher concentration of elements (K,Na,Zn,Ca,Mg,Fe) in the fly ash increase yield of agricultural crops.

The concentration of all elements except N were higher in flyash than soil therefore flyash as an amendment for agricultural soil can improve the physical and chemical properties of soil and also improve soil fertility and crop productivity.

Flyash deposition effect physiochemical properties of soil, the massive flyash materials have been a potential resource for the agricultural activities, practical value of flyash in agriculture as an effective and safe fertilizer on soil amendment can be established after repeated field experiments. Prem kishor, *et al.,* (2010), Worlikar *et al.,* (2014), explained the use of flyash in agriculture to improve soil fertility and its productivity.

Numerous studies report the impact of flyash addition on the yields of different crops with either depressions or enhancements in yield. Whereas depression in yield have been largely reported to occur due to B toxicity P and Zn deficiency, improvements have been attributed principally to enhancements in B supply in B deficient soils improvements in sulphur supply and available water capacity. Significant higher grain as well as straw yield of rice recorded with the application of flyash addition higher than 20 percent decreased the yield. Residual wheat crop also registered higher yield by using up to 20 percent flyash amendment by Saxana and Ashokan, (1998),.

According to Agarwal, (1998), Incorporation of flyash in different compositions, without adding any fertilizer, improves plant growth. Favorable growth response of a few crops on flyash amended soil has been reported by many workers (Agarwal and Gupta, (1993). Fly ash application to the soil increases seed germination, seedling growth, dry matter production and photosynthetic pigments of all crops tested, much due to the increase availability of nutrients present in it. Thus land application of flyash can be favorable practice for flyash disposal especially in fertile and nutrient deficient soils or highly alkaline or acidic soil to amend them, which can solve the problem of its deposal and utilization to a great extent (Agarwal, 1998). Flyash directly affect the

seed germination if used as fertilizer by mixing with soil in very low concentration Dubey, *et al.,* (1982). Study of germination in palak and mung bean by (Katiyar, *et al.,* 2012). Agrawal and Gupta, (1993), studied impact of flyash amended soils on pigment characteristic of seedlings of maize, sorghum, wheat, gram & soyabean.

Wong and Wong, (1990), with *Brassica chinensis* and *Brassica parachinensis* and Pandey, *et al.,* (1994), with *Helianthus annuus* have found that low concentration of flyash was beneficial for their cultivation. Khan and Khan, (1996), found that tomato plants respond positively to flyash soil amendments showing luxuriant grown up to 60-70% and above which it had a deleterious effect.

Singh, *et al.,* (2008), have been studied the effects of flyash incorporation on heavy metal accumulation, growth and yield responses of *Beta vulgaris* plants. Flyash severely affect tree plants by changing the chemical and biochemical composition of soil (Banerjee, *et al.,* 2003). Faizan and Khan *(*2004), evaluated the growth and productivity of wheat by the application of flyash and results showed maximum growth enhancement at >50% level of flyash.

The growth performance and biochemical responses of three rice cultivars grown in fly ash amended soil reported exhibited toxicity of flyash at higher context (>50%) and it was reflected in the reduction of photosynthetic pigments and growth parameters where as lower content of flyash enhanced growth of plants in these parameters (Dwivedi, *et al.,* 2005). Singh, *et al.,* (2008), reported that flyash respond negative relationship with the yield of *B. vulgaris.* Ravikumar, *et al.,* (2008), studied the potential of earthworm in composting of different organic residues blended with flyash and its effect on recovery of nutrients and soil enzyme activity. Metal resistant plant growth promoting bacteria present in applied flyash enhance the growth of *Brassica juncea* (Kumar, *et al.,* 2009). The best result of application impact of coal flyash and water hyacinth on cultivation of Tomato, in terms of plant growth maturation period, quality and quantity of produce were obtained with composts, containing

40%(v/v) of coal flyash of total volume and flyash application is advantageous in cultivation of tomato by Punjwani, *et al.,* (2011) .Yield parameters showed positive response of flyash amendment as they increase significantly in comparison with unamended ones in mung bean, palak and chilli by Katiyar, *et al.,(* 2012). In conjunction with organic manure and microbial inoculants, flyash can enhance plant biomass production from degraded soil. Non judicious application of flyash deteriorates soil quality as well as depressed crop growth was observed by Shukla, *et al.,* (2003), in studies on bio-utilization of flyash using leguminous crops.

Singh, *et al.,* (1997), also reported that leguminous plants could grow well on flyash amended soil without manifestation of any injury symptom and seed germination enhanced by 6-8% at lower rate of flyash application in *Vicia faba* plants. Singh and Agrawal, (2010), with *Vigna radiata* have found that both growth and yield shows positive responses when soil amended with different concentration of flyash in a field experiment. Studies reveals that application of coal ash at 25 % (v/v) in concentration on lentil crops shows best performance where as higher coal ash levels affected the crop adversely (Faizan and Khan, 2004).

Impact of flyash amendment of seed germination, growth and yield of *Vigna mungo* studied by Agarwal, *et al.,* (2004), result showed that flyash application favored plant growth, plant height, number of leaves, branches and number of nodules per plant were increased up to 25% flyash amendment. Pandey, *et al.,* (2005), studied the germination and growth response of *Cajanus cagan* L. in flyash amended soil and reported seed germination, growth behavior and nodulation frequency at higher exposure concentration. It delayed the nodulation as lesser number of nodules was recorded at higher amendments.

Rai*, et al.,* (2003), also observed performance of seed germination and growth of *Vicia faba* L. in soil. The performance of the plant in soil amended by different concentrations of flyash has been studied, result revealed that while flyash amendment to the soil improved the growth

performance at initial stages with application of lower concentrations, it was inhibitory at higher exposure concentration flyash delayed the nodulation as lesser number of nodules was recorded at higher amendments.

A revegetation trial was conducted to evaluate the feasibility of growing a legume species on flyash ameliorated with combination of various organic amendments, blue green algae biofertilizers and rhizobium inoculation, a significant enhancement in plant biomass photosynthetic pigments, protein content and in-vivo nitrate reductase activity were found in the plants grown in ameliorated flyash in comparison to plant growing in unamended flyash or garden soil. Result showed that potential growth of *Prosopis juliflora* in plantation on flyash landfills and to reduce the metal contents of flyash by bioaccumulation in its tissues. (Rai, *et al.,* 2004, Ankita Naval *et al.,* 2014). Various workers as Cheng and Chu, (2007), Ram, (2008), Bhdoria, (2009), Verma and Sharma, (2009), Singh, (2010), performed there researches on the utilization of thermal flyash and its impact on different agricultural crops and plant species were carried out through different projects.

Shankar, *et al.,* (2006), demonstrated the influence of chromium and cadmium (in flyash) on germination, seedling growth and photosynthetic pigments of soybean, and reported that there was a gradual retardation of germination and growth parameters.

Changes in soil properties caused by flyash may directly or indirectly affect microbial activity and the root growth of plants. The high amount of deposition of flyash during sowing season reduced seed germination as well as seedling growth and development. Due to continuous deposition of flyash on agriculture lands the crop productivity has been drastically reduced. The excess levels of soluble elements released from flyash induce hazardous effects in plant root and the rhizosphere. Increased pH resulted in a loss of applied and indigenous N. significant reduction in formation of nodules in leguminous plants was

noticed which may be attributed to flyash toxicity (B,As,Se,Mo,Al,Cd). (Kumari V, 2009).

Flyash mission, now known as Flyash Utilization Programme (FAUP) in varying agro-climatic conditions and different soil-crop combinations supported with laboratory investigations have shown significant increase in yields of edible part as well as biomass without any adverse impact on soil health or crop produce (Kumar, *et al.,* 2005). The large scale use of flyash in agriculture and waste development holds a potential to increase on an average 15% yield of grains, oil seeds, sugarcane, cotton and 25-30% of vegetables resulting in another green revolution.

Effect of certain heavy metals on seed germination and seedling growth performance of legume plant *Cyamopsis tetragonoloba L* observed by Jain, *et al.,* (2009). Response of onion (*Allium cepa L.)* grown in flyash amended soil during kharif season was studied by Singh, *et al.,* (2014).

Seed germination of maize, sorghum wheat and gram was tested in two soil types treated with flyash additions (0-100%wt basis). Percentage germination in most crops increased in soils treated with ash (up to 10%) and decreased with higher fly ash rates except in gram, which tolerated up to 30% flyash addition. Agarwal, (1993), Kalra, *et al.,* (1997), Lentil seed showed 50% germination in 72-94hr (0-40%ash treatment) except for 30 and 40% ash treatments showed considerable reduction in germination. Mustard crop was adversely affected in terms of delay as well as reduced germination count. It is evident that initially in all the flyash mixed soils the germination percentage was higher as compared to control soils. Germination of wheat and gram grown in flyash mixed soil exhibit a promotive response to germination. The maximum germination rate observed in was in15 and 20% in flyash amended soil Sorghum and Maize in different type of soils, and higher flyash rates were inhibitory for seed germination. Gupta and Agarwal, (1993),Observation on the effect of flyash amended soil on seedling growth of two Varieties of *Glycine max*

*L,* by Gupta and Agarwal, (1993), show that in term of height the maximum was recorded at 10% flyash amendment and minimum was observed in pure flyash. Flyash application in soil affected the photosynthetic pigments in leaves, protein and lysine contents of the grains of all the varieties. Foliar pigments, protein and lysine contents were found to be increased at 20 and 40% flyash levels while gradually reduced with the increase in flyash level in soil i.e. 60, 80 and 100% flyash. Chlorophyll and carotenoid are the pigments active in the process of photosynthesis. Plants grown in flyash amended soil showed increased value of these pigments. Effects of flyash on chlorophyll and carotenoid content of Maize, Sorghum, and Soyabean showed percentage increase in 5%, 10%, 15% and 20% in flyash amended soils. Agarwal and Gupta, (1993), The quantitative enhancement of photosynthetic pigment in *Glycine max L, at* 5% and 10% flyash incorporation could be possibly related to nutrients like sulphate, magnesium and zinc. Agarwal and Gupta, (1993),. The role of magnesium in the chlorophyll structure is well known and documented (Bogorad, 1966, Nason and Mc Elory, 1963). Similarly Power, (1930), demonstrated that the amount of chlorophyll in alpha-alpha was increased up to 18% by sulphur application. The positive effect of industrial waste flyash was studied on daily growth rate (DGR), chlorophyll,carotenoids, protein, carbohydrate, lipid and phycocolloids (agar and algin) content of four economically important seaweeds by Sornalakshmi V and V Kumar, (2014). Sharma *et al.,* (2011) perform biochemical estimation of primary metabolites in *Ocimum spp*.

Ascorbic acid being a strong reluctant, activates many physiological and defense mechanisms. Maximum value of ascorbic acid was observed at 10% flyash in *Glycine max L,* with increase in flyash concentration the reduction of ascorbic acid concentration was gradual in both the varieties of *Glycine max L.*

Seedling phenol content in *Glycine max L, was* observed maximum in pure flyash and minimum in 5% flyash mixed soil. These observations

showed that phenol concentration increased in response to flyash concentration and has directly correlated with flyash level in the soil.

Plant of *Vigna radiata* were grown in soil with different amounts of flyash (10 and 25%), observed that addition of flyash initially increased the rate of growth, toxic symptoms were observed for 25% flyash. Result from analysis of antioxidants (carotenoid, ascorbic acid, non protein thiol and free proline) revealed that these increased more in plants grown in 10% flyash than in those grown in garden soil. So it may be grown in 10% flyash amended soils. Gupta and Sinha, ( 2009),

The carbohydrate, also known as saccharides (sugar on sweetness), and are widely distributed molecules and occur as food reserves in organs of plants. They are an important sources of energy required for the various metabolic activities of plants. Carbohydrate contents have been studies in different plant species like *Balanites aegyptica* by Vijayvergiya and Vijay,(2006) *Cassia obtusifolia* and *Cassia siamea* (Sharma, *et al.,* 2006), *Terminalia catappa* (Nagesh, *et al.,* 2007),Comparative studies of carbohydrate contents in two species of *Araucaria* have been carried out by Unnikrishnan, *et al.,*( 2007).

Protein is macro-molecules with high molecular weight. Variation in protein contents is also considered as an important biochemical event during growth and differentiation of cells in plants, (Audichya, 1999). Protein contents have been estimated in various plant species by various workers such as *Lens culinaris, Vicia faba, Cicer arietinum, Phaseolus lunatus, Phaseolus vulgaris, Pisum sativum and Glycine max L,* (Borhade, *et al.,* 1984, Kay, 1979, Chatrath, *et al.,* 1996).

Ascorbic acid an important primary plant product play significant role in germination, growth, metabolism and flowering of plants. There are several reports of ascorbic acid accumulation in plants, Kaur, (1997).

Phenols are the plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acid stems from their potential

protective role, through ingestion of fruits and vegetable, against oxidation damage diseases. Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years.

Proline accumulation is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses. Proline plays a critical role in protecting plants under stress. Proline accumulation under salt stress in both root and leaf tissues (Aziz, *et al.,* 1999). The accumulation of solutes like Glycine and proline has linked to water stress, salinity and other abiotic plant stress. (Ashraf and Harris, 2004).

The most convenient method of obtaining rhizobia from nature is by isolation from root nodules. Contrary to popular belief, many of the bacteroids in nodules are viable (Pohlman, 1931, Pribac and Ardelean, 2008), is impractical to isolate rhizobia directly from the soil because of their fastidious growth requirements and the presence of numerous less fastidious fast growing soil microorganisms. Nodules, on the other hand, generally contain only rhizobia. Some nodules, however, may contain more than one strain of rhizobium (Lindemann, 2008), or may even contain other bacteria. Nodules used for isolation should be in good physical condition to reduce the chance of bacteria other than rhizobia being present. The portion of the nodule containing rhizobia can be located by noting the area with the reddish pigment leg hemoglobin. If the nodule is not potentially capable of fixing $N_2$, the bacteroid area may not be red but will likely differ in color from the remainder of the nodule. A nodule contains rhizobia within it but has many other microorganisms on its surface. These surface micro organisms must be prevented from contaminating the rhizobia portion of the nodule by surface sterilizing the nodule.

Rhizobia are well known for their capacity to establish a symbiosis with legumes. Legumes are unique plants which have the ability to work with certain bacteria i.e. Rhizobia to gather available nitrogen from the soil atmosphere and convert it to usable ammonia nitrogen and make it available to the plant. They inhabit root nodules, where they reduce atmospheric nitrogen and make it available to the plant. Biological nitrogen fixation is a component of sustainable agriculture and Rhizobial inoculants have been applied frequently as bio-fertilizers. Each major legume group is nodulated by different species of Rhizobium. Soybeans are nodulated by *Rhizobium japonicum* (Krichner and Buchanan, 1926), Fred and his associates (1932), recognized eight cross inoculants group in legumes. The genus *Rhizobium* was erected by Frank, (1890), based on its characters to form nodules on roots of legume plants. This property is the only valid test in the identification of the organism. Apart from it some diagnostic features of Rhizobium could be conveniently not only determine and identify the organism but also delineate different species (Graham and Parker, 1964, Vincent, 1970, Gaur, 1975, Mahana, 1981) *Rhizobium japonicum syn. Bradyrhizobium- japonicum* is associated with the root nodules of Soybean and fixes 100 kg nitrogen/ha/year, (Purohit and Kumar, 1998). Therefore the attempt has been made to study the morphological and biochemical characters of the bacterium. (Singh, Y, *et al.,* 2011)

Flyash has great potentiality in agriculture due to its efficacy in modification of soil health and crop performance. The high concentration of elements (K, Na, Zn, Ca, Mg and Fe) in flyash increases the yield of many agricultural crops. But the use of flyash in agriculture is limited compare to other sector. An exhaustive review of numerous studies of last four decades took place in this study, which systematically covers the importance, scope and apprehension regarding utilization of flyash in agriculture. This study also identified some areas, like soil fertility and its response on cereal oil seed and vegetable crops. Agricultural lime application contributes to global warming as Intergovernmental Panel on Climate Change (IPCC) assumes that all the carbon in agricultural lime is

finally released as $CO_2$ to the atmosphere. It is expected that use of flyash instead of lime in agriculture can reduce net $CO_2$ emission and also reduce global warming (Premkishor, *et al.,* 2010).

Though a number of studies have been carried out on different plants in various part of world, but review of studied literature indicate that no study was done on impact of fly ash on wild legumes and their root nodule bacteria, so our study may be a small start.

CHAPTER – 4

# MATERIAL AND METHODS

# MATERIAL AND METHODS

The present study was conducted along the area of KTPS Kota for collection of flora. For collection and analysis of samples various methods were used. For data collection and analysis complete methodology is divided in different parts as following-

## (1) Floristic Survey

Survey and identification of vegetation found near KTPS. The present work is based on the result of two years study of vegetation near Kota thermal power station. Systematic survey of study sites was done, field visit were arranged in such a way as to cover all selected sites at more or less regular intervals. Survey of selected sites has been done periodically; five different plots were established for the study of ecosystem at each site. The land selected was not previously in use for agriculture purposes and hence the impact of human activity was eliminated, the dominant plant species in the site were marked, recorded and tried to collect them in flowering and fruiting stage, polythene bags were used for carrying the plants. Polythene bags were easily transported or carried in the field and plant could be kept fresh for a long period. After the collection, plant material has been placed in newspaper between blotters and cardboard wooden end board for the pressing. After drying provisional identification was made with the help of flora of Rajasthan (Shetty and Singh, 1991) and flora of Rajasthan (South and South East region) by Sharma, (1997) and Bhandari, (1990). The identification was later on confirmed in department of botany government college, Kota.

### 1-A. Collection of Flyash Samples From Selected Site-

Flyash samples were collected from Kota Thermal Power Station. Flyash from KTPS Kota was derived from sub-bituminous black coals. A representative bulk sample of freshly precipitated (unweathered) flyash

was taken from the hopper of power station. The entire sample was taken at ones (Feb 2011), to reduce scope of any type of change in flyash composition. After collection, the dry ash was thoroughly mixed and stored in plastic lined containers at room temperature before use. Ash collected from ESP was relatively finer in texture, lower in pH and richer in nutrients comparatively ash from dumping site.

### 1-B. Collection of Soil Samples-

Soil sampling is the most vital step for analysis, The soil samplewas collected from garden near R K Puram area (top layer 200mm thickness). The particular soil collected was somewhat loamy in texture, yellowish brown in colour. Soil was collected during Jan 2011. After collection samples were sun and air dried for 7-7days each and stored at room temperature before use.

**Sterilization of soil** - Soil was collected from the field (garden) sterilized $121^0$C for 15 min at 15 Ibs pressure, the sterilized soil was cooled to room temperature and disposed in to the sterile pots. Air dry samples of soil and flyash were mixed accordingly i.e. control (0% flyash), 5%,10%, 15%,20%, 25% and 40% flyash by weight. Flyash was mixed with soil with a tumbler to provide a homogeneous mixture.

## (2) Physio-Chemical Characters of Soil and Flyash

The following characteristics of the sample were selected for testing based on their importance in influencing plant growth and their role as promoting or limiting factors. (Table-4)

### 2-A. PHYSICAL CHARACTERS

#### A-1. Soil Texture-

The frame work of soil consists principally of mineral and organic particle of various sizes. The relative proportion of different soil particles, Sand, silt and clay is known as soil texture.

Procedure- In the field texture is determined by feel or rubbing the soil between the thumb and the finger. It is a rapid procedure and proficiency is gained through experience, trial and comparison with samples of known textural class. Texture was confirmed through mechanical analysis in laboratory.

## A-2. Water Holding Capacity-

Water holding capacity of soil depends upon the texture and structure of soil components,

Requirements- Oven, filter paper, balance, tin cigarette box with perforated bottom or brass, petriplate.

Procedure- Firstly soil was dried in oven and crush it properly, then take a tin cigarette box with perforated bottom and put a filter paper inside the box at the perforated bottom and took the weight of box, then fill the box gradually with the dried soil and put this soil filled box in petridish filled with water, then wait for 8-10min and after that again take the weight of box, then put this container with in an oven at $105^0$C for about 24 hours and then once again take its weight. Now take a similar filter paper utilized in box and dip it in water to find out the amount of water absorbed by the filter paper. Then calculate the water holding capacity of soil.

$$\text{Percentage Water Holding Capacity} = \frac{\textit{Amount of water in the soil}}{\textit{Weight of the oven dry soil}} \times 100$$

Amount of Water in Soil = Weight of wet box filled with soil - (weight of box+ weight of wet fitter paper + weight of oven dry soil)

## A-3 Particle Density –

The mass of a unit volume of soil is called particle density (Dp). It was determined by measuring the mass and volume of soil solids.

Requirements - Pycnometer (R.D.bottle), volumetric flask, conical flask and balance

Procedure - Weigh Pycnometer empty ($W_1$) and then fill with water and weigh again($W_2$), then put 10g of air dry soil in to the small beaker and add few ml of water and boil (expel all air), now empty the Pycnometer and fill it with the soil transferring from the beaker with a jet of water. Allow cooling and filling completely by adding water and weight ($W_3$), the weight of the soil divided by the weight of water displaced gives the true density of the soil.

Weight of water displace by soil = $(W_2+10) - W_3$

Particle density of soil = $\frac{10}{(W2+10)-W3}$ g/cm$^2$

## A-4 **Bulk density** –

The bulk density or apparent specific gravity of soil is the mass of a unit volume of soil bulk including pore space.

Requirements - Large weighing bottle or specific gravity bottle of 50ml capacity, Balance, water and burette.

Procedure **-** Weight a large weighing bottle of about 50ml capacity without the stopper ($W_1$) then fill up with soil, flush up to brim tapping the bottle about 20 times and weight ($W_2$). Now remove the soil and now fill the bottle with water by burette and note the exact volume of water (V) needed to fill the bottle, the bulk density is obtained by dividing the weight of the soil with volume of the soil.

Bulk Density= $\frac{(W2-W1)}{V}$ g/cm$^2$

## A-5 Porosity –

Porosity of soil is the fraction of soil volume not occupied by soil particles. It can be measured with the help of bulk density and particle density using following formula.

Percent pore space (porosity) = 100- ( $\frac{Db}{Dp}x100$)

Db=bulk density g/cm$^2$ DP=particle density g/cm$^2$

## A-6 pH –

The pH value is a measure of the hydrogen ion activity of soil water system and expresses the activity and alkalinity of soil. The pH is very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of the soil. The pH of a solution has been defined as the negative logarithm of the hydrogen ion activity which in dilute solution can be expressed as concentration, in gram mole per liter. The pH was determined in soil-water suspension of ratio 1:2. Method used was Electronic pH meter method. The instrument commonly used in this method is a glass electrode pH meter with calomel reference electrode introducing salt bridge.

Principle - A glass surface in contact with hydrogen ions of the solution under test, acquired an electrical potential which depends on the concentration of $H^+$ ions. A measure of the electrical potential is, therefore give $H^+$ ion concentration or pH of the solution.

Requirements - Glass electrode pH meter, beaker, glass rod, distilled water

Reagents & Standard buffer solutions - These may be of pH4.0, 7.0 or 9.2 in pure water.

Soil water suspensions (1:2) **-** Weigh 40g of soil put into a 250ml flask and add 80 ml of distilled water in it. Stopper the flask and shake the mixture on the reciprocating shaker for one hour.

Procedure - Firstly take soil suspension sample, allow pH meter to warm up for 15min, place known standard buffer solution in beaker having pH 7 and pH 9.2 , adjust the instrument, then electrodes were immersed in to the beaker containing soil suspension, and pH was taken.

## A-7 Electrical conductivity-

Soil posse's at least small amount of various soluble salts. These may be acidic, neutral or basic. They may arise from different sources (rocks, ground water). Soluble salts present in soil dissociate in to their respective cations and anions when come in soil solution. These cations and anions carry current and impart conductivity. Thus the measurement of EC can be directly related to the soluble salt concentration. Amount of soluble salts in a sample are estimated from EC of aqueous soil extracts.

Principle - A simple Wheatstone bridge circuit is used to measure EC by null method.

Requirements - Conductivity meter and cell, Beaker

Standard potassium chloride solution (0.01M):0.7456g of dry AR grade KCl is dissolved in freshly prepared double distilled water and made one liter. At $25^0$C it gives an electrical conductivity of 1.413mmhos/cm (ds/m).The instrument is to be calibrated or checked with this solution.

Procedure – 25 gm of soil sample was dissolved in 50ml distilled water in a 100 ml beaker and shaken properly for fifteen minutes on a mechanical shaker and was allowed to stand or equilibrate for half an hour. The conductivity bridge was calibrated with the help of

standard KCl solution and cell constant was determined. Then electric conductivity of the supernatant liquid of the soil solution was measured with the help of Conductivity Bridge in (ds/m).

## 2-B. MACRONUTRIENTS

### B-1 Determination of Total Nitrogen by Autoanalyzer

Digestion- The sample is digested in $H_2SO_4$ to convert organic N to $NH_4^+$.N Digestion block digester with tractor auto temperature controller was used in digestion, Transfer 1to2g mineral soil low in N(60mesh) into a digestion tube and add 10ml concentration $H_2SO_4$ and mix by swirling, heat at $200^0$C in digestion block then add one Kjeltab, again heat for 15-20min until Kjeltab dissolves ($300^0$C), Then raise temperature to $375^0$C and heat until sample turn turquoise(45min), then remove the digestion tubes from block allow to cool for 5min, add about 50ml water and mix well until sample is in solution.

Principle- In the Kjelteb Auto Analyzer method, $NH_4$.N (librated by distillation of the digest with strong alkali) is absorbed in un standardized $H_3BO_3$ by titration against standard strong acid (HCl)

Requirement - Kjelteb Auto Analyzer

Reagent - 40% NaOH solution,

Receiving solution – Dissolve 100g $H_3BO_3$ in 10 litter water. Add 100ml bromocresol green solution. Add 70ml methyl red solution, then5ml of 4%NaOH.

Standard acid solution - (0.01M HCl)

Procedure - Bring the digest up to about 100ml, follow instruments for the Kjelteb Auto analyzer, set the alkali pump to deliver 30ml of 40%NaOH, then titrate with 0.01M NaOH. Calculate the readings.

## B-2 Determination of Available phosphorus

Phosphorus in soil ranges from 0.01 to 0.3 per cent and occurs in several forms and combinations. Extraction method (Olsen *et. al.,* 1954) was adopted to determine available phosphorus. Phosphorus, among the major plant nutrients, plays a key role in the development of the plant, in influencing the maturity of the crops and also in quality and quantity of the crop. In soil phosphorus exists in the form of various types of orthophosphates. A very small fraction of these is available to plants at a given time. Available phosphorus content of soil mainly of Ca, Al and Fe –P. In the neutral or alkaline soil particularly, Ca-P is the dominant fraction.

Principle - After extraction from the soil, phosphate in the extract is measured by the reaction of phosphate with ammonium molybdate in an acid medium to form molybdophosphoric acid. The molybdophasphoric acid is then reducing to a blue colored complex through reaction with ascorbic acid. Absorbance readings are taken at a 730nm wave length using a spectrophotometer. A standard curve constricted from absorbance readings of standards is used to deduce phosphate concentration of sample.

Requirements - Spectrophotometer, shaker

Reagents - Sodium bicarbonate ($NaHCO_3$) 0.5M extracting solution: Dissolve 42g of $NaHCO_3$ in 1000ml of demonized or distilled water. Mixed thoroughly adjust the pH of the solution to 8.5 with 1M NaOH solution. Darco-G-60 or equivalent grade phosphorus free charcoal, Ammonium molybdate solution, Ascorbic acid solution, Antimony potassium tartrate solution, Sulphuric acid 2.5M,40%$Sncl_2$ solution(stannous chloride),100mg phosphorus solution and 2mg$L^{-1}$ phosphorus working solution.

Procedure - 2.5 gm of soil sample, a pinch of Darco-G-60 and 50ml of Olsen reagent were mixed in a 100 ml conical flask and mixed thoroughly on a mechanical shaker. After filtrate 5 ml of ammonium molybdate solution containing 400 ml of 10N HCL per liter was gradually added. $CO_2$ evolved was driven out by slowly shaking. When frothing completely ceased, distilled water was added, washing done the sides, to bring the volume to about 22 ml there after 1ml of freshly diluted $Sncl_2$ solution was added, shaken a little and volume was made 25 ml then intensity of blue color was read at 600ml (red filter). A blank without soil was run under identical manner.

Preparation of standard curve for phosphorus- In a series of 25 ml volumetric flask 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of 2mg $L^{-1}$ phosphorus solution was pipette out and 5 ml of Olsen reagent was added. Further gradually 5 ml of ammonium molybdate solution was added and proceed in same the same way described earlier to develop blue color. Intensity of blue color was measured and a standard curve was drawn by plotting concentration of phosphorus against readings.

Calculation-Available phosphorus(Kg/ha-1) = $\frac{Q \times V \times 2.24 \times 10^{-6}}{A \times S \times 10^{-6}} = \frac{Q \times V \times 2.24}{A \times S}$

Where, Q = quantity of P in (µg) read on X- axis against a sample a sample reading.

V = volume (ml) of Olsen reagent

A = volume (ml) of aliquot used for color development

S = Weight (gm) of soil sample taken

Thus, Available P (Kg ha-1) = Qx8.96

## B-3 Determination of Available Potassium

The Ammonium acetate method to determine available potassium is based on the determination of these two easily available fractions.

Instrument used- Flame photometer, mechanical shaker and pH meter.

Reagent Used - 1N ammonium acetate and standard K solution (1000 mg $L^{-1}$ K solution)

Procedure- 1gm of soil sample was weighted in a 100 ml conical flask, 25 ml of the neutral 1N ammonium acetate solution was added shaken for five minutes and the solution was filtered through What man No 1 filter paper. Concentration of K in the filtrate was measured using flame photometer.

Preparation of standard curve for potassium - Suitable volumes of standard K solution was diluted to get 100 ml of working standard containing 10, 15, 20, 25, 30 and 40 mg $KL^{-1}$. Reading of the flame photometer was recorded for each of working standards of K after adjusting blank to zero. A standard curve was drawn by plotting the reading against K concentrations.

Calculation- Available K (Kg $ha^{-1}$) = $C \times \frac{25}{5} \times \frac{10^6}{10^6} \times 2.24 = C \times 11.2$

Where, C stands for the concentration of potassium in the sample obtained on X- axis, against the reading.

## 2-C. MICRONUTRIENTS

Available metallic ions (Zn, Mn, Cu, Fe) The method commonly used for determining the available micronutrients in soil sample is given by Lindsay and Norvell, (1978).

Principle - DTPA as a chelating agent combines with free metal ions in the solution to form soluble complexes. Stability constants for the simultaneous complxing of Zn,Cu,Mn, and Fe shows DTPA as a most suitable extractant. Triethanolamine (TEA) is used as buffer because it burns clearly during atomization. (pH -7.3).

Apparatus - Analytic balances, Narrow mouth polyethylene bottles with stoppers, vials, funnels, Pipette, Reciprocating electric shaker, Whatman's paper(1/42),Atomic Absorption Spectrophotometer(AAS) and Hollow cathode lamps of Zn, Mn, Cu, Fe were required.

Reagents -Extracting solution-0.005 M DTPA, 0.01M $Cacl_2.2H_2O$ and 0.1M TEA adjusted to pH 7.3 dissolve 1.967 gm DTPA and 13.3 ml TEA in deionized or glass distilled water. Add 1.47gm $CaCl_2.2H_2O$ to about 500 ml deionized or add distilled water taken in one liter volumetric flask and add the DTPA. TEA mixture to it and make final volume to about 900 ml. Adjust pH to 7.3 using 1N HCl, make the final volume to one liter and thoroughly.

Stock Standard Solutions-The standard solutions of different micronutrients cation should be prepared preferably by using their foil or wire (A R grade). Dissolve 0.1gm the foil in dilute HCl (1+1) and make the volume to one liter with deionization water to obtain 100µg/ml (i.e. mg/L or ppm) solution of every micronutrient cation.

Alternatively, analytical grade salts can be used to prepare stock standard solutions of different micronutrients.

Working Standard solutions -

**i. Zinc-**

Transfer 10ml of stock standard solution to 100ml volumetric flask and dilute up to the mark with DTPA extracting solution to have a stock solution of 10µgZn/ml (10ppm).Take 0,1,2,4,6 and 8ml of stock solution(10µgZn/ml) to a series of 100ml volumetric flask and dilute each to the mark with DTPA extracting solution.

This will give standard solutions having zinc concentration 0, 0.1, 0.2, 0.4, 0.6 and 0.8 µg/ml (ppm).

### ii Iron-

Transfer 0,1,2,4,6 and 8ml of stock solution(100 µgFe/ml or 100ppmFe) to a series of 100ml volumetric flask and dilute each to the mark with DTPA extracting solution. This will give standard solution having iron concentration of 0, 1,2,4,6 and 8 µg/ml (ppm).

### iii Copper-

Transfer 0, 1,2,4,6 and 8ml of stock solution (100µg Cu/ml or 100ppm Cu) to a series of 100ml volumetric flask and dilute each to the mark with DTPA extracting solution. This will give standard solution having copper concentration of 0, 1,2,4,6 and 8 µg/ml (ppm).

### iv Manganese-

Transfer 0, 1,2,4,6 and 8ml of stock solution (100µgMn/ml or 100ppmMn) to a series of 100ml volumetric flask and dilute each to the mark with DTPA extracting solution. This will give standard solution having manganese concentration of 0, 1,2,4,6 and 8 µg/ml (ppm).

**A**- Extraction of soil samples- 10g air dried sample transferred in to 100ml conical flask and add20ml of DTPA extracting solution shake by electric shaker for two hours at $25^0$C, then filter the content by whatman filter paper. Keep the filtrate in bottle to be analyzed for Zn,Cu,Fe,Mn with an atomic absorption spectrophotometer.

**B**- Analysis of extracts -The micronutrient cation Zn,Cu,Fe,Mn in the soil extracts, obtained by the above described procedure can be determined with the use of atomic absorption

spectrophotometer, which is calibrated to display the concentration of a micronutrient in ppm directly in the soil extract.

## (3) Roll Towel Experiment (Germination Testing)

Germination testing is considered as the most important quality test in evaluating the planting value of seed lot. The ability of produce normal seedlings and plants later on is measured in terms of germination test. Testing of seeds under field conditions is normally unsatisfactory as the results cannot be reproduced with reliability. Laboratory methods then have been conceived wherein the external factors are controlled to give the most uniform rapid and complete germination. Testing conditions in the laboratory have been standardized to enable the test results to be reproduced within limits as nearly as possible as determined by random simple variations.

### 3-A General Requirements for Germination

Seed require certain conditions for normal germination the most important requirements are substrata, moisture, temperature and light.

- **Suitable Substratum-** The substratum serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substratum are paper, (filter paper, blotter or towel, Kraft paper) sand (washed sterilized) and soil (pH 6.0-7.5).

- **Adequate Moisture -** High concentration of water at cellular level is necessary for the seed to start germination. The moist substrata are sufficient to rehydrate, too much water would allow fungal growth and decay of seeds.

- **Favorable Temperature -** Germination occurs under different ranges of temperatures provided the seed is given adequate

moisture, seeds of most of the plants germinate in the temperature ranges of 10-35$^0$C.

- **Light -** Some crops is not required during germination test. However presence of light is desirable to enable the evaluation of seedling easier and with greater certainty.

## 3-B Procedures

Working sample-certified seeds were collected from seed certification agency, Seed Testing Laboratory, Bajrang Nagar, Kota (Raj). 100 seeds are counted at random from the well-mixed pure seed as replicates of 100 seeds are normally used. We have selected four different legume plants that are *Cyamopsis tetragonoloba* L *(RMG1002), Medicago sativa* L *(T9), Trigonella foenum graecum* L *(SWATI1), Glycine max* L *(JS335)* for study of germination experiments. 30 seeds of each plant were taken and soaked in to distilled water for 12 hr followed by 4 hr soaking in to pre prepared solutions of different concentrations of flyash control, 20 %,40%,60%,80%100% (only spry of ash) then wash the treated seeds with distilled water thrice and these counted seeds were placed between paper towels (10seed/towel) and arrange these rolled towels properly in the seed germinator, and set the germinator at optimum range of temperature and humidity according to crop requirement. Paper substrates are used for the following methods-'TP' (Top of paper), 'BP' (Between paper), Methods 'using soil'.In present study we use BP method (Plate-7,8).

- **BP (Between Paper) –**

The seeds are germinated between two layers of paper. This may be achieved by loosely covering the seeds with an additional layer of paper or by placing the seeds in rolled towels packed by rubber bands and the rolled towels are to be placed inside the germinator

in an upright position. (95-99% relative humidity)(Table-5)(Plate-9-12)

❖ **Moisture and Aeration**-

The substrate must all times contain sufficient moisture and air to meet the requirements for germination. The initial quality of water to be added will depend on the nature and dimension of the substrate and also on the size and species of the seed to be tested.

## 3-C Categories of seedling

Normal seedling is one which shows the capacity for continued development into mature plant when grown in good quality soil and under favorable conditions of water supply, temperature and light. According to International Seed Testing Association (1985) seedlings to be classified as Normal seedling, Abnormal seedling, Ungerminate/nongerminate seedlings.

**A** **Normal Seedling -** Seedling with all their essential structures well developed complete in all proportion and healthy.

**B** **Abnormal Seedling** - An abnormal seedling is one which does not have the capacity to develop in to a normal plant when grown in the under favorable conditions because one or more the essential structures is irreparably defective. These are damaged seedling (any of the essential structure is missing), deformed or unbalanced seedling (week and unbalanced development), decayed seedling (essential structures are diseased or decayed due to primary infection).

**C** **Ungerminated Seed –** Seed which have not germinated by the end of test period when tested under favorable

conditions they may be hard seed, fresh seed, dead seed. In present studies non germinated seeds are of third category i.e. dead seeds, it is conformed as dead seed collapses and a milky paste comes out when pressed at the end of the test. (Plate-13)

## Table- 1: Germination procedure for 'Roll Towel Method'

| Crop- Botanical name | Common name | substrata | Temperature ($^0$C) | First count | Final count | Other treatments |
|---|---|---|---|---|---|---|
| ***Cyamopsis tetragonoloba (RMG1002)*** | guar | BP soil | 20-30 | 4 days | 10 days | Non |
| ***Medicago sativa (T9)*** | rijca | BP,TP soil | 20 | - | 10 days | Non |
| ***Trigonella foenum graecum (SWATI1)*** | methi | BP,TP soil | 20-30 | 5 days | 15 days | Non |
| ***Glycine max (JS335)*** | soya bean | BP soil | 25 | 5 days | 8 days | Non |

Source-International Seed Testing Association (1985)

# (4) Pot experiment

For Germination Trey we have selected four legume plants that are *Cyamopsis tetragonoloba* L (RMG1002*), Medicago sativa* L (T9)*, Trigonella foenum graecum* L (SWATI1*), and Glycine max* L (JS335*)* for study of growth parameters. For Biochemical analysis, microbiological study some plants were grown in Pots filled with different concentration of soil and flyash as-control (100%soil), 5% (5%FA+95%soil), 10% (10%FA+90%soil), 15% (15%FA+85%soil), 20% (20%FA+80%soil), 25% (25%FA+75%soil) and 40%.(40%FA+60%soil).

## 4- A. Collection of Data from Germination Tray / Pot Experiments –

Vegetative and nodulation characters of plant (2-3 months). Every amendment have established in triplets. Ten seed were sown in each pot during year 2011-2012, for nodulation studies, seeds of selected legumes were raised in earthen pots using sterilized garden soil and fly ash in different concentrations. Three pots were maintained for each amendment. The pots were watered regularly and pot was maintained with at least 10 plants. Random samples of plants were taken from each replicate pot of different flyash concentrations at 35 and 90 days after sowing for analysis of growth parameters and biochemical analysis. (Plate-14-17)

Data of nodule initiation for all the plants was recorded by observing the root system of one plant of each pot after each 5 days from day of sowing. one plant of each pot were gently uprooted for the studies on nodulation characters like size, shape, color, number, distribution, fresh weight, dry weight of root.

Plants containing intact roots were carefully dug out at random from each pot, thoroughly washed (i.e. placed them on sieves of 1mm mesh size under running tap water) to remove soil particles. Lengths of root and shoot were separately measured and added for total plant length. The roots and shoots of plants were separated and oven dried at 80 $^{0}$C till

constant weight. For total plant biomass, dry weight of root and shoot were added.

Among the all three replicate pots of a plant, the plant with highest number of nodules/plant and biomass was selected for microbiological studies of root nodule bacteria.

## 4 -B. Biochemical Analysis

**a** - 35-50days old plant samples was taken for spectrophotometric studies.

**b** - Whole plant was taken for sample analysis.

**c** - Fresh plant material was taken for analysis.

**d** - Chlorophyll content (chl a, chl b, carotenoids, Total chlorophyll), Carbohydrate, Protein, Phenol, Ascorbic acid, Proline etc

**e** - Fully expended fresh leaved plants were sampled randomly from each replicate pot for various biochemical analyses at 35 and 50 days, and kept in deep freezer for further estimation of photosynthetic pigments, carbohydrate, protein, phenol, ascorbic acid and proline contents.

## 4-B-i CHLOROPHYLL CONTENT –

Procedure- Chlorophyll and carotenoids contents were extracted from the leaf disc with80% acetone and estimated by method adapted by Arnon (1949).The pigment in each sample of 100mg fresh leaves, cut into small pieces and extraction with 80% acetone the homogenate was centrifuged at 3000 rpm for 15 min, the process was repeated till the residue was completely devoid of pigments. the extract was made up to 10 ml with 80% acetone. The optical density tract was made up to 10 ml with 80% acetone. The optical density at 450,510,645 and 663 nm wave length using spectrophotometer against 80% acetone as blank. the

amount of chl a and chl b and total chlorophyll was calculated by following formula given by Arnon,(1949) the value of chl a, chl b, total chlorophyll and cartenoids were expressed in terms of mg/1 mg of fresh weight.

**Chl a .mg/gm** = (12.7 x $OD_{663}$- 2.69 $OD_{645}$ x V) /a x1000 x W

**Chl b mg/gm** = (22.9 x $OD_{645}$-4.68 $OD_{663}$ x V) /a x1000 x W

**Total Chl** = (20.2 x $OD_{645}$ +8.02 x $OD_{663}$) /a x 1000 x W

Where, OD = optical density, V= volume of extract, a= length of light path in cell (1 cm), W = fresh weight in gm

**4-B-ii CARBOHYDRATE CONTENT**

Requirements - Spectrophotometer (systronic UV-VIS), Phenol (5% solution in water), Sulphuric acid (concentrated), Standered glucose(100mg/100ml),

Procedure-In the present studies total soluble sugar contents of different plant samples collected from growing the four experimental plants in different concentrations of fly ash. The amount of total soluble sugar was estimated by phenol sulphuric acid reagent method (Dubois *et al,* 1956). This method is based on the principle that in hot acidic medium glucose is dehydrated to form hydroxyl methyl furfural which combines with phenol forming a coloured complex.

Alcoholic extract of plant sample was used for estimation of total soluble sugar. To 1.0ml of extract, 1.0ml of 5% phenol was added and mixed. To this, 5.0ml sulphuric acid was rapidly added to tubes (gently agitated)and placed at water bath at 26-36$^0$C for 20 minutes. The optical density of characteristic yellow orange color was measured at 490 nm in spectrophotometer (systolic UV-VIS) against blank. Blank was prepared by 1.0ml of 80% ethanol 1m of 5% phenol solution and 5.0ml of 96% sulphuric acid. The quantity of sugar was expressed as mg/g fresh weight of sample. The standard curve was prepared by using known concentration of glucose and their respective optical density which

followed Lambert Beers low. All samples were analyzed in the same way and contents of total soluble sugar and starch were calculated by computing optical density of each of sample with slandered curve.

### 4-B-iii PROTEIN CONTENT

Requirement - Spectrophotometer (systronic UV-VIS), Alkaline sodium carbonate (2% $Na_2CO_3$ in 0.1NaOH), Copper sulphate-sodium pottassium tartrate,(Alkaline solution prepared fresh), Folin-Ciocalteau reagent, Bovin serum albumin.

Procedure- In the present studies protein content was estimated by Folin-Lowry's method (1951). Protein reacts with folin ciocalteau reagent to give a coloured complex. This color is produced by the reduction of phosphomolybdate by tyrosin and tryptophan of protein by action of alkaline copper. In this method plant residue (sample+trichoroacitic acid) homogenized and dissolved in .1N NaOH and water, then alkaline copper reagent was added to the dissolved residue and 0.5ml of folin–ciocalteau reagent was added rapidly and mixed immediately, 10 min after optical density at 750nm was measured against blank, the amount of protein in samples was calculated with a standard curve prepared from bovine serum albumin.

### 4-B-iv ASCORBIC ACID CONTENT

Ascorbic acid was estimated titramatrically (Olson and Hodges1987). Ascorbic acid reduces the 2, 6- dichlorophenol indophenols dye to a colorless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. The dye is pink colored in acid medium. Oxalic acid (4%) is used as the titrating medium.

Requirements - Micro burette, pipette, measuring flask, conical flask, beaker.

Oxalic acid 4%

Dye solution- Prepared by weigh 42mg sodium bicabarbonate in to a small volume of distilled water. Dissolve 52mg 2, 6-dichlorophenol indophenols (sodium salt) in it and make up the volume up to 200ml with distilled water.

Stock standard solution- Dissolve100mg ascorbic acid in 100ml of 4%oxalic acid solution in a standard flask. (1mg/ml)

Working standard- Dilute 10ml of stock solution to 100ml with 4%oxalic acid. The concentration of working standard is 100µg/ml.

The assay method is titramatrically method i.e. pipette out 10ml of working standard solution into 100ml flask,then add 20ml of 4%oxalic acid and titrate against the dye($V_1$), the end point is the pink colour which persist for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. Now extract the sample (0.5-5g) in 4%oxalic acid with the help of mortar and pestle, filter with muslin cloth and make up to a known volume (100ml) and pipette out 10ml of this sample, add oxalic acid and titrate against the dye ($V_2$). Amount of ascorbic acid mg/100gm of sample was calculated.

**4-B-v PHENOL CONTENT-**

Requirement - Spectrophotometer, folin ciocalteau reagent, $Na_2CO_3$, catechol.

Procedure - Total phenol content in each sample was estimated by the spectrophotometer method of Malik and Singh 1980. Aliquot of the extracts were taken in a 10 ml glass tube and made up to a volume of 3ml with distilled water. Then o.5 ml folin ciocalteau reagent(1:1with water) and 2ml $Na_2CO_3$ (20%) were added, sequentially in each tube, A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The test solution was warmed for 1 minute, cooled and absorbance was measured at 650nm against the reagent used as a

blank. A standard calibration plot was generated at 650nm using known concentrations of catechol. The concentrations of phenols in test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample. Estimation of Phenols with Folin-ciocalteau reagents is based on the reaction between phenol and an oxidizing agent's phosphomolybdates which results in the formation of a blue complex, the intensity of colored complex is measured in a colorimeter (650nm) or in spectrophotometer (725nm).

**4-B-vi PROLINE CONTENT-**

Requirement - Colorimeter, Proline, ninhydrin, glacial acetic acid, toluene

Procedure - For Proline colorimetric determination a 1:1:1 solution of Proline, ninhydrin, and glacial acetic acid was incubated at $100^0$c for one hour, the reaction was arrested in an iced and the cromophore was extracted with 4ml toluene and its absorbance at 520nm was determined in a spectrophotometer. The method was calibrated for each determination with standard Proline solution within the detection range of the method.(0-39µgm$^{-1}$).Proline accumulation under salt stress in both root and leaf tissues(Aziz *et al*1999). The accumulation of solutes like Glycine and Proline has linked to water stress, salinity and other abiotic plant stress. (Ashraf and Harris 2004).

# (5) Nodulation Studies-

**5-A Collection and Preservation of Nodules**.

Plant roots of legumes bearing nodules were collected from control and 25% concentration of fly ash amended soil of 40-60days of plantation. Roots were uprooted and washed in running tap water to obtain root nodule. Data on plant height, root length, dry biomass, number of lateral roots and nodules/plant were collected. The portion of the nodule-containing rhizobia can be located by noting the area with the reddish

pigment leg-hemoglobin. A nodule contains rhizobia within it but has many other microorganisms on its surface. These surface microorganisms must be prevented from contaminating the rhizobia portion of the nodule by surface sterilizing the nodule.

Isolation is easiest from fresh nodules cut from the root about 0.5 cm on either side of the nodule with small scissors or a sharp knife. Preservation of nodules for a few weeks before isolation of rhizobium may be accomplished by keeping the nodules in capped vials in a freezer. Nodule stored in this manner will dehydrate by desiccant (silica gel or anhydrous $CaCl_2$) and may be kept for weeks at room temperature before isolations are made. Rehydrate the nodules for isolation by soaking them for a few minute in sterile water.

**5-B Culture Media-**

The complex medium generally used for growth of rhizobia is a modification of medium 79 described by Fred and Waksman (1928). The medium is referred to as yeast extract mannitol agar (YEMA) and contains 10gm of mannitol,0.5 gm of potassium monohydrate phosphate ($K_2HPO$,), 0.2 gm of magnesium sulfate heptahydrate ($MgSO_4$ $7H_2$ O), 0.1 gm of sodium chloride (NaCI), 0.01 gm of calcium carbonate ($CaCO_3$) 0.5 gm of yeast extract powder,15gm of agar, and 1,000 ml of distilled water. Leave the agar out to prepare yeast extract-mannitol broth (YMB). Adjust the pH to7.0 with 1N hydrochloric acid (HCI) before autoclaving.

Isolation and purification of Rhizobium bacteria. Each major legume group is nodulated by different species of Rhizobium. Fred and his associates (1932) recognized eight cross inoculants group in legumes. The genus Rhizobium was erected by Frank, (1890) based on its characters to form nodules on roots of legume plants. This property is the only valid test in the identification of the organism. Apart from it some diagnostic features of Rhizobium could be conveniently not only determine and identify the organism but also delineate different species (Graham and Parker, (1964) Vincent, (1970), Gaur, (1975), Mahana,

(1981).Therefore the attempt has been made to study the morphological and biochemical characters of the bacterium. Host legumes nodulated by specific species of root-nodule bacteria. *Trigonella faenum-graecum* L is nodulated by *Rhizobium meliloti. (Sinorhizobium melitoti). Glycine max* L is nodulated by *Brabyrhizobium japonicum. Cyamopsis tetragonoloba* L is nodulated by *Rhizobium leguminosarum. Medicago sativa* L is nodulated by *Rhizobium meliloti (Sinorhizobium melitoti*).

## 5-C Isolation of Rhizobial Strains-

Plant roots of legumes bearing nodules were collected from 25% concentration of fly ash amended soil pots healthy pink nodules were collected carefully washed with sterile water followed by surface treatment with 95%alcohol and again with sterile water. The washed nodules were surface sterilized by immersing in 0.1% HgCl2 solution for 5min. Then nodules were washed with sterilized water 5times to get the sterilizing agent. The surface sterilized nodules were crushed in sterilized petriplates this nodule suspension was then serial diluted (10-5 to 10-7) streaked on the sterilized YEMA medium plates. The plates were incubated for 3to8 days at $28^0$C. Rhizobium was obtained from these nodules. Isolated colonies of rhizobia were transferred on YEMA medium slopes and stored in the refrigerator for further studies. Rhizobia colonies on YEMA may appear in 2 to 4 days for the fast growing rhizobia and from 3 to 9 days for the slow-growing rhizobia depending on the physiological state of the rhizobia at the time of plating.

## 5-D Study on Microbiology Character-

The identity of the isolates as rhizobium was established by characterization tests including Gram staining, growth on YEMA medium on Congo red (2.5 ml/1 of 0.1% solution), after confirmation as rhizobium strain, pure cultures maintained on basal media were used in the study. all the 8 samples 4 plants x 2 (control + 25%FA amended soil) were screened for their ability to produce different enzymes involved in

biochemical reaction following standard methods (Dubey and Maheswari 2002, Aneja 1996).

## 5-E Characterization of Authenticated Isolates-

The morphological and cultural as well as physiological or bio-chemical characteristics of the authenticated isolates were studied following the procedure given by Aneja (1996).

## (6) Morphological and Cultural Characteristics-

**6-A Morphological Characteristics-** The size, shape, motility and Gram stain reaction of rhizobial cells were observed under microscope using standard procedure.

**I Gram Staining of Bacteria –**

The gram negative bacteria cell wall is thin complex multilayered structure and contains relatively a high lipid contains, in addition to protein and muco-peptides, the higher amount of lipid is readily dissolved by alcohol resulting in the formation of large pores in the cell wall which do not close appreciably on dehydration of cell wall protein, thus facilitating the leakage of crystal violate iodine (CV-I) complex and resulting in the decolonization of bacterium, which later takes the counter stain and appear red.

Make a thin smear of culture on glass slides dried the smear and heat fix, cover the smear one by one with crystal violet (60 sec), gram iodine (60 sec) 95% $C_2H_5OH$ (20 sec) and safranin (40 sec). Air dried slides after washing with D/W and observed under microscope. (Table-10-13)(Plate-18, 22, 26, 30)

**II Motility –**

SIM agar may also be used to detect motile organisms. Motility is recognized when culture growth (turbidity) of flagellated

organisms is not restricted to the line of inoculation. Growth of non motile organisms is confined to the line of inoculation.

**6- B. Colony characteristics-**

The configuration, margin, elevation and colour of the colonies of the test isolates grown on standard YEMA plates were observed.

**Cultural Characteristics**-Strains was spread over YEMA agar plates strains were also streaked on YEMA agar. The inoculated plates were incubated at 28˚C for 48 hours and observed for colony shape, size, colour and texture. The shape, color, opacity, margin and elevation of the colonies of the test isolates grown on standard YEMA plates were observed. The results are shown in (Plate-18, 22, 26, 30).

## (7) Biochemical Characteristics –

Biochemical methods were performed to differentiate the unknown cultures and for this, various tests were performed.

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja (1996). The biochemical tests were carried out in growth medium at 28˚C for 48 hours incubation. All the tests were carried out with 03 replicates.

**Identification of Rhizobium**- To study the fast or slow growing nature of the isolates they were grown on freshly prepared YEMA plates containing bromo-thymol blue adjusting the pH to 6.8.

**7-A Indole Test –**

To determine the ability of organism to degrade the amino acid tryptophan. The tube containing (SIM Agar), Peptone-30gm, Beef extract-3gm, Ferrous ammonium sulphate-0.2gm, Sodium thiosulphate-0.025,

Agar-3.5gm, Distilled water-1000ml, pH-7.3 were inoculated by isolated by Rhizobium culture. After 24 to 48 hours of inoculation on addition of Kovac's reagent,change in colour of media was observed, and it also observed that after addition of Kovac's reagent a red colour ring formed.(Plate-19,23,27,31)

**7-B Methyl Red –**

To determine the ability of microorganisms to oxidize glucose with the production and stabilization of high concentration of acid end products. The tubes containing (MR-VP Broth) Peptone-7.0gm, Dextrose-5.0gm, Potassium phasphate-5.0gm, Distilled water-1000ml, pH-7.3 were inoculated by isolated Rhizobium culture. After 24 to 48 hours of incubation on addition of Methly red reagent, change in colour of the medium was observed and observed that red colour was appeared after the addition of methyl-red reagent. (Plate-19,23,27,31)

**7-C Voges-Proskauer-**

The voges proskauer test determines the capability of organism to produce non acidic or neutral end products.The tubes containing (MR-VP Broth) Peptone-7.0gm, Dextrose-5.0gm, Potassium phasphate-5.0gm, Distilled water-1000ml, pH-7.3 was inoculated by isolated Rhizobium culture. After 24-48 hours of incubation on addition of Barrit's reagent, change in colour of the medium was observed and find that there was no colour change was observed.(Plate-19,23,27,31)

**7-D Citrate Utilization** –

To detect the ability to ferment citrate as sole carbon source. The tube containing(Simmons Citrate Agar) Ammonium di hydrogen phosphate-1.0gm, Di potassium phosphate-1.0gm, Sodium chloride-5.0gm, Sodium citrate-2.0gm, Magnesium sulphate-0.2gm, Agar-15gm, Bromothymol Blue-0.08gm, Distilled water-1000ml, pH-6.9 were inoculated by Rhizobium culture, After 24-48 hours of incubation change in the colour of the media was observed and result showed change in

colour of medium. Citrate Utilization ability was determined, by replacing mannitol from YEMA agar with equal amount of sodium citrate and bromothymol blue. The plates with modified media were inoculated then incubated for 48 hours. (Plate-20,24,28,32)

**7-E Urease Production –**

To determine the ability to degrade urea by means of the enzyme. The tube containing (Urea Broth) Yeast extract-0.1gm, Potassium di hydrogen phosphate-9.1gm, Di potassium hydrogen phosphate-9.5gm,Phenol red-0.01gm, Distilled water1000ml,pH-6.8(*after sterilization add 20% urea solution previously sterilized by filtration) were inoculated by isolated bacterial culture ie Rhizobium. After 24-48 hours of incubation, change in the colour of the media was observed and change in colour of media was observed. (Plate-20,24,28,32)

**7-F $H_2S$ Production** –

To determine the ability of rhizobium to produce hydrogen sulfide from substrate such as sulfur containing amino acids.

SIM agar medium prepared, pour in to SIM Agar deep tubes and autoclaved, After cooling aseptically incubate in to its appropriately labeled tube by means of stab inoculation, incubate all cultures for 24-48 hours at $37^0$C.change in colour of media was observed.(Plate-20,24,28,32)

**7-G Nitrate Reduction**-

To determine the ability of rhizobium to reduce nitates ($NO_3$ ) to nitrites. The tube containing (Trypticase nitrate broth) Trypticase-20gm, Di sodium phosphate-2.0 gm, Dextrose-1.0 gm, Potassium nitrate-1.0 gm, Agar-1.0 gm,Distilled water-1000 ml, pH-7.2 were inoculated by isolated bacterial culture. After 24-48hours of inoculation on addition, solution A (Sulphanilic acid) and Solution B (α-naphthylamine) and zinc power,

change in colour of media was observed and colour of media found changed. (Plate-21,25,29,33)

**7-H Catalase Activity** –

To determine the ability of some rhizobium to degrade hydrogen peroxide by producing the enzyme catalase. Slide containing 2-3 drops of (Trypticase soya broth) Trypticase-15gm, sodium chloride-5.0gm, Distilled water-1000ml, pH-7.3 were inoculated by 24-48 hours isolated Rhizobium culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Bubbles were appeared on the slide. Different isolates which were 48 hour old were flooded with hydrogen peroxide and observed for liberation of effervescence of oxygen around the bacterial colonies according to Graham and Parker (1964). (Plate-21, 25, 29, 33)

# CHAPTER – 5

# OBSERVATION AND RESULTS

# OBSERVATION AND RESULTS

The observations and results of present study are divided in to four parts. The first part deals with survey of study site, collection and analysis of flyash and soil sample and selection of four legumes for further study. The second part deals with seed germination test and morphological studies of flyash treated seeds (selected legumes) to find out appropriate concentrations of for pot experiments. Third part includes ecomophological and biochemical study of selected legumes grown in different soil amendments with flyash. Fourth part deals with isolation purification and culture of rhizobium bacteria from control and 25% flyash amendments, along with biochemical testing to analyze impact of flyash on various Rhizobium strains.

## (1) SURVEY AND COLLECTION

### 1-A Flora of Area of Kota Thermal Power Station (KTPS)

The present work is based on the result of two years systematic survey of vegetation near Kota thermal power station. The aim of this study is to find out the resistant species in polluted area, tolerant species may be used as bio-monitor for monitoring the quantitative and qualitative level of pollutants. The dominant plant species in the site were marked recorded and collected. Observation of injury on sensitive plant has provided a means of monitoring air pollution, however under acute pollution defoliation takes place. These responses may reflect in periodic phenomenon of plants. Foliar damage was morphological criteria for knowing about the impact of emissions of Kota thermal power station plants. It was observed that study area experienced the condition of drought except a wet spell of two or three month's i.e. late June to early September, herbaceous annuals had luxuriant growth during the rainy

period and slowly disappear except few native wild vegetation cover as *Croton bonplandianum Baill, Chenopodium album* L, *Euphorbia hirta* L, *Phyllanthus fraternus Web, Alternanthera pungens Kunth, Tridex procombens* L. (Table-2). It is observed that the vegetation around thermal power station, though not exhibiting the apparent symptoms of pollution injury, but constantly under stress due to pollution. The result indicates that ground vegetation i.e. herbs and the shrub seem to be more sensitive to pollution induced changes then the trees. Herbal species were the most susceptible while already established woody species could cope-up with the pollution. Though under stress, they did not show any permanent visible injury. In the comparative study between two decades some common plants were identified that were remain unchanged e.g. *Cassia siamea L, Cassia fistula L, Azadirachta indica Juss, Delonix regia L, Bauhinia variegate L, Albizzia lebbeck L, Ipomoea fistulosa, Thevetia peruviana Pers, Zizyphus nummularia W.A, Solanum xanthocarpum S.W, Chenopodium album, Euphorbia hirta L, Phyllanthus fraternus W, Croton bonplandianum Bail, Alternanthera pungens Kunth, Tridex procombens L*.(Table-2)(Plate-4-6) These plants may provide a natural sink for pollutants of KTPS.

To know Comparative status of plant diversity at Kota Thermal Power Station survey was conducted and the result were compare with the data collected during the review of literature. The review of literature indicates that the dominant tree species observed were *Cassia siamea L, Cassia fistula L, Azadirachta indica Juss,* and *Albizzia lebbeck L* while Mangifera *indica L* and *Azadirachta indica Juss* show more resistance to emissions as compared to other tree species. *Cassia siamea L, Cassia fistula L, Azadirachta indica Juss, Delonix regia L, Bauhinia variegate L, Albizzia lebbeck L* were find near the campus while *Anoegissus spp Roxb, Acacia nilotica L, Zizyphus nummularia Wt,&Arn* were dominant far away from main plant and near to fly-ash dumping area (Table-2,3). in present study, 38 species of shrubs and herbs were observed in which 7 shrubs present dominantly were includes *Ipomoea fistulosa, Lantana*

# Table-2 : Flora of KTPS (2011-2012)

| Sr. No. | NAME OF PLANTS | FAMILY |
|---|---|---|
| *1* | ***Abutilion indicum* L** | ***Malvaceae*** |
| *2* | ***Acacia nilotica* L** | ***Mimosaceae*** |
| *3* | ***Acyranthes aspera* Linn** | ***Amaranthaceae*** |
| *4* | ***Acalypha indica* L** | ***Euphorbiaceae*** |
| *5* | ***Ageratum conyzoides* L** | ***Compositae*** |
| *6* | ***Albizzia lebbek* L Benth** | ***Mimosaceae*** |
| *7* | ***Alianthus excelsa* Roxb** | ***Simaraubaceae*** |
| *8* | ***Alternanthera pungens* Kunth** | ***Amaranthaceae*** |
| *9* | ***Amaranthus spinosus* L** | ***Amaranthaceae*** |
| *10* | ***Amaranthus hybridus* L** | ***Amaranthaceae*** |
| *11* | ***Anogeissus. acunminata* Roxb.Ex.D.C** | ***Combretaceae*** |
| *12* | ***Azadiracta indica* A.Juss.** | ***Meliaceae*** |
| *13* | ***Bauhinia varigata* L** | ***Caesalpiniaceae*** |
| *14* | ***Callistemon citrinus* Curtis** | ***Myrtaceae*** |
| *15* | ***Cassia siamea* Lamk** | ***Caesalpiniaceae*** |
| *16* | ***Casuarina equisetiflolia* L** | ***Casuarinaceae*** |
| *17* | ***Celosia argentea* L** | ***Amaranthaceae*** |
| *18* | ***Chenopodium album* Linn** | ***Chenopodiaceae*** |
| *19* | ***Coccinia cordifolia* L** | ***Cucurbitaceae*** |
| *20* | ***Commelina attenuate Koenig ex vahl*** | ***Commelinaceae*** |
| *21* | ***Convolvulus microphyllus* Steb** | ***Convolvulaceae*** |
| *22* | ***Corchorus spp*** | ***Tiliaceae*** |
| | | **Cont....** |

| | | |
|---|---|---|
| ***23*** | ***Croton banplandianum* Baill** | ***Euphorbiaceae*** |
| ***24*** | ***Dalbergia* sissoo Roxb** | ***Fabaceae*** |
| ***25*** | ***Delonix regia Baj.*** | ***Caesalpiniaceae*** |
| ***26*** | ***Eclipta prostrate* L** | ***Compositae*** |
| ***27*** | ***Eukalyptus rudis* Endl.** | ***Myrtaceae*** |
| ***28*** | ***Euphorbia hirta* L** | ***Euphorbiaceae*** |
| ***29*** | ***Ficus benghalensis* L** | ***Moraceae*** |
| ***30*** | ***Ficus religiosa* L** | ***Moraceae*** |
| ***31*** | ***Gomphrena celosoides* Mart.** | ***Amaranthaceae*** |
| ***32*** | ***Holoptelea integrifolia*** | ***Ulmaceae*** |
| ***33*** | ***Ipomoea fistulosa mart.exchoisy*** | ***Convolvulaceae*** |
| ***34*** | ***Ipomoea spp*** | ***Convolvulaceae*** |
| ***35*** | ***Indigofera spp*** | ***Papilionaceae*** |
| ***36*** | ***Lantana camera* L** | ***Verbenaceae*** |
| ***37*** | ***Lantana indica*** | ***Verbenaceae*** |
| ***38*** | ***Launaea asplenifolia* Willd** | ***Compositae*** |
| ***39*** | ***Mangifera indica* L** | ***Anacardiaceae*** |
| ***40*** | ***Malvastrum coromandelianum* L** | ***Malvaceae*** |
| ***41*** | ***Mitragyna parvifolia* Roxb** | ***Rubiaceae*** |
| ***42*** | ***Oxalis corymbosa* L** | ***Oxalidaceae*** |
| ***43*** | ***Parkinsonia aculeate* L** | ***Caesalpiniaceae*** |
| ***44*** | ***Peristophe paniculata* L** | ***Acanthaceae*** |
| ***45*** | ***Phoenix sylvestris* L** | ***Arecaceae*** |
| ***46*** | ***Phyllanthus fraternus* Webster** | ***Euphorbiaceae*** |
| | | **Cont....** |

| | | |
|---|---|---|
| ***47*** | ***Polygonum barbatum* L** | ***Polygonaceae*** |
| ***48*** | ***Rumax dentatus* L** | ***Polygonaceae*** |
| ***49*** | ***Saraca indica* Roxb** | ***Caesalpiniaceae*** |
| ***50*** | ***Sida cordifolia* L** | ***Malvaceae*** |
| ***51*** | ***Solanum xanthocarpum* Schrad and Wendl.** | ***Solanaceae*** |
| ***52*** | ***Sonchus asper* L** | ***Compositae*** |
| ***53*** | ***Syzygiun qumini* L** | ***Myrtaceae*** |
| ***54*** | ***Thevetia peruviana* Pers.** | ***Apocynaceae*** |
| ***55*** | ***Tridex procombens* L** | ***Compositae*** |
| ***56*** | ***Tectona grandis* L** | ***Verbenaceae*** |
| ***57*** | ***Tephrosia hamiltonii Drumm*** | ***Papilionaceae*** |
| ***58*** | ***Vernonia albicans* DC** | ***Compositae*** |
| ***59*** | ***Zizyphus nummularia Wt and Arn.*** | ***Rhamnaceae*** |

# PLATE - 4

(A) *Ageratum conyzoides*

(B) *Rinkosia spp*

(C) *Calotropis procera*

(D) *Ziziphus spp*

(E) *Convolvulus microphyllus*

(F) *Cassia spp*

**Showing Vegetation Near Kota Thermal Power Station (KTPS)**

# PLATE - 5

(A) *Alysicarpus spp.*

(B) *Lantana camera*

(C) *Launia asplenifolia*

(D) *Malvastrum coromandeliamum*

(E) *Tephrosia hamiltonii*

(F) *Ipomia spp*

**Showing Vegetation Near Kota Thermal Power Station (KTPS)**

# PLATE - 6

(A) *Euphorbia hirta*

(B) *Amaranthus hybridus*

(C) *Chenopodium alba*

(D) *Croton bonplandianum*

(E) *Tridex procombens*

(F) *Acyranthus aspera*

**Showing Vegetation Near Kota Thermal Power Station (KTPS)**

## Table-3 : Comparative Status of Plant Diversity at K.T.P.S. (1988-2011)

| Sr. No. | FAMILES | PHASE -1 | PHASE-2 |
|---|---|---|---|
| 1 | Acanthaceae | - | 1 |
| 2 | Amaranthaceae | 2 | 7 |
| 3 | Annonaceae | 1 | - |
| 4 | Anacardiaceae | 2 | 1 |
| 5 | Arecaceae | - | 1 |
| 6 | Apocynaceae | - | 2 |
| 7 | Ascelpideaceae | 1 | 1 |
| 8 | Boraginaceae | 1 | - |
| 9 | Capparadaceae | 1 | - |
| 10 | Caryophyllaceae | 2 | - |
| 11 | Casurinaceae | - | 1 |
| 12 | Chenopodiaceae | - | 1 |
| 13 | Cleamaceae | 1 | - |
| 14 | Compositeae | 7 | 6 |
| 15 | Commelinaceae | 1 | 1 |
| 16 | Combretaceae | - | 1 |
| 17 | Convolvulaceae | 4 | 2 |
| 18 | Cucurbitaceae | - | 1 |
| 19 | Cyperaceae | 1 | - |
| 20 | Fabaceae | 6 | 4 |
| 21 | Euphorbiaceae | 4 | 5 |
| 22 | Liliaceae | 1 | - |
| | | | Cont.... |

| | | | |
|---|---|---|---|
| **23** | **Malvaceae** | **2** | **2** |
| **24** | **Mimosaceae** | **4** | **2** |
| **25** | **Meliaceae** | **1** | **1** |
| **26** | **Moraceae** | **-** | **2** |
| **27** | **Myrataceae** | **1** | **1** |
| **28** | **Nyctaginaceae** | **1** | **-** |
| **29** | **Nyctanthaceae** | **1** | **-** |
| **30** | **Oxalidaceae** | **-** | **1** |
| **31** | **Papaveraceae** | **1** | **-** |
| **32** | **Polygonaceae** | **-** | **2** |
| **33** | **Poaceae** | **5** | **-** |
| **34** | **Rhamnaceae** | **1** | **1** |
| **35** | **Rubiaceae** | **-** | **1** |
| **36** | **Scrophilariaceae** | **1** | **-** |
| **37** | **Solanaceae** | **1** | **1** |
| **38** | **Simaraubaceae** | **-** | **1** |
| **39** | **Tiliaceae** | **-** | **1** |
| **40** | **Verbenaceae** | **-** | **3** |
| **41** | **Ulmaceae** | **-** | **1** |
| **42** | **Zygophylaceae** | **1** | **-** |
| | **Total** | **27** | **29** |

*coromandelian Roxb, Thevetia peruviana pers, Ziziphus nummularia Wt & Arn, Solanum xanthocarpum S.W, Abutilion indicum L* (Table-2,3). Dominant herbs were *Amaranthus spinosus L, Acyranthes aspera L, Chenopodium album L, Euphorbia hirta L, Phyllanthus fraternus W, Croton bonplandianum Baill, Alternanthera pungens Kunth, Tridex procombens L.*

Table-3 Show family wise distribution pattern of plants, changes were clearly observable during two decades, 27 families out of 42 were available, while during recent study, 29 families were observed, although it is not the remarkable difference, but change in dominant family's distribution pattern was found.

## (2) PHYSICAL AND CHEMICAL PARAMETERS OF SOIL AFTER FLYASH AMENDMENTS

A clear change in texture towards coarse to fine particle size along with change in colour i.e. darkest brown to greyish brown with higher percentages of flyash was observe with increasing percentage of flyash amendments. (Table-4)

Decrease soil particle density pattern was observed highest density (1.49g/cc$^2$) was found in unamended soil followed by 5%, 10%, 15%, 20%, 25%, minimum value of density found in 40% flyash amendment. (Table-4) (Figure-1C).

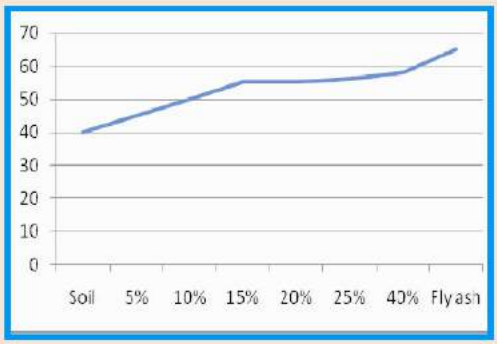
Water holding capacity increases with increased percentages of flyash application (range 40-58%) the maximum value was observed in 40% flyash amended soil, while minimum value observed in untreated soil. (Table-4) (Figure 1, A).

Porosity of soil increased with greatly along with flyash application, ranges from 22-45% that is almost double in control to 40% flyash

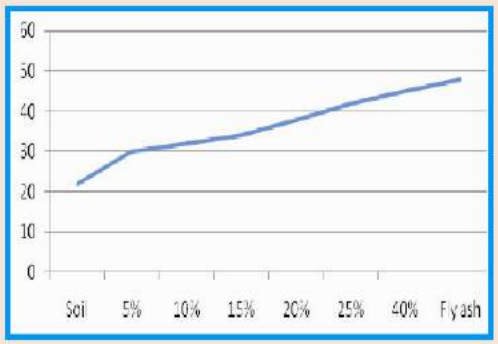
## Table-4 : Comparative Analysis of Physical and Chemical Parameters of Soil After Flyash Amendment

| Parameter | Soil | Fly ash | 5% | 10% | 15% | 20% | 25% | 40% |
|---|---|---|---|---|---|---|---|---|
| Texture | Loam | Silt | Loam | Loam | Silty Loam | Silty Loam | Silty Loam | Silty |
| Water Holding Capacity (%) | 40 | 65 | 45 | 50 | 55 | 55 | 56 | 58 |
| Porosity (%) | 22 | 48 | 30 | 32 | 34 | 38 | 42 | 45 |
| Density (gram /cc$^{2)}$ | 1.49 | 0.92 | 1.3 | 1.28 | 1.25 | 1.22 | 1.21 | 1.18 |
| pH | 6.9 | 8.15 | 7 | 7.12 | 7.15 | 7.16 | 7.16 | 7.2 |
| ElectricalConductivity (dSm-1) | 0.3 | 0.65 | 0.3 | 0.27 | 0.34 | 0.38 | 0.38 | 0.40 |
| Nitrogen (kg/ hect) | 188.16 | - | 200.7 | 225.7 | 222.5 | 223.2 | 218.9 | 215 |
| Phosphorus (kg/ hect) | 12.05 | - | 13.87 | 16.37 | 14.62 | 14.67 | 14.23 | 13.82 |
| $K_2O$ (kg/ hect) | 210.73 | -` | 314.28 | 324.53 | 324.33 | 323.98 | 324.22 | 366.72 |
| Zinc (ppm) | 6.030 | - | 5.756 | 5.656 | 5.654 | 5.759 | 5.843 | 6.487 |
| Manganese (ppm) | 12.28 | - | 12.28 | 12.32 | 12.39 | 12.37 | 12.44 | 12.42 |
| Ferrous (ppm) | 13.63 | - | 13.27 | 13.68 | 14.93 | 15.04 | 14.69 | 15.21 |
| Copper (ppm) | 2.43 | - | 3.44 | 4.916 | 4.98 | 5.27 | 5.32 | 5.22 |

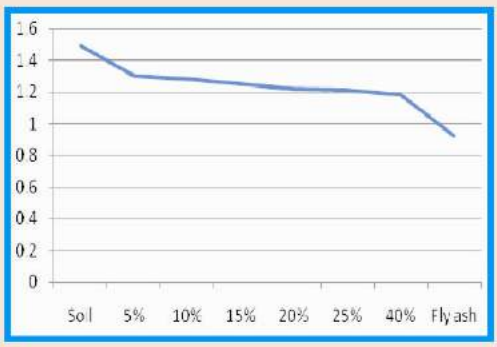
# FIGURE - 1



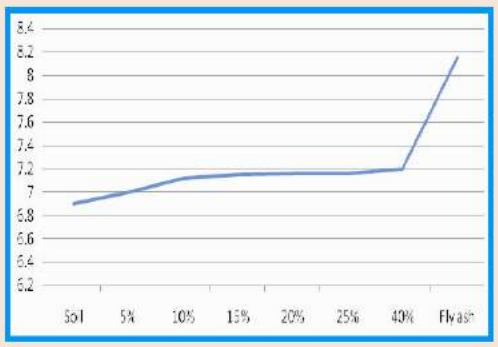

(A) Water Holding Capacity (WHC) %



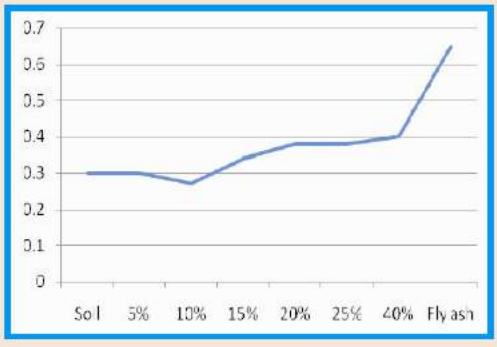

(B) Porosity %



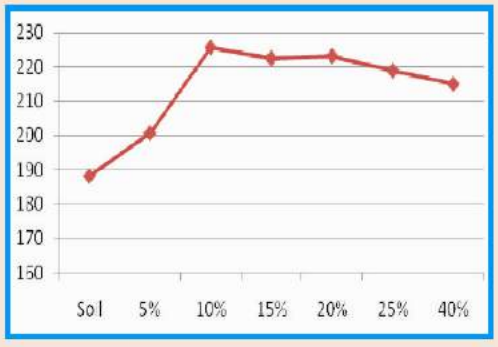

(C) Density (gram/cc)




(D) pH



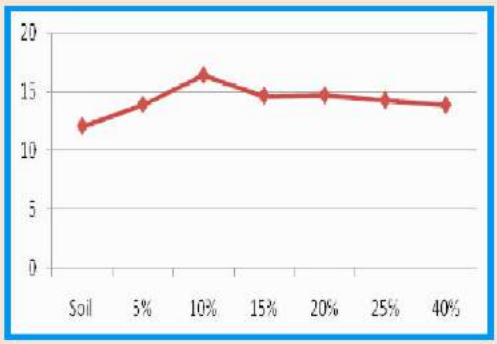

(E) Electrical Conductivity 'EC' $dsm^{-1}$



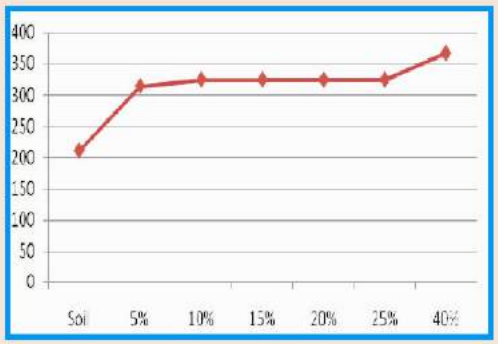

(F) Nitrogen 'N' Kg/hact

**Graph Showing Comparative Study of Physio-Chemical Characters of Soil after Flyash Amendment**

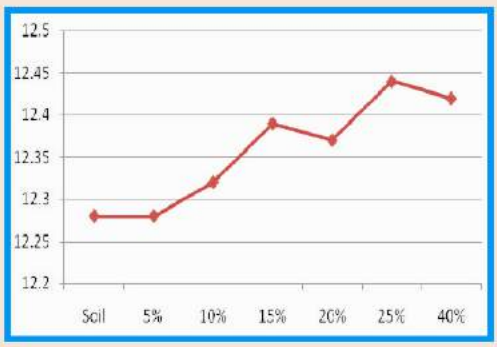
# FIGURE - 2



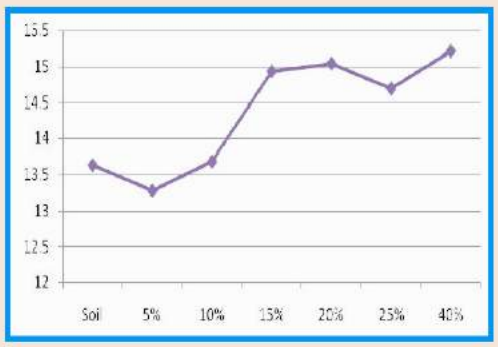

(A) Phosphorus 'P' Kg/hact



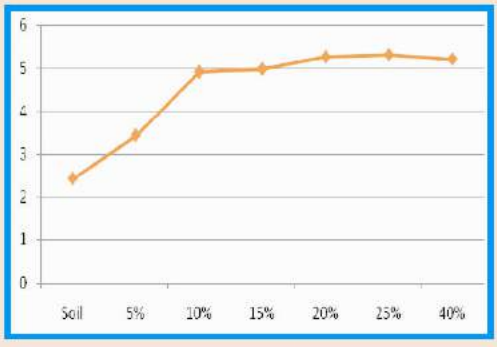

(B) Potassium 'K' Kg/hact



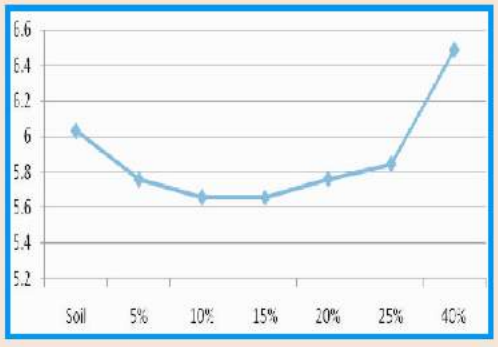

(C) Manganese 'Mg' ppm




(D) Ferrous 'Fe' ppm




(E) Copper 'Cu' ppm




(F) Zinc 'Zn' ppm

**Graph Showing Comparative Study of Physio-Chemical Characters of Soil after Flyash Amendment**

amendment. Increases in porosity in turn decrease the bulk density of soil. (Table-4) (Figure-1B,).

The pH of soil was significantly influenced by flyash application. The table indicates that application of flyash to soil in increased percentages marked increases pH of amended garden loam soil from 6.9-7.2 (Table-4) (Figure-1,D).

Electrical Conductivity also increased with percentage increase in flyash application due to increase the salt content of soil over control. (Table-4) (Figure 1,E).

Flyash (unweathered and fresh) generally contain sufficient concentration of nutrients, however N are usually in small amounts and it is medium in available K and high in available P. Flyash incorporation increased the available N,P,K, content slightly. (Table-4) (Figure-1F,2AB)

Availability of Zn, Mg, Fe, Cu also increased slightly in comparatively control to 40% flyash amendment. But in over all study patterns marked increase was only observed in Fe, Cu whereas Zn, Mg remained almost unchanged with fluctuating results. (Table-4) (Figure 2C-F)

## (3) ECOMORPHOLOGICAL STUDIES-

### 3-A. Comparative Study of Seed Germination Characters in Selected Legume Plants in Response to Flyash Treatment. (Roll Towel Method)

Germination of *Cyamopsis tetragonoloba L, Medicago sativa L, Trigonella foenum graecum L, Glycine max L,* was performed in seed germinator by roll towel method after 20%, 40%, 60%, 80%, 100% of flyash treatment exhibit a promotive response only in lower concentrations of flyash to germination. (Table-5, Plate-9-13). Observation on the effect

## Table –5 : Comparative Study of Germination Parameter of Selected Legume Plant in Response of Flyash Treatment

| | Fly ash Concentration | Number of Seed | Root Length (Avg) | Shoot Length (Avg) | Number of Normal Seedling (Avg) | Number of Abnormal Seeds | Number of Non Germinate | Germination Percentage | Germination Time |
|---|---|---|---|---|---|---|---|---|---|
| ***Cyamopsis tetragonoloba L ( RMG1002)*** | **CONTROL** | **30** | **3.78** | **8.17** | **18** | **4** | **8** | **60.0** | **4 Days** |
| | **20% Fly ash** | **30** | **6.56** | **9.13** | **18** | **4** | **8** | **60.0** | **4 Days** |
| | **40% Fly ash** | **30** | **5.44** | **8.83** | **18** | **6** | **6** | **60.0** | **4 Days** |
| | **60% Fly ash** | **30** | **6.44*** | **9.94** | **21** | **7** | **2** | **70.0** | **4 Days** |
| | **80% Fly ash** | **30** | **7.1** | **9.65** | **20** | **5** | **5** | **66.7** | **4 Days** |
| | **100% Fly ash** | **30** | **7.05** | **10.3** | **15** | **8** | **7** | **50.0** | **4 Days** |
| | | | | | | | | | |
| ***Glycine max L (JS 335)*** | **CONTROL** | **30** | **12.9** | **12.35** | **20** | **10** | **0** | **66.7** | **5 Days** |
| | **20% Fly ash** | **30** | **8.9** | **8.2** | **15** | **15** | **0** | **50.0** | **5 Days** |
| | **40% Fly ash** | **30** | **9.17** | **11.89** | **16** | **14** | **0** | **53.3** | **5 Days** |
| | **60% Fly ash** | **30** | **6.09** | **9.18** | **17** | **13** | **0** | **56.7** | **5 Days** |
| | **80% Fly ash** | **30** | **5.21** | **7.86** | **9** | **21** | **0** | **30.0** | **5 Days** |
| | **100% Fly ash** | **30** | **4.9** | **7.89** | **7** | **23** | **0** | **23.3** | **5 Days** |

**Cont....**

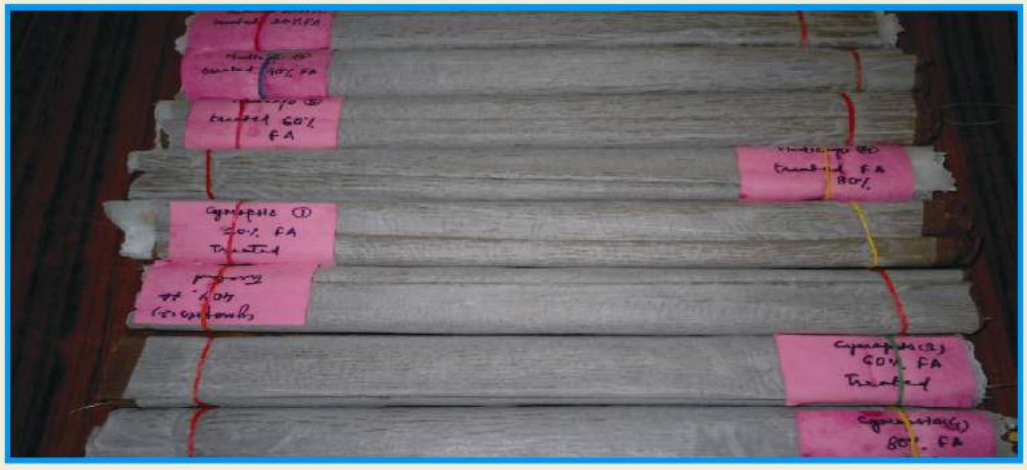
**Table –5 :** **Cont....**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ***Medicago sativa L (T9)*** | **CONTROL** | **30** | **1.73** | **4.45** | **30** | **0** | **0** | **100.0** | **6 Days** |
| | **20% Fly ash** | **30** | **1.925** | **5.187** | **29** | **1** | **0** | **96.7** | **6 Days** |
| | **40% Fly ash** | **30** | **1.514** | **5.014** | **29** | **1** | **0** | **96.7** | **6 Days** |
| | **60% Fly ash** | **30** | **1.48** | **4.88** | **29** | **1** | **0** | **96.7** | **6 Days** |
| | **80% Fly ash** | **30** | **1.31** | **4.03** | **29** | **1** | **0** | **96.7** | **6 Days** |
| | **100% Fly ash** | **30** | **1.55** | **4.075** | **29** | **1** | **0** | **96.7** | **6 Days** |
| | | | | | | | | | |
| ***Trigonella foenum graecum L (SWATI 1)*** | **CONTROL** | **30** | **2.09*** | **7.33** | **30** | **0** | **0** | **100.0** | **5 Days** |
| | **20% Fly ash** | **30** | **8*** | **9** | **29** | **1** | **0** | **96.7** | **5 Days** |
| | **40% Fly ash** | **30** | **5.28*** | **6.25** | **28** | **2** | **0** | **93.3** | **5 Days** |
| | **60% Fly ash** | **30** | **3.5*** | **5.13** | **28** | **2** | **0** | **93.3** | **5 Days** |
| | **80% Fly ash** | **30** | **3.88** | **5.25** | **27** | **3** | **0** | **90.0** | **5 Days** |
| | **100% Fly ash** | **30** | **3.86** | **5.2** | **27** | **3** | **0** | **90.0** | **5 Days** |

# PLATE - 7

(A) Seed Germinator

(B) Placing Roll Towel in Seed Germinator



(C) BP Method of Placing Seed in Roll Towel

## Showing Seed Germination Experiments 'Roll Towel Method'

# PLATE - 8

(A) *Cyamopsis tetragonoloba* L

(B) *Glycine max* L

(C) *Medicago sativa* L

(D) *Trigonella foenum graecum* L

**Seeds of Selected Legume Plants for Study**

# PLATE - 9

(A) Control

(B) 20% FA

(C) 40% FA

(D) 60% FA

(E) 80% FA

(F) 100% FA

**Showing Percentage Germination of *Cyamopsis tetragonoloba* L in Different Concentration of Flyash (FA) Treatment Roll Towel Method**

# PLATE - 10

(A) Control

(B) 20% FA

(C) 40% FA

(D) 60% FA

(E) 80% FA

(F) 100% FA

**Showing Percentage Germination of *Glycine max* L in Different Concentration of Flyash (FA) Treatment Roll Towel Method**

# PLATE - 11

(A) Control

(B) 20% FA

(C) 40% FA

(D) 60% FA

(E) 80% FA

(F) 100% FA

**Showing Percentage Germination of *Medicago sativa* L in Different Concentration of Flyash (FA) Treatment Roll Towel Method**

# PLATE - 12

(A) Control

(B) 20% FA

(C) 40% FA

(D) 60% FA

(E) 80% FA

(F) 100% FA

**Showing Percentage Germination of *Trigonella foenum graecum* L in Different Concentration of Flyash (FA) Treatment Roll Towel Method**

# PLATE - 13

(A) *Cyamopsis tetragonoloba* L

(B) *Glycine max* L

(C) *Medicago sativa* L

(D) *Trigonella foenum graecum* L

**Showing Abnormal Seed of Four Experimental Plant Present in Different Concentrations of Flyash (FA)**

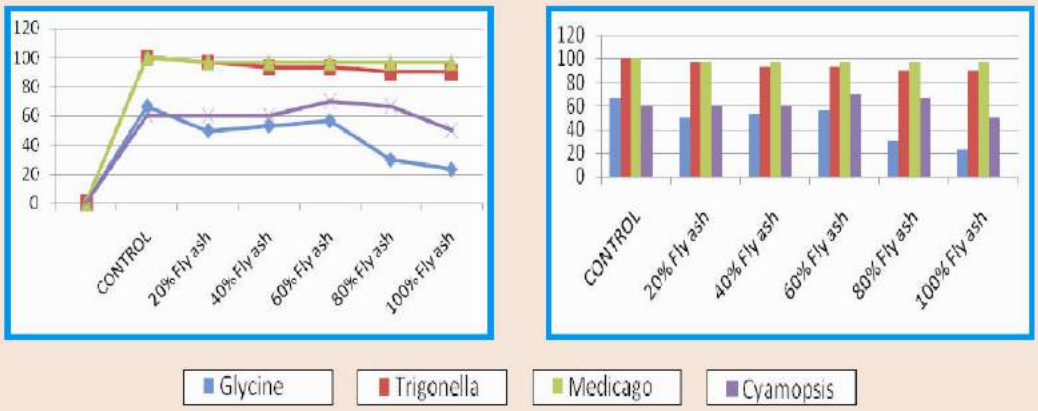
of flyash treatment on seedling growth of *Cyamopsis tetragonoloba L, Medicago sativa L, Trigonella foenum graecum L, Glycine max L*, shows favorable seedling growth response in *Cyamopsis tetragonoloba L* and *Trigonella foenum graecum L* while opposite result obtain in *Glycine max L* and *Medicago sativa L*. (Table-5, Plate-7-13, Figure-3A-F)

In *Cyamopsis tetragonoloba L,* no change was observed in 10-20% flyash treatment over control. Highest germination percentage obtained in 60% flyash treatment (70%), with decline order of germination percentage along with increased percentage of flyash treatment. Minimum germination found in 100% flyash treatment. Cyamopsis *tetragonoloba L* has maximum number of non germinated seeds. (Table-5, Plate-9)

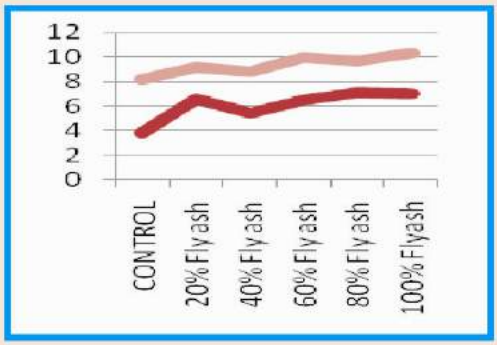
Increasing trend was recorded in *Cyamopsis tetragonoloba L,* with increased concentration of flyash treatments in terms of height. In other plants lower concentration of flyash show favourable results in comparative to higher (<40%). In *Cyamopsis tetragonoloba L,* root length was recorded highest in 80 %( 7.1cm) followed by 100% flyash treatment while minimum was in control (3.78cm). But curling of root starts after 40% flyash treatment, maximum curling was observed in 60% flyash (Table-5)(Plate-9). The length of shoot increased with increased flyash treatments. Exceptionally reduction in value of shoot length was observed in 40% treatment (8.83cm). Maximum value of shoot length was observed in 100%flyash (10.1 cm) followed by 9.94 in 60% flyash treatment. (Table-5, Plate-9) (Figure-3 A,C)

In *Medicago sativa* L*,* decreasing trend in germination percentage was observed in flyash treatments over control, with highest percentage of germination was obtained in controls (100%) germination (Table-5, Plate-11). The flyash treatment in *Medicago sativa L* show increase root length in 20 %( 1.925 cm) flyash treatment after that reduction in root length was recorded minimum value was obtained in 80% (1.3 cm) flyash treatment. Similarly shoot length also show maximum value in 20 %

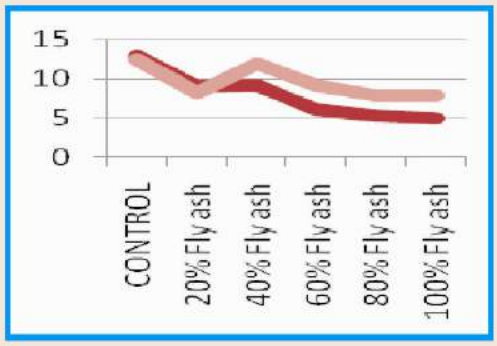
# FIGURE - 3



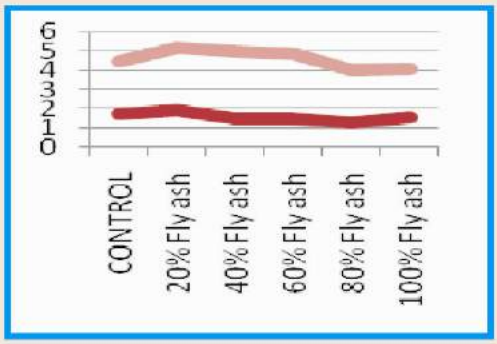

**(A & B) - Graph Showing Germination Percentage in Studied Plant by 'Roll Towel Method'**



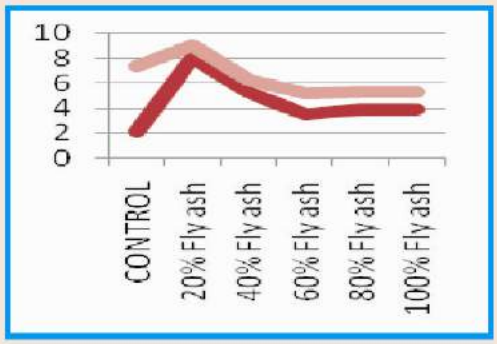

**(C) *Cyamopsis tetragonaloba* L**




**(D) *Glycine max* L**




**(E) *Medicago sativa* L**




**(F) *Trigonella foenumgraecum* L**


**Graph Represent Comparative Study of Root Length and Shoot Length of Selected Legume Plants**

(5.18cm) treatment followed by 40 % (5.01cm). Minimum value was obtained in 80% treatment (4.03). (Table-5, Plate-11) (Figure-3 A,E)

*Trigonella foenum graecum* L decreasing trend in germination percentage in 20%, 40%, 60%, 80%, and 100% flyash treatments were observed. (Table-5, Plate-12). *Trigonella* foenum graecum L, show maximum curling symptoms with drastic increase in length of root in 20% (8cm) flyash treatment in comparative to control (2.09cm) . In 40%,60%, 80%,100% treatment reduction in root length was observed in comparative to 20% treatment but higher in comparative to control, highest value of shoot length was also observed in 20% flyash treatment (9cm) followed by control (7.33cm). Minimum value was found in 60% treatment (5.1cm) followed by100 %( 5cm). (Table-5, Plate-12) (Figure-3 A,F)

In *Glycine max* L the inhibitory effect of flyash started from very early, it is evident from the results that flyash highest germination was in control which decreases with percentage increase of flyash. In *Glycine max L* highest number of abnormal seed was recorded. (Table-5, Plate-10). *Glycine max L s*how negative growth responses in terms of height, root length decrease with increase flyash treatments, maximum root length observed in control (12.9cm) followed by (9.1cm) in 40% flyash treatment, while minimum length was obtained in 100% flyash,(4.9cm). Similar pattern is found in shoot length where maximum length was recorded in control (12.35cm) followed by (11.89cm) in 40%. Minimum value found in80% (7.86cm) followed by 100% (7.89cm) flyash treatment. (Table-5, Plate-10)(Figure-3A,D)

Application of flyash showed significant effect on germination percentage, root and shoot length of seeds of selected legumes. The maximum germination percentage was observed in *Trigonella foenum graecum L,* followed by *Medicago sativa L,* while minimum germination

percentage was observed in *Glycine max* L, that is lesser then germination percentage of all four plants (Figure-3A-F)

## 3-B. Comparative Study of Seed Germination and Seedling growth Parameters in Selected Legume Plants in Response to Flyash Amendments. (**Germination Tray Experiments**)

The performance of selected plants i.e.*Cyamopsis tetragonoloba L, Medicago sativa L, Trigonella foenum graecum L, Glycine max L,* in soil amended by different concentrations (0%, 5%, 10%, 15%, 20%, 25%, 40%) of fly ash has been studied through germination tray experiments, (Table-6, Plate-14, 15) (Figure-4,5,6)

### B-1. Germination Percentage-

In *Cyamopsis tetragonoloba L* highest germination percentage obtained in control (88.8%) followed by 5%(83.3%), with decline order of germination percentage with increased percentage of flyash treatment exceptionally 15% flyash amended soil show minimum value of germination percentage(70%),that is equals to minimum value was obtained from 40% flyash amended soil (70%). In *Medicago sativa L,* trend in increment in germination percentage was observed in flyash treatments over control, with highest percentage of germination was obtained in 10% and 15% flyash amended soils (93.2%).while minimum germination percentage was obtained in 40% flyash incorporation (82%) followed by (90%)in control. In *Trigonella foenum graecum L,* favourable result was obtained with higher flyash concentrations. Result obtained were 98.6 in control, 98 in 5%,, 99 in10%,, 98.2 in15%, 98 in 20%, 98.6 in 25%, 92.2 in 40% germination in *Trigonella foenum graecum L.* As show that minimum value obtained in 40% flyash addition while maximum was in 10% flyash. In *Glycine max L,* no clear trend was obtained, as germination percentage in control was 73% which decline with 5% and

## Table – 6 : Comparative Study of Germination & Seedling Growth Parameters of Selected Legume Plant in Response of Flyash Amendment

| | Fly ash Concentration | Number of Seed | Root Length (Avg.) | Shoot Length (Avg.) | Germination Percentage (%) | Fresh Weight (mg/pt) | Dry Weight (mg/pt) |
|---|---|---|---|---|---|---|---|
| ***Cyamopsis tetragonoloba L (RMG1002)*** | **CONTROL** | **30** | **4.7** | **39.6** | **88.8** | **1.27** | **0.065** |
| | **5% Fly ash** | **30** | **4.23** | **37.17** | **83.3** | **1.45** | **0.076** |
| | **10% Fly ash** | **30** | **4.9** | **31.6** | **76.6** | **1.51** | **0.070** |
| | **15% Fly ash** | **30** | **5.37** | **25.5** | **70** | **1.66** | **0.080** |
| | **20% Fly ash** | **30** | **5** | **27.4** | **76.2** | **1.74** | **0.083** |
| | **25% Fly ash** | **30** | **5** | **26.6** | **76.4** | **1.72** | **0.101** |
| | **40% Fly ash** | **30** | **3.75** | **24.4** | **70** | **1.72** | **0.102** |
| | | | | | | | |
| ***Glycine max L (JS 335)*** | **CONTROL** | **30** | **6.13** | **16.83** | **73** | **0.984** | **0.101** |
| | **5% Fly ash** | **30** | **5.63** | **18.12** | **63.3** | **1.001** | **0.112** |
| | **10% Fly ash** | **30** | **5.39** | **15.87** | **63.3** | **0.919** | **0.100** |
| | **15% Fly ash** | **30** | **5.34** | **18.5** | **73.3** | **0.914** | **0.104** |
| | **20% Fly ash** | **30** | **5.35** | **15.7** | **76.6** | **0.912** | **0.101** |
| | **25% Fly ash** | **30** | **6.34** | **17** | **80** | **0.912** | **0.100** |
| | **40% Fly ash** | **30** | **6.25** | **18.75** | **53.3** | **0.909** | **0.099** |

Cont....

**Table – 6:-** **Cont....**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Medicago sativa L (T9)*** | **CONTROL** | **30** | **1.9** | **3.7** | **90** | **0.872** | **0.05** |
| | **5% Fly ash** | **30** | **2.7** | **3.5** | **92.4** | **0.900** | **0.07** |
| | **10% Fly ash** | **30** | **2.5** | **3.2** | **93.2** | **0.904** | **0.06** |
| | **15% Fly ash** | **30** | **2** | **3.4** | **93.2** | **0.914** | **0.08** |
| | **20% Fly ash** | **30** | **2.2** | **3.5** | **92.3** | **0.913** | **0.07** |
| | **25% Fly ash** | **30** | **2.7** | **3.9** | **92.4** | **0.918** | **0.09** |
| | **40% Fly ash** | **30** | **2.2** | **2.9** | **82** | **0.923** | **0.10** |
| | | | | | | | |
| ***Trigonella foenum graecum L (SWATI 1)*** | **CONTROL** | **30** | **5.32** | **4.54** | **98.6** | **0.338** | **0.010** |
| | **5% Fly ash** | **30** | **5.52** | **4.37** | **98** | **0.367** | **0.018** |
| | **10% Fly ash** | **30** | **5.2** | **4.37** | **99** | **0.380** | **0.020** |
| | **15% Fly ash** | **30** | **6.3** | **4.49** | **98.2** | **0.405** | **0.022** |
| | **20% Fly ash** | **30** | **6.08** | **5.06** | **98** | **0.0411** | **0.024** |
| | **25% Fly ash** | **30** | **7.28** | **5.40** | **98.6** | **0.425** | **0.027** |
| | **40% Fly ash** | **30** | **7.5** | **6.2** | **92.2** | **0.430** | **0.029** |
| | | | | | | | |

# PLATE - 14

(A) Calculation for Germination Percentage

(B) Legumes Showing Growth (i)

(C) Legumes Showing Growth (ii)

**Showing Germination Tray Experiments for Eco-morphological Study**

# PLATE - 15

(A) *Cyamopsis tetragonoloba* L

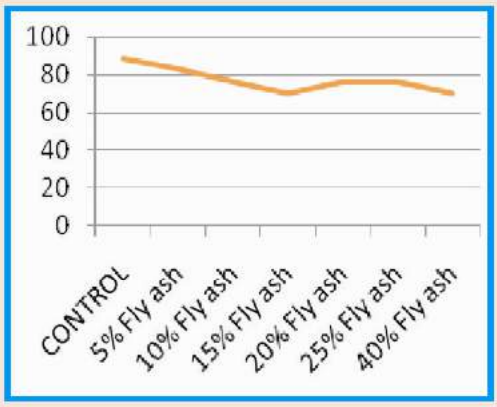
(B) *Glycine max* L

(C) *Medicago sativa* L

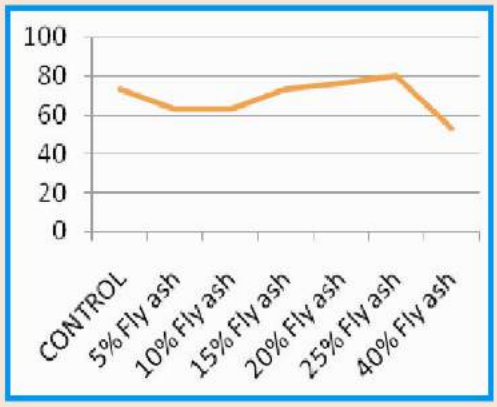
(D) *Trigonella foenum graecum* L

**Showing Germination Tray Experiments for Eco-morphological Study**

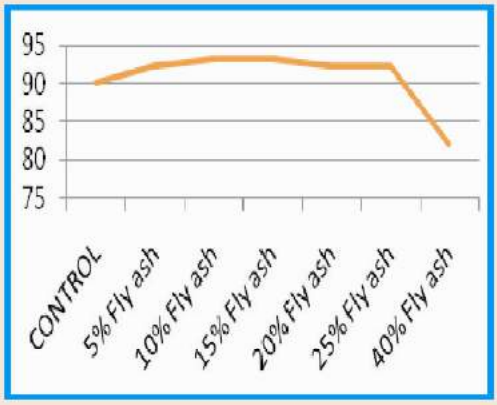
# FIGURE - 4



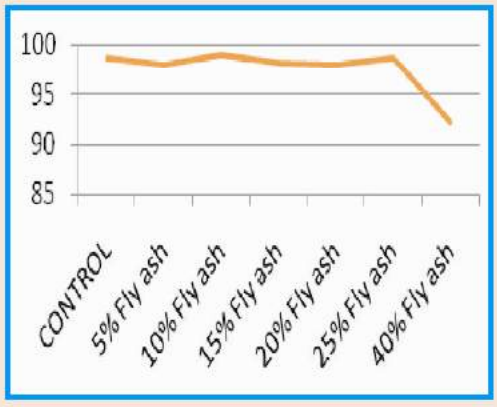

(A) *Cyamopsis tetragonaloba* L




(B) *Glycine max* L



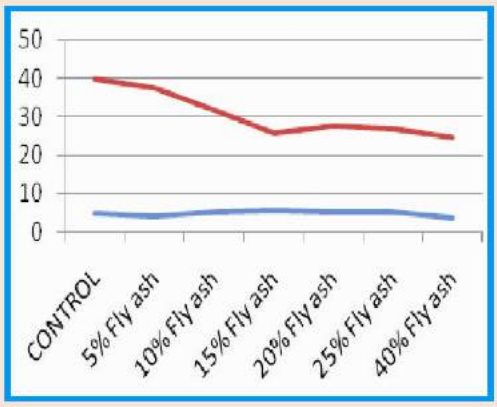

(C) *Medicago sativa* L



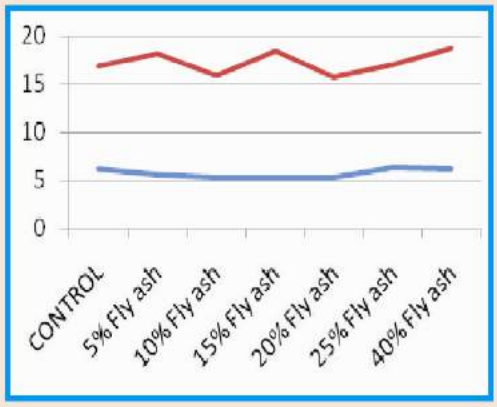

(D) *Trigonella foenum graecum* L

**Comparative Study of Response of Flyash Treatment on Germination Parameter of Selected Plant**

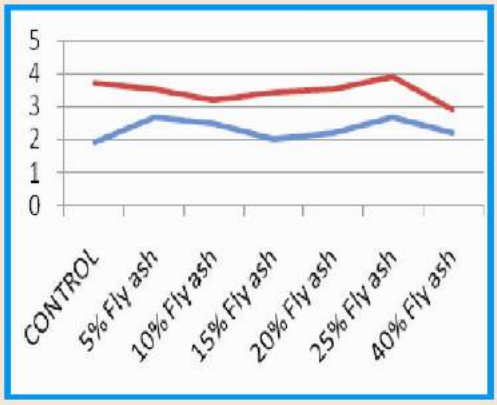
# FIGURE - 5



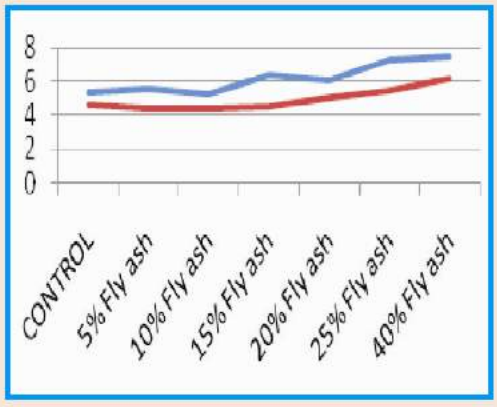

(A) *Cyamopsis tetragonaloba* L




(B) *Glycine max* L



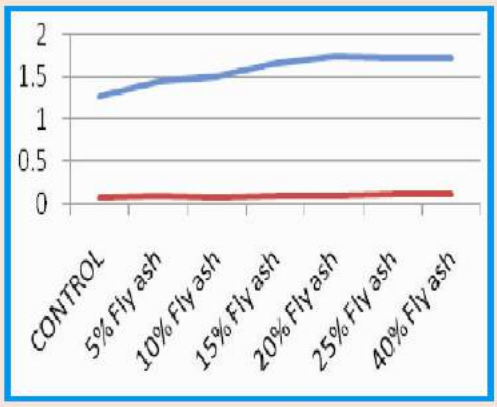

(C) *Medicago sativa* L



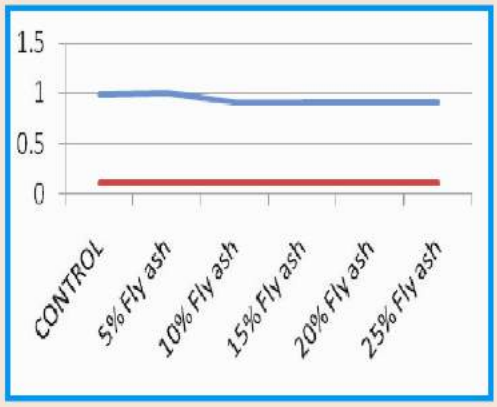

(D) *Trigonella foenum graecum* L


**Comparative Study of Response of Flyash Treatment on Root Length/ Shoot Length Parameter of Selected Plant**

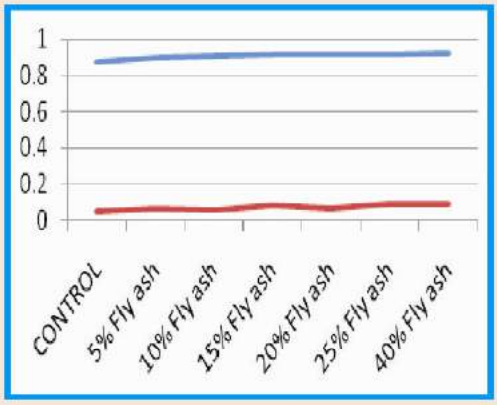
# FIGURE - 6



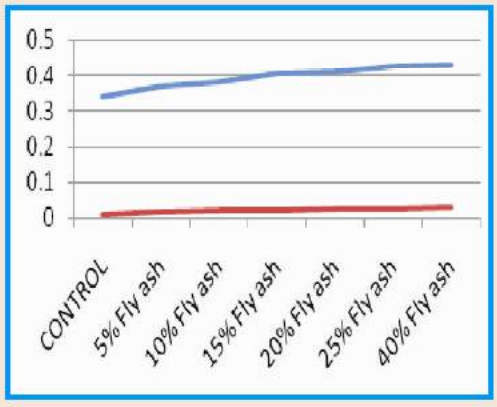

(A) *Cyamopsis tetragonaloba* L




(B) *Glycine max* L




(C) *Medicago sativa* L




(D) *Trigonella foenum graecum* L


**Comparative Study of Response of Flyash Treatment on Fresh Weight / Dry Weight Parameter of Selected Plant**

10% flyash incorporation (63.3%) and improved in 15%, 20%, 25% flyash i.e. 73.3, 76.6, 80% respectively. Minimum value obtain in 40% flyash incorporation (53.3%). (Table-6, Plate-14,15) (Figure-4 A-D)

## B-2. Root Length –

In *Cyamopsis tetragonoloba L* root length show increased growth pattern in flyash amended soil highest root length recorded in 15% flyash amended soil (5.37cm followed by 20% (5cm) and 25% (5cm), minimum root length was found in 40% flyash addition(3.75cm), it is less in comparative to control (4.7cm). In 5% flyash addition slight reduction in length was also observed (4.23cm). *Medicago sativa L,* highest value of root length observed in both 5% (2.7cm) and 25 % (2.7cm) flyash amended soil, with variable value (2-2.5 cm) obtained in 10%, 15%, 20% and 40% concentrations while least value is obtained in control (1.9cm). *Trigonella foenum graecum L.* A continuous increment was observed in root length of *Trigonella foenum graecum L* with increased concentration of flyash (5.32-7.5cm) except 10% (5.2cm) flyash amended soil which show slight reduction, maximum value was obtained in 40% flyash amended soil i.e. 7.5 cm in comparison to 5.32cm in control. In *Glycine max L,* almost similar values obtained in 5%,10%, 15%, 20% (5.34-5.63 cm) flyash amended soil, while max value was obtained in 25% concentration (6.34) followed by 40%(6.25) and control (6.13). (Table-6, Plate-14,15) (Figure-5A-D)

## B-3. Shoot Length-

In *Cyamopsis tetragonoloba L* show gradual decline in value of shoot length with higher concentrations of flyash, minimum value of shoot length obtained at 40% (24.4cm) flyash incorporation while maximum value was recorded in control (39.6cm),followed by 5% (37.17cm) and10% (31.6cm) successively. In *Medicago sativa L,* almost similar values were obtained in control, 5%, 10%, 15%, 20% (3.7, 3.5, 3.2, 3.4,

3.5cm respectively) flyash incorporation with slight increment in 25%flyash incorporated soil over all others (3.9cm). On the contrary minimum value was obtained from 40% flyash amendment (2.9cm). In *Trigonella foenum graecum L,* similar to root length a continuous increment was observed in shoot length too with increased concentration of flyash, maximum value was obtained in 40% flyash incorporated soil(6.2cm), with slight reduction in value of shoot length in 5% and10%(4.37cm) flyash treated soil over control(4.54cm). In *Glycine max L,* no clear trend was observed with increasing concentration of flyash, in control, 5%, 10%15%20%, 25% flyash amendment 16.83, 18.12, 15.87, 18.5, 15.7, 17cm respectively. The maximum value was obtained by 40% (18.75 cm) flyash incorporation and minimum value was obtained by 20% flyash treated soil (15.7cm). (Table-6, Plate-14, 15) (Figure-5A-D)

## B-4. Fresh and Dry weight-

In *Cyamopsis tetragonoloba L* increase growth trend was observed in terms of fresh weight minimum fresh weight was observed in control (1.27mg/pt) maximum fresh weight found in 20%(1.74mg/pt), followed by 25%and 40%(1.72mg/pt).The observations were recorded in case of dry weight were, maximum in 40%(0.102mg/pt) and minimum was in control (0.065mg/pt). In *Medicago sativa L,* significant increment was observed in fresh weight with increase flyash concentration starting with minimum in control (0.872mg/pt) to maximum 40 %( 0.923mg/pt). In case of dry weight maximum value obtain in 40 % (0.10mg/pt) while minimum in control (0.05mg/pt) followed by 10% ( 0.06mg/pt), 5% and 20 % (0.07mg/pt). In *Trigonella foenum graecum L,* also show significant increase in biomass, in term of fresh weight maximum growth was observed in 40 % (0.430mg/pt), 25 % (0.425). While minimum was observed in control (0.338mg/pt) and 10% in (0.367mg/pt). In case of dry weight maximum weight was recorded in 40 %( 0.029mg/pt), while minimum in control (0.010mg/pt). Seedling growth in terms of fresh in *Glycine max L,* was observed maximum in 5 %( 1.001mg/pt) and control

(0.984mg/pt) after that slight reduction was observed in plant fresh weight, minimum weight observed in pure flyash i.e. 40% (0.909mg/pt). In case of dry weight almost similar results were observed in all concentrations of flyash (0.112mg/pt)in 5%,(0.104mg/pt) in15%flyash treatment, while minimum value observed in 40 %( 0.10mg/pt).(Table-6,Plate-14,15) (Figure-6A-D)

## (4) BIO-CHEMICAL PARAMETRS OF SELECTED PLANTS

### 4-a Chlorophyll Content in Studied Legume Plant Grown in Flyash Amendments

The amount of chl a and chl b and total chlorophyll was calculated by following formula given by Arnon,(1949), the value of chl a, chl b, total chlorophyll and carotenoids were expressed in terms of mg/1 mg of fresh weight.(Table-7) (Figure-7 A-D). In *Cyamopsis tetragonoloba L* maximum value of chl a was obtained in plants grown in 40% flyash amended soil (1.188mg) while minimum in 5%(0.590mg). chl b highest value observed in 25%(0.521mg) and minimum was in 40%(0.134mg).carotenoids maximum value was in 40%(0.435mg)and minimum was in 5%(0.230mg). The value of total chlorophyll was observed highest in 40 %( 1.320mg), followed by 10 %( 1.290mg), control (1.280). (Table-7, Plate-16, 17). In *Medicago sativa L,* maximum value of chl a was obtained in 5% flyash (1.287mg) and minimum was in 10 %( 1.259mg), chl b maximum value was in 40 %( 0.211mg) and minimum was in 25 %( 0.141mg), carotenoids maximum observed in 40%(0.553mg) and minimum was in control and 5%(0.487mg),The value of total chlorophyll was maximum in 15%(1.500mg) and control(1.490mg). (Table-7, Plate-16,17). In *Trigonella foenum graecum L,* maximum value of chl a was obtained in 15% flyash (1.303mg) and minimum was in 5 %( 0.762mg), chl b maximum value was in 25%( 0.456mg) and minimum was in 10 %( 0.141mg) , carotenoids maximum observed in control (0.534mg) and minimum was in 5%(0.271mg),The value of total chlorophyll was maximum in

## Table – 7 : Comparative Account of Pigments in Selected Legume After Flyash Treatment

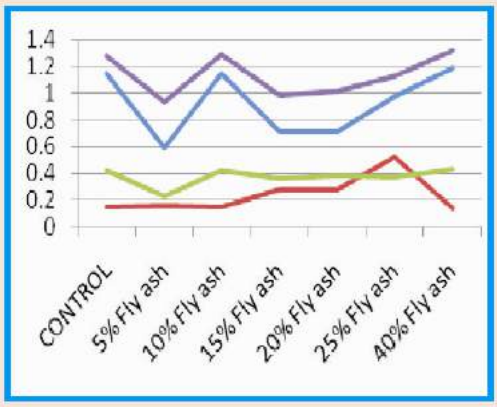
| | Fly ash Concentration | Chlorophyll a | Chlorophyll b | Carotenoid | Total Chlorophyll |
|---|---|---|---|---|---|
| *Cyamopsis tetragonoloba L ( RMG1002)* | CONTROL | 1.15 | 0.148 | 0.422 | 1.28 |
| | 5% Fly ash | 0.59 | 0.15 | 0.23 | 0.935 |
| | 10% Fly ash | 1.15 | 0.146 | 0.424 | 1.29 |
| | 15% Fly ash | 0.714 | 0.275 | 0.365 | 0.989 |
| | 20% Fly ash | 0.715 | 0.28 | 0.38 | 1.02 |
| | 25% Fly ash | 0.978 | 0.521 | 0.371 | 1.13 |
| | 40% Fly ash | 1.188 | 0.134 | 0.435 | 1.32 |
| | | | | | |
| *Glycine max L (JS 335)* | CONTROL | 0.866 | 1.516 | 0.425 | 2.381 |
| | 5% Fly ash | 0.880 | 1.515 | 0.426 | 2.39 |
| | 10% Fly ash | 0.860 | 1.471 | 0.430 | 2.388 |
| | 15% Fly ash | 1.078 | 1.509 | 0.426 | 2.39 |
| | 20% Fly ash | 0.855 | 1.525 | 0.428 | 2.38 |
| | 25% Fly ash | 0.876 | 1.521 | 0.421 | 2.40 |
| | 40%Flyash | 0.864 | 1.470 | 0.430 | 2.33 |

Cont....

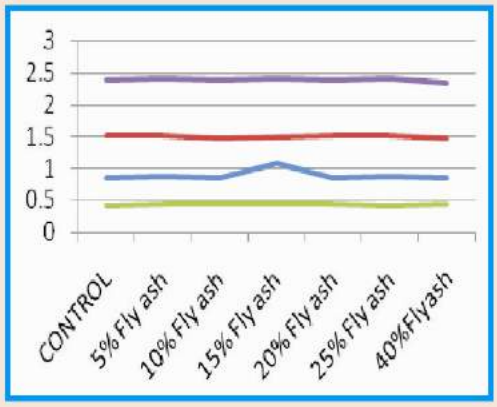
**Table – 7 :** **Cont....**

| | | | | | |
|---|---|---|---|---|---|
| ***Medicago sativa L (T9)*** | **CONTROL** | **1.284** | **0.207** | **0.487** | **1.49** |
| | **5% Fly ash** | **1.287** | **0.176** | **0.487** | **1.46** |
| | **10% Fly ash** | **1.259** | **0.178** | **0.534** | **1.43** |
| | **15% Fly ash** | **1.264** | **0.146** | **0.494** | **1.50** |
| | **20% Fly ash** | **1.263** | **0.165** | **0.519** | **1.42** |
| | **25% Fly ash** | **1.266** | **0.141** | **0.520** | **1.47** |
| | **40%Flyash** | **1.285** | **0.211** | **0.553** | **1.47** |
| | | | | | |
| ***Trigonella foenum graecum L (SWATI 1)*** | **CONTROL** | **1.287** | **0.340** | **0.534** | **1.30** |
| | **5% Fly ash** | **0.762** | **0.308** | **0.271** | **0.859** |
| | **10% Fly ash** | **1.231** | **0.280** | **0.388** | **1.20** |
| | **15% Fly ash** | **1.303** | **0.433** | **0.519** | **1.38** |
| | **20% Fly ash** | **1.281** | **0.398** | **0.487** | **1.34** |
| | **25% Fly ash** | **1.276** | **0.456** | **0.532** | **1.38** |
| | **40%Flyash** | **0.984** | **0.361** | **0.296** | **1.07** |

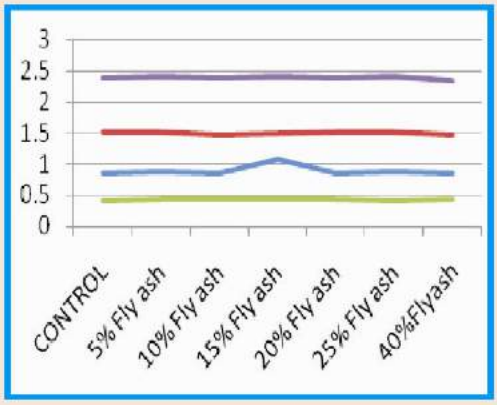
# FIGURE - 7



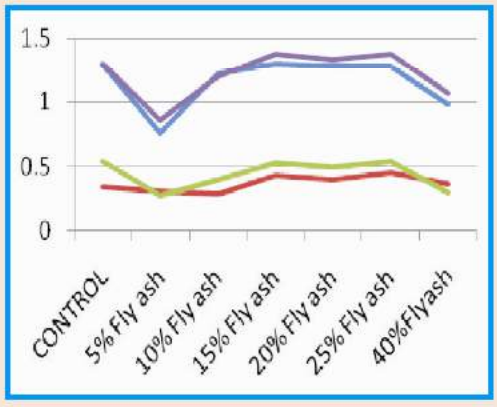

(A) *Cyamopsis tetragonaloba* L




(B) *Glycine max* L




(C) *Medicago sativa* L




(D) *Trigonella foenum graecum* L

**Comparative Study of Total Chlorophyll in Studied Plants after Flyash Treatment**

# PLATE - 16

(A) Showing Set Experimental Concentrations of Flyash/Soil

(B) Legume Plant Showing Growth in Pot (i)

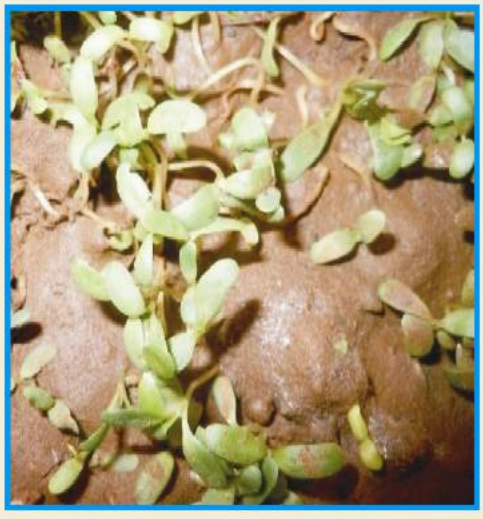
(C) Legume Plant Showing Growth in Pot (ii)

## Showing Pot Experiment (A)

# PLATE - 17

(A) *Cyamopsis tetragonoloba* L

(B) *Glycine max* L



(C) *Medicago sativa* L

(D) *Trigonella foenum graecum* L

**Showing Pot Experiment (B)**

15%(1.38mg) and 25%(1.38mg) and minimum was in5%(0.859mg). (Table-7, Plate-16,17). In *Glycine max* L, maximum value of chl a was obtained in 15% flyash (1.078mg) and minimum was in 20 %(0.855mg), chl b maximum value was in 20%( 1.525mg) and minimum was in 40 %( 1.470mg) , carotenoids maximum observed in 10%&40% (0.430mg) and minimum was in 25%(0.421mg),The value of total chlorophyll was maximum in 25%(2.400mg) and minimum was in 40%(2.330mg). (Table-7, Plate-16,17)

## 4-b. Biochemical Parameters of Studied Legume Plant Grown in Flyash Amendments.

### i. Protein

To determine the effect of flyash on plants protein content was measured. In *Cyamopsis tetragonoloba* L highest protein content was obtained in plants grown in 25 %( 160µg/gm) flyash amended soil and minimum was in control, 5% and 10% (100 µg/gm). (Table-8,Figure-8A). In *Medicago sativa* L, highest protein content was obtained in plants grown in 15 and 25 %( 2800µg/gm) flyash amended soil and minimum was in control (120 µg/gm). (Table-8, Figure-10A). In *Trigonella foenum graecum* L, increasing trend in protein content was found from control to 25 %( 180 µg/gm) while minimum protein was observed in 5% (80%). (Table-8, Figure-11A). In *Glycine max* L slight reduction in protein content was observed initially i.e. (192.5 µg/gm) in 5% and the maximum protein observed in control (205 µg/gm) followed by (204) in 40%. (Table-8, Figure-9A)

### ii. Phenols

In *Cyamopsis tetragonoloba* L maximum phenol content was obtained in 40 %( 122.3mg/gm) flyash and minimum was in control (60.9mg/gm).(Table-8, Figure-8B). In *Medicago sativa L,* maximum

## Table – 8 : Comparative Study of Effect of Flyash on Biochemical Parameters of Selected Legume Plant

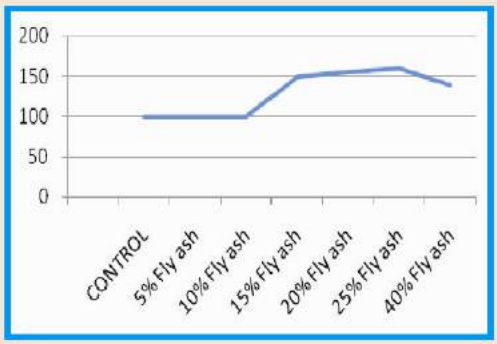
| | Fly ash Concentration | Protein Content (µg/gm) | Phenol Content (mg/gm) | Carbohydrate Content (mg/gm) | | Proline (µg/gm) | Ascorbic acid (mg/100gm) |
|---|---|---|---|---|---|---|---|
| | | | | 480(A) | 490(A) | | |
| *Cyamopsis tetragonoloba L (RMG1002)* | CONTROL | 100 | 60.9 | 9.29 | 7.95 | 10 | 32.4 |
| | 5% Fly ash | 100 | 78.7 | 9.81 | 8.27 | 10 | 36.6 |
| | 10% Fly ash | 100 | 86.4 | 12.8 | 12.27 | 10 | 39.6 |
| | 15% Fly ash | 150 | 98.5 | 19.8 | 16.9 | 15 | 39.5 |
| | 20% Fly ash | 155 | 97.4 | 13.6 | 11.3 | 15 | 40.1 |
| | 25% Fly ash | 160 | 105 | 13.0 | 10.8 | 15 | 39.3 |
| | 40% Fly ash | 140 | 122.3 | 10.9 | 9.26 | 14 | 68.2 |
| | | | | | | | |
| *Glycine max L (JS 335)* | CONTROL | 205 | 131.3 | 20.049 | 16.828 | Traces | 36.4 |
| | 5% Fly ash | 192.5 | 100 | 20.057 | 16.80 | ‘ | 37.77 |
| | 10% Fly ash | 199.7 | 87.5 | 20.057 | 16.849 | ‘ | 34.8 |
| | 15% Fly ash | 197.2 | 69.99 | 20.057 | 16.828 | ‘ | 39.8 |
| | 20% Fly ash | 195.8 | 64.65 | 20.033 | 16.828 | ‘ | 39.4 |
| | 25% Fly ash | 201.4 | 55.88 | 20.049 | 16.835 | ‘ | 42.2 |
| | 40% Fly ash | 204.0 | 62.88 | 20.041 | 16.828 | ‘ | 57.7 |

Cont....

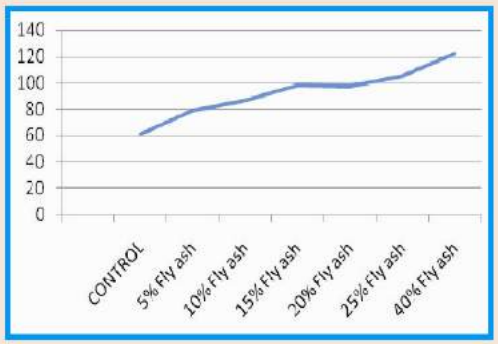
**Table – 8 :** **Cont....**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Medicago sativa L (T9)*** | **CONTROL** | **120** | **120.2** | **2.075** | **1.84** | **10** | **54.5** |
| | **5% Fly ash** | **200** | **121.9** | **3.73** | **3.29** | **15** | **54.8** |
| | **10% Fly ash** | **240** | **121.7** | **1.86** | **1.67** | **14** | **53.5** |
| | **15% Fly ash** | **280** | **123.7** | **2.435** | **2.22** | **10** | **52.9** |
| | **20% Fly ash** | **240** | **125.4** | **1.33** | **1.22** | **10** | **53.7** |
| | **25% Fly ash** | **280** | **125.8** | **2.73** | **2.52** | **10** | **54.4** |
| | **40% Fly ash** | **260** | **124.7** | **2.17** | **1.93** | **12** | **59.00** |
| | | | | | | | |
| ***Trigonella foenum graecum L (SWATI 1)*** | **CONTROL** | **86** | **217** | **19.82** | **16.90** | **10** | **68.2** |
| | **5% Fly ash** | **80** | **176.7** | **14.9** | **11.5** | **14** | **66 .2** |
| | **10% Fly ash** | **95** | **168.5** | **19.80** | **16.9** | **12** | **67.9** |
| | **15% Fly ash** | **105** | **171.9** | **19.82** | **16.9** | **18** | **70.3** |
| | **20% Fly ash** | **170** | **164.4** | **19.81** | **16.62** | **10** | **72.3** |
| | **25% Fly ash** | **180** | **167.6** | **19.82** | **16.90** | **9** | **84** |
| | **40% Fly ash** | **85** | **140.8** | **19.48** | **16.90** | **10** | **88** |
| | | | | | | | |

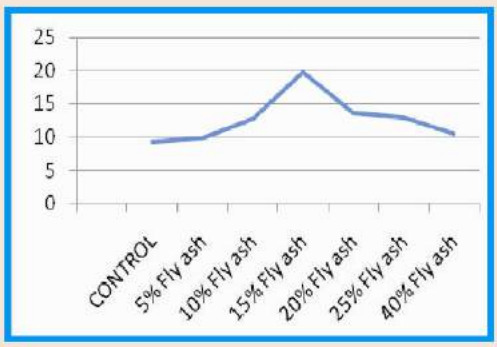
# FIGURE - 8



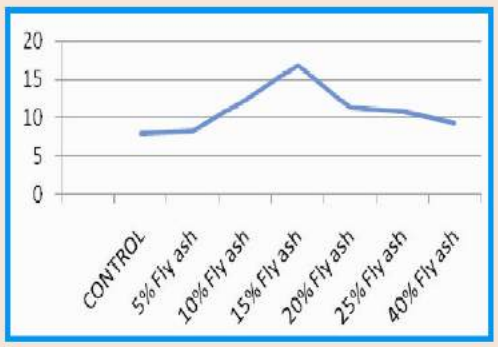

(A) Protien (µg/gm)



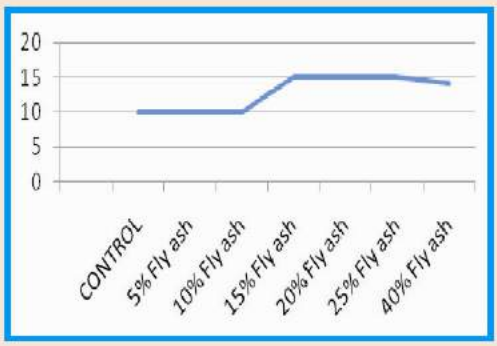

(B) Phenol (mg/ gm)



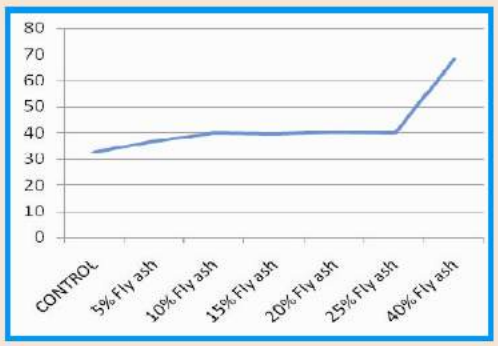

(C) Carbohydrate (mg/gm)480A




(D) Carbohydrate (mg/gm) 490A




(E) Proline (µg/gm)

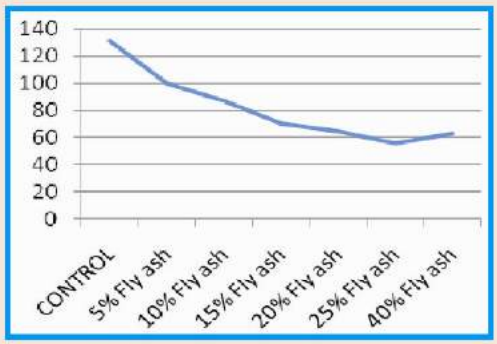



(F) Ascorbic acid (mg/100gm)

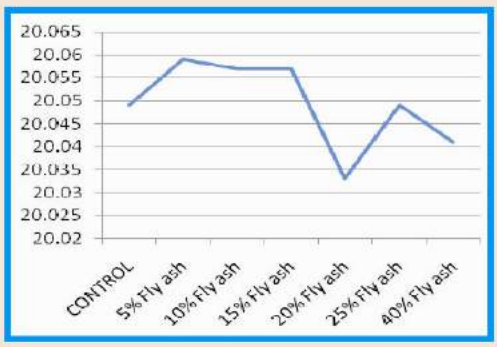
**Biochemical Parameters Studied in *Cyamopsis tetragonoloba* L Grown in Various Flyash Amendment**

# FIGURE - 9

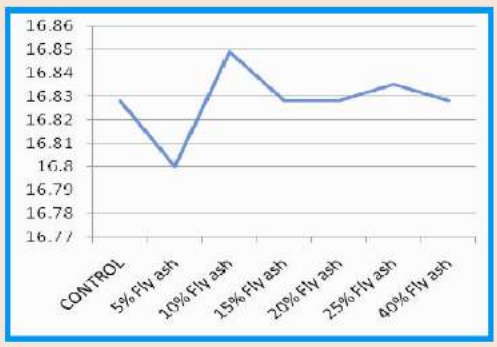

(A) Protien (µg/gm)



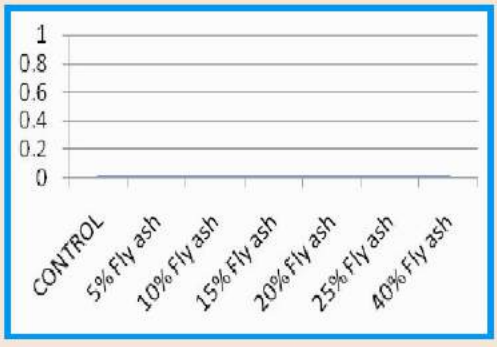

(B) Phenol (mg/ gm)



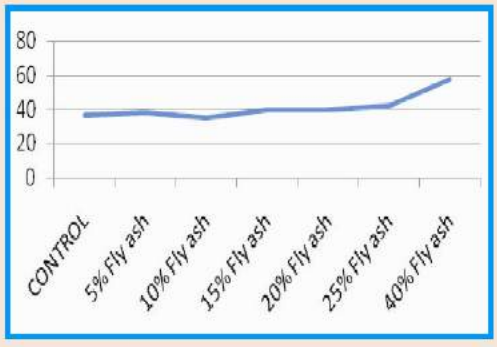

(C) Carbohydrate (mg/gm)480A




(D) Carbohydrate (mg/gm) 490A



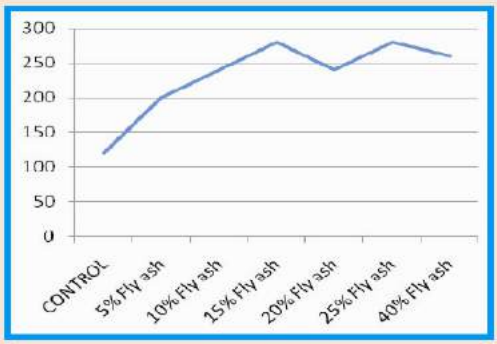

(E) Proline (µg/gm)



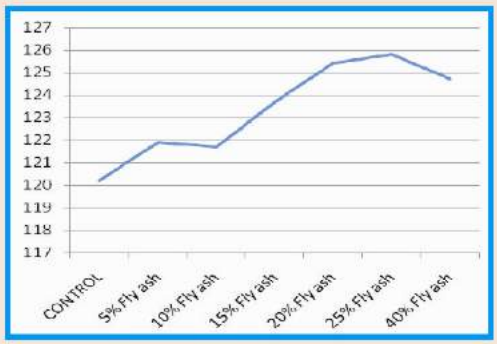

(F) Ascorbic acid (mg/100gm)

**Biochemical Parameters Studied in *Glycine max* L Grown in Various Flyash Amendment**

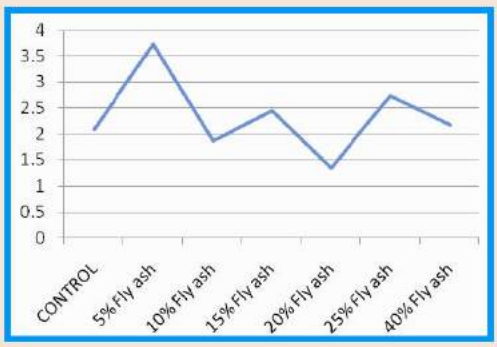
# FIGURE - 10



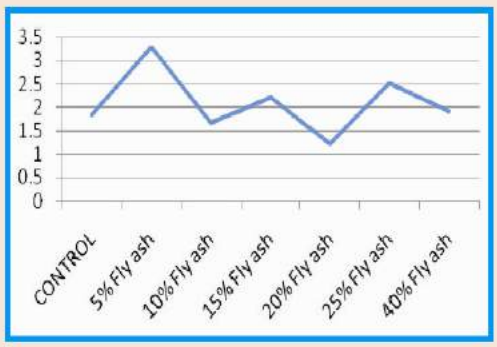

(A) Protien (µg/gm)



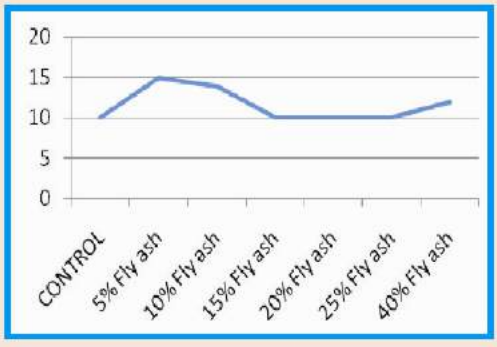

(B) Phenol (mg/ gm)



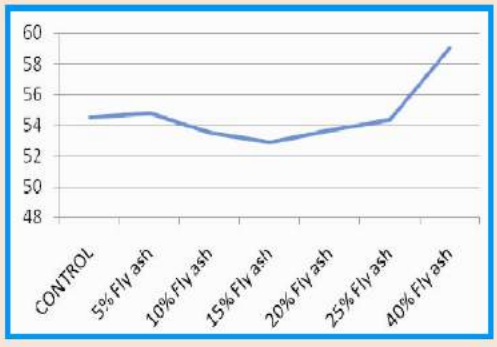

(C) Carbohydrate (mg/gm)480A




(D) Carbohydrate (mg/gm) 490A



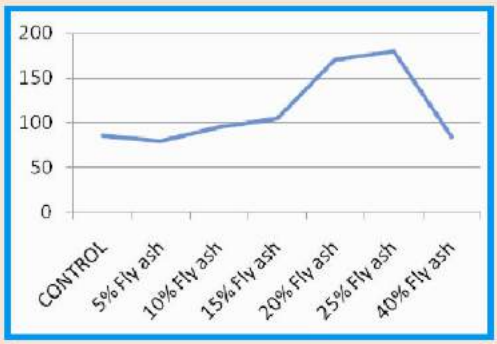

(E) Proline (µg/gm)



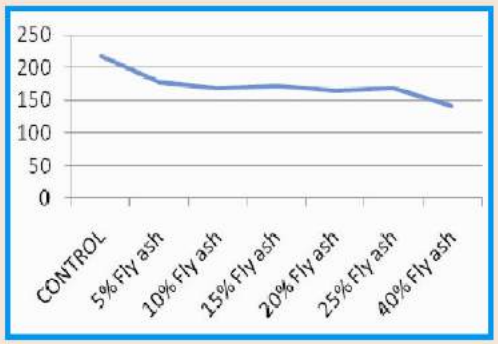

(F) Ascorbic acid (mg/100gm)

**Biochemical Parameters Studied in *Medicago sativa* L Grown in Various Flyash Amendment**

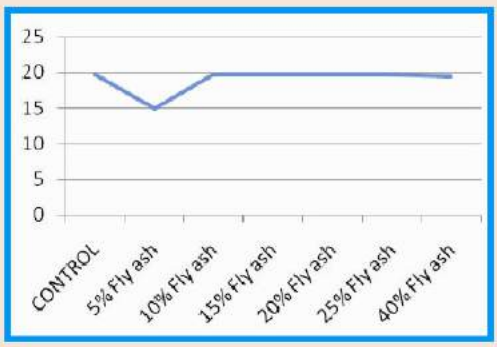
# FIGURE - 11



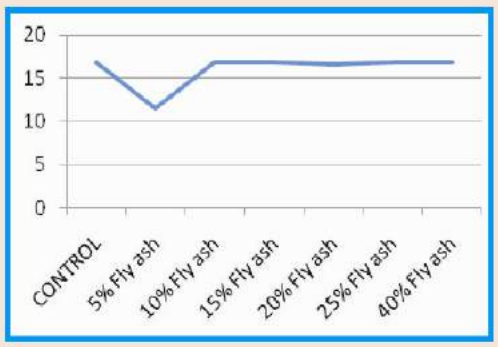

(A) Protien (µg/gm)



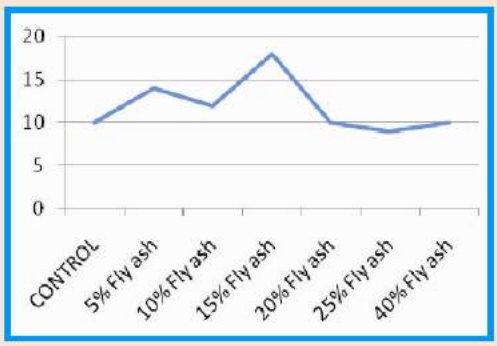

(B) Phenol (mg/ gm)



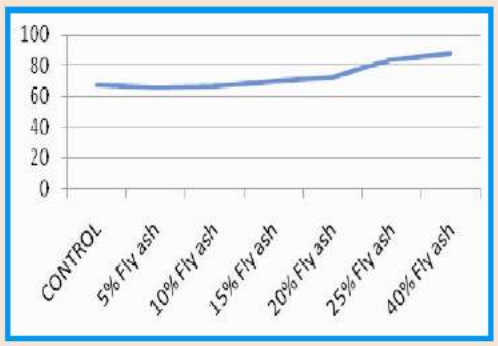

(C) Carbohydrate (mg/gm)480A




(D) Carbohydrate (mg/gm) 490A




(E) Proline (µg/gm)




(F) Ascorbic acid (mg/100gm)

**Biochemical Parameters Studied in *Trigonalla foenum graecum* L Grown in Various Flyash Amendment**

phenol content was obtained in 25 %( 125.8 mg/gm) flyash and minimum was in control (120.2 mg/gm). (Table-8,Figure-10B). In *Trigonella foenum graecum* L, minimum phenol content was in (140.8 mg/gm) in 40% and maximum phenol content was obtained in (217 mg/gm) control (Table-8, Figure-11B). In *Glycine max* L maximum phenol content was obtained in control (131.3 mg/gm) flyash and start decreasing up to 25% i.e. minimum (55.88 mg/gm). (Table-8, Figure-9B)

### iii. Carbohydrates

In *Cyamopsis tetragonoloba L* maximum value was obtain in 15% flyash (19.8mg/gm at 480 A) and (16.9mg/gm at 490A) and minimum was in control (9.29mg/gm at 480A and 7.95mg/gm at 490A). (Table-8, Figure-8C,D). In *Medicago sativa* L, maximum value was obtain in 5% flyash (3.73mg/gm at 480 A) and (3.29mg/gm at 490A) and minimum was in 20% (1.33mg/gm at 480A and 1.22mg/gm at 490A) (Table-8, Figure-10C,D). In *Trigonella foenum graecum* L, almost similar values were obtain in all amendments of flyash (19.4-19.8mg/gm at 480 A) and (16.6-16.9mg/gm at 490A) and the minimum was in 5% (14.9 mg/gm at 480A and11.50 mg/gm at 490A). (Table-8, Figure-11C,D). In *Glycine max* similar results were obtain in all amendments of flyash (20.03-20.05mg/gm at 480 A) and (16.00-16.04mg/gm at 490A) (Table-8, Figure-9C,D).

### iv. Proline

In *Cyamopsis tetragonoloba* L least variation was observed in lower amendments i.e. similar value was in control, 5%, 10%(10 µg/gm) after that proline content reaches up to (15 µg/gm) in higher percentages. (Table-8, Figure-8E). In *Medicago sativa* L, maximum value was observed in lower amendments i.e. almost similar value was in 5%, 10 %( 14-15 µg/gm) after that proline content ranges

from (10-12 µg/gm) in higher percentages.(Table-8, Figure-10E). In*Trigonella foenum graecum* L*,* proline content increase with increase concentration of flyash up to 15% (18 µg/gm)after that decrease in proline content was observed, minimum was in 25%(9 µg/gm). (Table-8, Figure-11E) In *Glycine max* L proline was observed in traces that could not be measured quantitatively(Table-8, Figure-9E).

**v. Ascorbic acid**

In *Cyamopsis tetragonoloba L* maximum value of ascorbic acid was in 40 %( 68.2 mg/100g) and minimum was in control (32.4 mg/100g). (Table-8, Figure-8F) In *Medicago sativa* L*, maximum* value of ascorbic acid was in 40 %( 59.0 mg/100g) and minimum was in 15% (52.9 mg/100g) (Table-8, Figure-10F). In *Trigonella foenum graecum* L, highest ascorbic acid content was measured among all four plants. Maximum value was observed in 40 %( 88 mg/100g) minimum amount was observed in 5% (66.2 mg/100g). (Table-8, Figure-11F) In *Glycine max* L increasing trend was observed the minimum value was observed in 10% (34.8 mg/100g) and maximum value was in 40 %( 57.7 mg/100g). (Table-8, Figure-9F)

## (5) NODULATION STUDIES

Data on plant height, root length, number of lateral roots and nodules per plant were collected. In *Cyamopsis tetragonoloba* L maximum plant height was observed in 10% flyash amendments (59cm), followed by 5% and control (57cm). Maximum root length was observed in 10 % (32cm), minimum plant length and root length was recorded in 40 % (46cm), (22cm) respectively. Number of lateral branches recorded maximum in 10 %( 17) and in others it ranges from (12-15). In contrary number of nodules found highest in control (14) followed by 5%, 10%,

15% (12) (11) and (11) respectively. In *Medicago sativa* L, maximum plant height was observed in 5% and 10% (22cm), minimum was in 40 % (11cm). maximum root length found in control (12cm), minimum in 40%(6cm). Number of lateral branches (root), Number of nodules/plant were maximum in 15 %( 9) and (9) respectively, while lateral branches (root) were in all others (6-7). In *Trigonella foenum graecum L,* maximum plant height was observed in 10% (28cm) and minimum was in 40% (20cm). Length of roots ranges (7-9cm) in all amendments, maximum was in control and 10% (9cm). Number of lateral branches were maximum in 10 %( 13cm) followed by control (12cm), number of nodules per plant were (12) in 10% and 15%. In *Glycine max* L maximum plant height was in 10 %( 41cm), similarly root length was maximum in 10 %( 22cm) followed by control (21cm), minimum was in 40% (15cm). Number of lateral branches were maximum in 10% (24), and minimum in 40%(16).number of root nodules per plant found maximum in control(20) and reduction was observed in higher flyash concentrations.(Table-9, Plate-18,22,26,30).

## (6) BACTERIAL STUDIES-

From the prior study it is concluded that up to 25% flyash amendment did not show any negative response especially in nodule development and growth, so the comparative study of four legume plants, grown in control with plants grown in 25% flyash amended soil was done. Comparative study includes isolation, culture and various microbiological tests.

For the study of impact of flyash on rhizobium bacteria, selected plants were grown in control soil and 25% flyash amended soil, nodule were collected, sterilized and rhizobium were inoculated on YEMA agar, After 24-48 hour inoculation on YEMA plates bacterial colonies were appeared, marked their location and re-streak again to avoid contamination. These isolated bacteria from (control and 25% flyash

## Table – 9 : Response of Flyash Amendments on Nodulation Parameter of Selected Plants Grown in Pot (40 to 60 days)

| | Fly ash Concentration | Number of Seed | Root Length (Avg.) | Plant Length (Avg.) | No. of Main Lateral Branched (Root) | No. of Nodules |
|---|---|---|---|---|---|---|
| ***Cyamopsis tetragonoloba L (RMG1002)*** | **CONTROL** | **10** | **30** | **57** | **12** | **14** |
| | **5% Fly ash** | **10** | **29** | **57** | **14** | **12** |
| | **10% Fly ash** | **10** | **32** | **59** | **17** | **11** |
| | **15% Fly ash** | **10** | **28** | **53** | **12** | **11** |
| | **20% Fly ash** | **10** | **30** | **56** | **15** | **9** |
| | **25% Fly ash** | **10** | **26** | **48** | **13** | **11** |
| | **40% Fly ash** | **10** | **22** | **46** | **12** | **7** |
| | | | | | | |
| ***Glycine max L (JS 335)*** | **CONTROL** | ***10*** | ***21*** | ***39*** | ***23*** | ***20*** |
| | **5% Fly ash** | ***10*** | ***19*** | ***34*** | ***22*** | ***14*** |
| | **10% Fly ash** | ***10*** | ***22*** | ***41*** | ***24*** | ***13*** |
| | **15% Fly ash** | **10** | **20** | **38** | **19** | **14** |
| | **20% Fly ash** | **10** | **17** | **39** | **17** | **12** |
| | **25% Fly ash** | **10** | **19** | **38** | **20** | **11** |
| | **40% Fly ash** | **10** | **15** | **36** | **16** | **10** |

**Cont....**

**Table – 9 :** **Cont....**

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Medicago sativa L (T9)*** | **CONTROL** | ***10*** | ***12*** | ***21*** | ***7*** | ***8*** |
| | **5% Fly ash** | ***10*** | ***11*** | ***22*** | ***6*** | ***5*** |
| | **10% Fly ash** | ***10*** | ***9*** | ***22*** | ***7*** | ***7*** |
| | **15% Fly ash** | ***10*** | ***11*** | ***19*** | ***9*** | ***9*** |
| | **20% Fly ash** | ***10*** | ***9*** | ***17*** | ***6*** | ***9*** |
| | **25% Fly ash** | ***10*** | ***8*** | ***18*** | ***7*** | ***8*** |
| | **40% Fly ash** | ***10*** | ***6*** | ***11*** | ***6*** | ***8*** |
| | | | | | | |
| ***Trigonella foenum graecum L (SWATI 1)*** | **CONTROL** | ***10*** | ***9*** | ***24*** | ***12*** | ***10*** |
| | **5% Fly ash** | ***10*** | ***7*** | ***22*** | ***9*** | ***9*** |
| | **10% Fly ash** | ***10*** | ***9*** | ***28*** | ***13*** | ***12*** |
| | **15% Fly ash** | ***10*** | ***8*** | ***27*** | ***8*** | ***12*** |
| | **20% Fly ash** | ***10*** | ***7*** | ***24*** | ***8*** | ***11*** |
| | **25% Fly ash** | ***10*** | ***7*** | ***22*** | ***9*** | ***10*** |
| | **40% Fly ash** | ***10*** | ***7*** | ***20*** | ***8*** | ***9*** |
| | | | | | | |

amended) four different legumes were identified by their staining, morphology and cultural characters, all the results were similar to the standard results given by Rhizobium. Colonies appearing white to somewhat translucent circular and rose on the agar surface. Isolated colonies of rhizobium were transferred on YEMA medium slopes and stored in the refrigerator for further studies.

**6-A. Morphological Characters** - Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L, *Glycine max* L, grown in control and 25% flyash amended soils and were examined through gram reaction (Plate- 18,22,26,30), Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Cyamopsis tetragonoloba* L grown in control and 25% flyash amended soils and were observed Gram negative, pink colour short rods, these rods were arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, these are fast growing bacteria with less than 6 hr generation time. (Plate-18). *Glycine max L*, grown in control and 25% flyash amended soils and were examined through gram reaction (Plate-22),after staining Gram negative, pink colour short rods were observed, these rods were also arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Medicago sativa* L *and Trigonella foenum graecum* L grown in control and 25% flyash amended soils and were examined through gram reaction (Plate-26,30) after staining Gram negative, pink colour short rods were observed, these rods were arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods,

**6-B. Motility Test—** The tube contains agar at pH 7.3 were inoculated by isolated rhizobium from all four legume plants. The pattern of growth in the motility agar stab culture of rhizobium of *Glycine max* L was observed

after 48-72 hr of incubation, and observed that bacteria move slowly from the stab line in to medium. The pattern of growth in the motility agar stab culture of rhizobium of *Cyamopsis tetragonoloba* L was observed after 48-72 hr of incubation, and observed that bacteria move fast comparative to other from the stab line in to medium. Rhizobium of *Medicago sativa* L, *and Trigonella foenum graecum* L move slow but comparative faster than rhizobium of *Glycine max* L*.*

**6-C. Cultural Characters**-Two to three days old culture grown on YEM agar plate examined for colony characters, colonies of *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L*, Glycine max* L*,* were circular, convex, whitish pink and glistering with entire margin. (Table-10,11,12,13,Plate-18,22,26,30). Fast growing rhizobia as *Cyamopsis tetragonoloba L* produce white, semitranslusent, circular, mucilaginous colonies while slow growing strains *Glycine max L* produce white, opaque, circular, granular colonies, which do not exceed 1mm in diameter after prolonged incubation. Culture grown on YEM agar plate examined for colony characters, the size of colony was 2-4mm in 3-5days incubation, colonies of *Cyamopsis tetragonoloba L* were circular, pin head like, convex, whitish pink and glistering with entire margin. 5-10 days old culture grown on YEMA agar plate examined for colony characters, colonies of *Glycine max L.* were circular, convex, whitish pink and glistering with entire margin. These are slow growing bacteria having more than 12 hr generation time. The colony were not exceed more than 1mm in diameter in 5-7days incubation on YEMA. Three days old culture grown on YEM agar plate examined for colony characters, colonies of *Medicago sativa L* were circular, convex, whitish pink and glistering with entire margin. Two to three days old culture grown on YEM agar plate examined for colony characters, colonies of *Trigonella foenum graecum L* were circular, convex, whitish pink and glistering with entire margin.( Table-13,Plate-18,22,26,30).

## Table-10 : Cultural Morphological and Biochemical Character of *Rhizobium leguminosarum* (*Cyamopsis tetragonoloba* L)

| Sr. No. | Characters | Result |
|---|---|---|
| 1. | Shape | Circular |
| 2. | Color | White creamish |
| 3. | Opacity | Opaque/ Semitransparent |
| 4. | Margin | Regular/entire |
| 5. | Elevation | Convex/ Raised |
| 6. | Shape | Rod shaped |
| 7. | Oxygen demand | Aerobic |
| 8. | Motility | Motile |
| 9. | Spore formation | Non spore forming |
| 10. | Gram's nature | Gram Negative |
| 11. | Production of Indole from tryptophan | Negative |
| 12. | Methyl red test | Negative |
| 13. | Voges-Proskauer test | Negative |
| 14. | Citrate utilization as source of carbon | Positive |
| 15. | Production of ammonia from urea | Positive |
| 16. | Production of Hydrogen peroxide | Negative |
| 17. | Nitrate Reduction | Positive |
| 18. | Catalase test | Positive |

## Table-11: Cultural Morphological and Biochemical Character of *Rhizobium japonicum* *(Glycine max L)*

| Sr. No. | Characters | Result |
|---|---|---|
| 1. | Shape | Circular |
| 2. | Color | Whitish pink and glistering |
| 3. | Opacity | Opaque/ Semitransparent |
| 4. | Margin | Regular/entire |
| 5. | Elevation | Convex/ Raised |
| 6. | Shape | Rod shaped |
| 7. | Oxygen demand | Aerobic |
| 8. | Motility | Motile |
| 9. | Spore formation | Non spore forming |
| 10. | Gram's nature | Gram Negative |
| 11. | Production of Indole from tryptophan | Negative |
| 12. | Methyl red test | Negative |
| 13. | Voges-Proskauer test | Negative |
| 14. | Citrate utilization as source of carbon | Positive |
| 15. | Production of ammonia from urea | Positive |
| 16. | Production of Hydrogen peroxide | Negative |
| 17. | Nitrate Reduction | Positive |
| 18. | Catalase test | Positive |

## Table-12 :Cultural Morphological and Biochemical Character of *Rhizobium meliloti* *(Medicgo sativa L)*

| Sr. No. | Characters | Result |
|---|---|---|
| 1. | Shape | Circular |
| 2. | Color | White creamish |
| 3. | Opacity | Opaque/ Semitransparent |
| 4. | Margin | Regular/entire |
| 5. | Elevation | Convex/ Raised |
| 6. | Shape | Rod shaped |
| 7. | Oxygen demand | Aerobic |
| 8. | Motility | Motile |
| 9. | Spore formation | Non spore forming |
| 10. | Gram's nature | Gram Negative |
| 11. | Production of Indole from tryptophan | Negative |
| 12. | Methyl red test | Negative |
| 13. | Voges-Proskauer test | Negative |
| 14. | Citrate utilization as source of carbon | Positive |
| 15. | Production of ammonia from urea | Positive |
| 16. | Production of Hydrogen peroxide | Negative |
| 17. | Nitrate Reduction | Positive |
| 18. | Catalase test | Positive |

## Table-13: Cultural Morphological and Biochemical Character of *Rhizobium meliloti* *(Trigonella faenum graecum L )*

| Sr. No. | Characters | Result |
|---|---|---|
| 1. | Shape | Circular |
| 2. | Color | White creamish |
| 3. | Opacity | Opaque/ Semitransparent |
| 4. | Margin | Regular/entire |
| 5. | Elevation | Convex/ Raised |
| 6. | Shape | Rod shaped |
| 7. | Oxygen demand | Aerobic |
| 8. | Motility | Motile |
| 9. | Spore formation | Non spore forming |
| 10. | Gram's nature | Gram Negative |
| 11. | Production of Indole from tryptophan | Negative |
| 12. | Methyl red test | Negative |
| 13. | Voges-Proskauer test | Negative |
| 14. | Citrate utilization as source of carbon | Positive |
| 15. | Production of ammonia from urea | Positive |
| 16. | Production of Hydrogen peroxide | Negative |
| 17. | Nitrate Reduction | Positive |
| 18. | Catalase test | Positive |

## (7) BIOCHEMICAL TEST OF RHIZOBIUM

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja (1996) from *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L*, Glycine max* L, (4 plants X 2(control/25%FA amended)=8) experimental sets. The biochemical tests were carried out in growth medium at 28˚C for 48 hours incubation. All the tests were carried out with 03 replicates.

**7-A. Indole Test -** The tube containing SIM agar medium at pH 7.3 were inoculated by isolated bacteria, After 24-48 hr of inoculation on addition of Kovac's reagent, change in colour of media was observed, (Table-10,11,12,13,16 Plate-19,23,27,31(EF) and it also observed that after addition of Kovac's reagent a red colour ring formed in all four sets of plants.

**7-B. Methyl Red - The** tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated Rhizobium culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in colour of the medium was observed, (Table-10-13&16 Plate-19,23,27,31(AB)and observed that red colour was appeared after the addition of methyl-red reagent all four sets of plants.

**7-C. Voges Proskauer-** The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated Rhizobium culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B,, change in colour of the medium was observed,(Table-10-13&16 Plate-19,23,27,31(CD)and find that there was no colour change was observed all four sets of plants.

**7-D. Citrate Utilization-** The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by Rhizobium culture. After 24-48 hours of incubation change in the colour of the media was observed and result showed change in colour of medium all four sets of plants. (Table-10-13&16 Plate-20,24,28,32(AB)

**7-E. Nitrate Reduction-** The tube containing (Trypticase nitrate broth)at pH-7.2 were inoculated by isolated bacterial culture. After 24-48hours of inoculation on addition, solution A ( Sulphanilic acid) and Solution B(α-naphthylamine) and zinc power, change in colour of media was observed and colour of media found changed in all four sets of plants (Table-10-13, &16)( Plate-21,25,29,33(AB).

**7-F. Hydrogen Sulphide-** SIM agar medium prepared, pour in to SIM Agar deep tubes and autoclaved, After cooling aseptically incubate in to its appropriately labelled tube by means of stab inoculation, incubate all cultures for 24-48 hours at $37^0$C. Change in colour of media was absent in all four sets of plants (Table-10-13&16) ( Plate-20,24,28,32(CD).

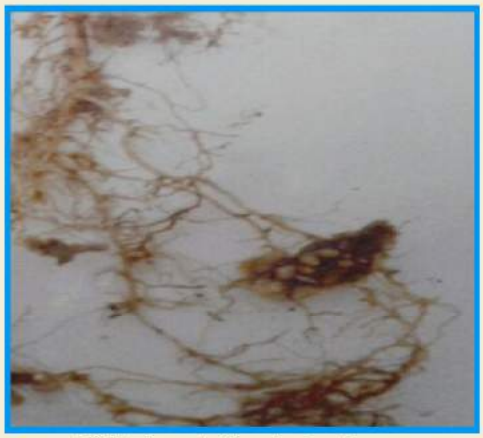
**7-G. Urease-** The tube containing (Urea Broth) at pH-6.8 (*after sterilization add 20% urea solution previously sterilized by filtration) were inoculated by isolated bacterial culture ie Rhizobium. After 24-48 hours of incubation, change in the colour of the media was observed and change in colour of media was obtained all four sets of plants. (Table-10-13&16)( Plate-20,24,28,32(EF)

**7-H. Catalase Test-** Slide containing 2-3 drops of (Trypticase soya broth) at pH-7.3 were inoculated by 24-48 hours isolated Rhizobium culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Bubbles were appeared on the slide in all four sets of plants. Different isolates which were 48 hour old were flooded with hydrogen peroxide and observed for liberation of effervescence of oxygen around the bacterial colonies according to Graham and Parker (1964)., (Table-10-13&16)( Plate-21,25,29,33(CD)

All four experimental sets of plant *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L*, Glycine max* L, (control/25%FAamended) show positive reaction to Citrate utilization, Urease test, Catalase test, Nitrate reduction test, though intensity of colour and duration is very in all four.

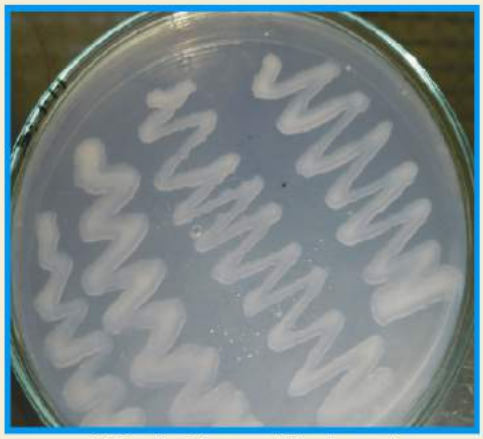
To study the fast or slow growing nature of the isolates they were grown on freshly prepared YEMA plates containing bromo-thymol blue adjusting the pH to 6.8. *Rhizobium leguminosarum* of *Cyamopsis tetragonoloba* L was found fast growing followed by *Rhizobium meliloti of Trigonella faenum graecum* L. *and Medicago sativa* L*, while Brabyrhizobium japonicum* of *Glycine max* L *was* slow growing strain.

# PLATE - 18



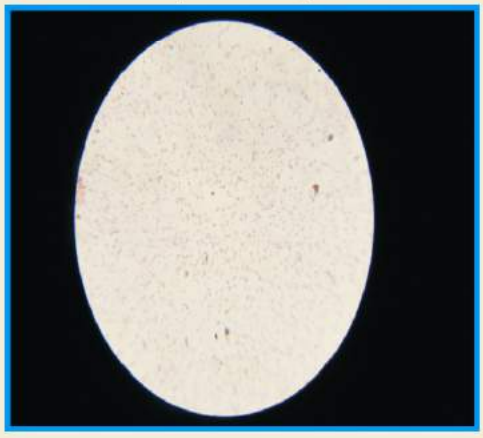
(A) Root Nodule from *Cyamopsis tetragonoloba* L (Control)

(B) Root Nodule from *Cyamopsis tetragonoloba* L (25% FA Treated)

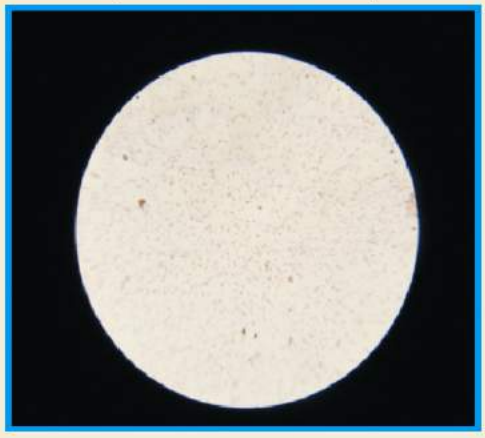


(C) Culture Plate of *Cyamopsis tetragonoloba* L (Control)

(D) Culture Plate of *Cyamopsis tetragonoloba* L (25% FA Treated)



(E) Microscopic View of Bacteria Showing Gram Staining Reaction (Control)



(F) Microscopic View of Bacteria Showing Gram Staining Reaction (25% FA Treated)

**Various Stages of Rhizobium Culture in *Cyamopsis tetragonoloba* L**

# PLATE - 19

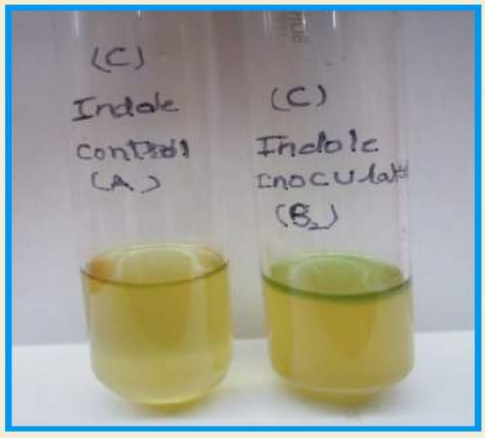

(A) Methyl Red Test
(Control)


(B) Methyl Red
(25% FA Treated)


(C) Voges Proskauer Test
(Control)


(D) Voges Proskauer Test
(25% FA Treated)


(E) Indole Test
(Control)




(F) Indole Test
(25% Treated)

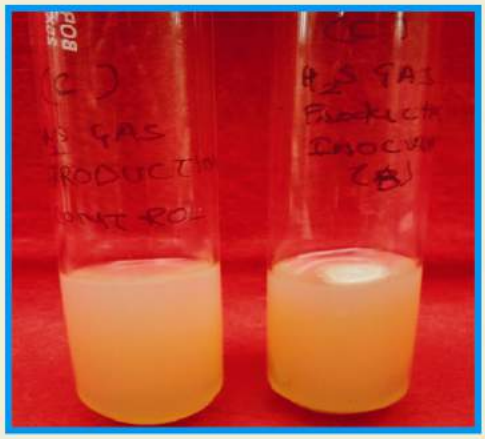
**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Cyamopsis tetragonoloba* L**

# PLATE - 20

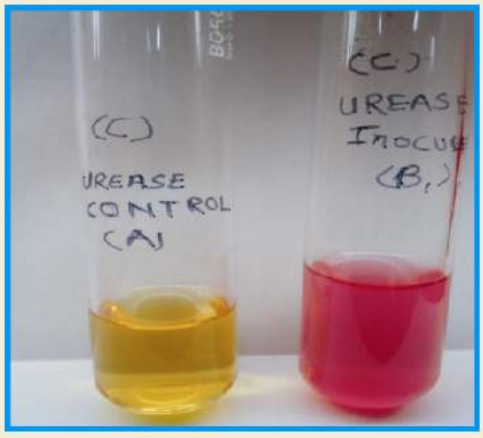
(A) Citrate Utilization Test (Control)

(B) Citrate Utilization Test (25% FA Treated)

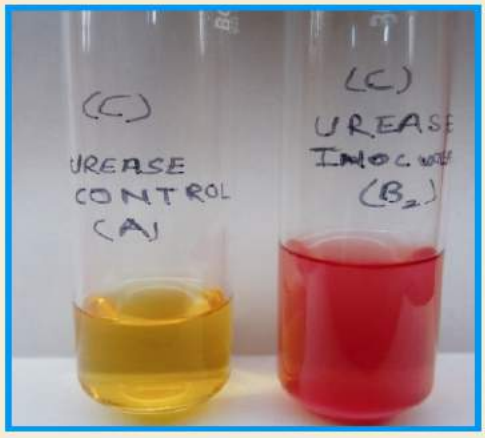
(C) Hydrogen Sulphide Production Test (Control)



(D) Hydrogen Sulphide Production Test (25% FA Treated)



(E) Urease Production Test (Control)



(F) Urease Production Test ( 25 % FA Treated)

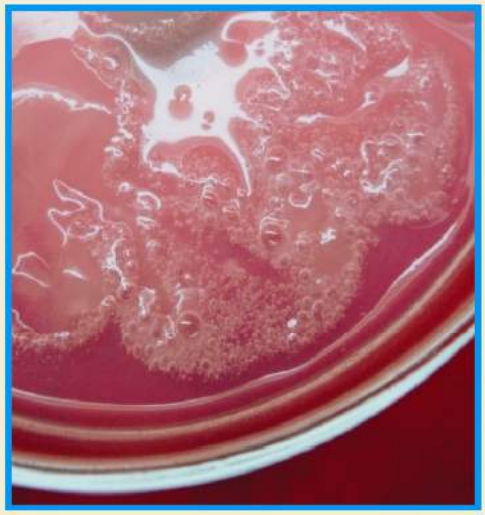
**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Cyamopsis tetragonoloba* L**

# PLATE - 21


(A) Nitrate Reduction Test
(Control)


(B) Nitrate Reduction Test
(25% FA Treated)

(C) Catalase Test
(Control)



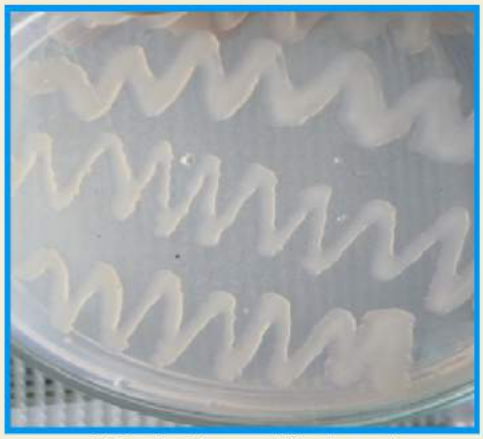
(D) Catalase Test
(25% FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Cyamopsis tetragonoloba* L**

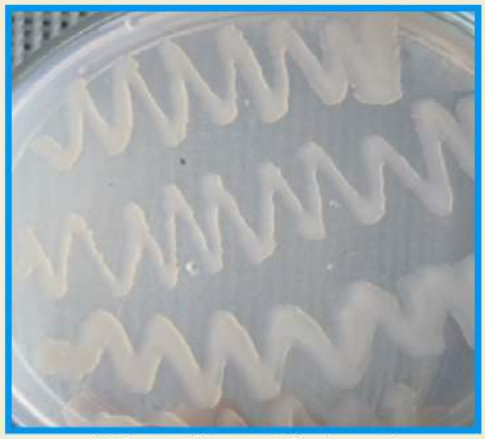
# PLATE - 22

(A) Root Nodule from *Glycine max* L (Control)

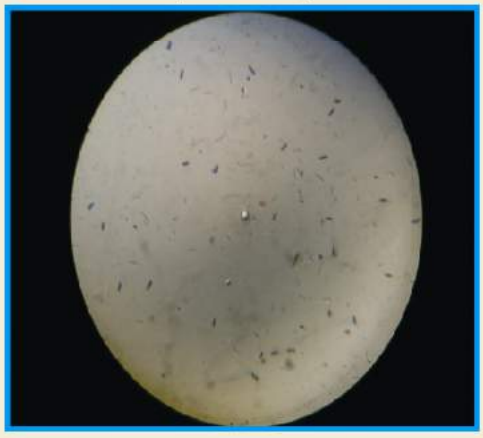
(B) Root Nodule from *Glycine max* L (25% FA Treated)



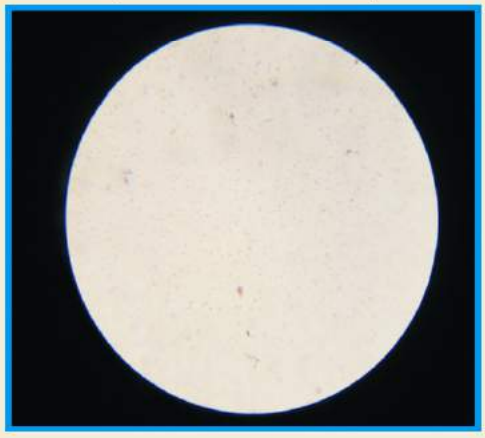
(C) Culture Plate of *Glycine max* L (Control)



(D) Culture Plate of *Glycine max* L (25% FA Treated)



(E) Microscopic View of Bacteria Showing Gram Staining Reaction (Control)



(F) Microscopic View of Bacteria Showing Gram Staining Reaction (25% FA Treated)

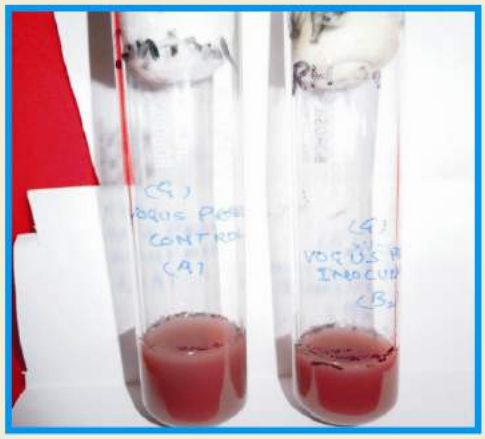
**Various Stages of Rhizobium Culture in *Glycine max* L**

# PLATE - 23

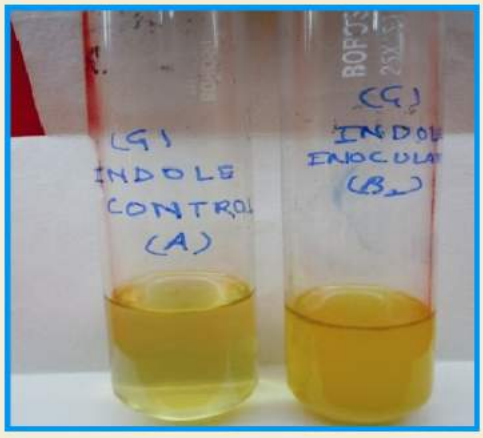
(A) Methyl Red Test
(Control)

(B) Methyl Red
(25% FA Treated)

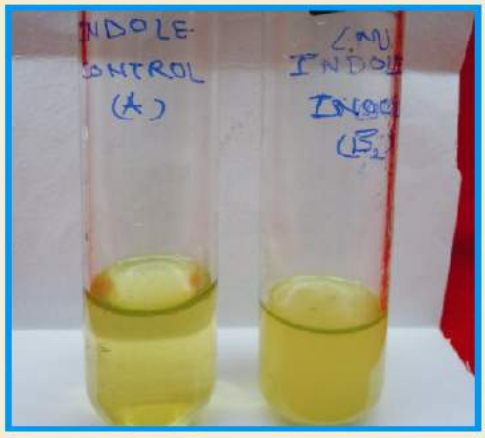
(C) Voges Proskauer Test
(Control)



(D) Voges Proskauer Test
(25% FA Treated)



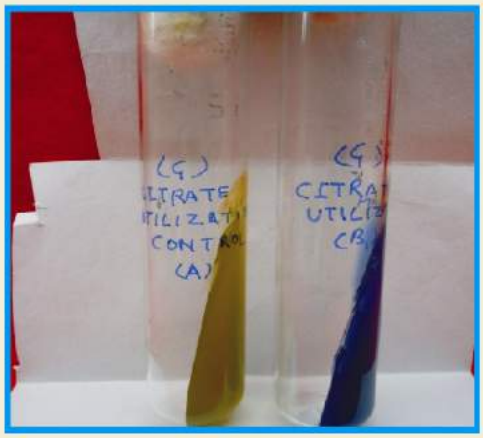
(E) Indole Test
(Control)



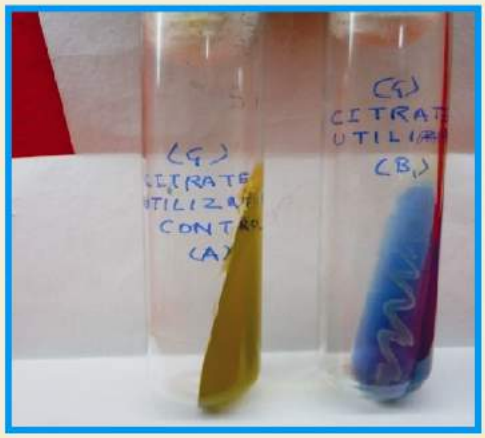
(F) Indole Test
(25% Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Glycine max* L**

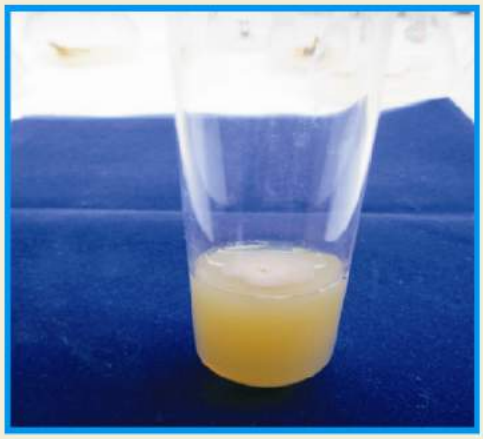
# PLATE - 24



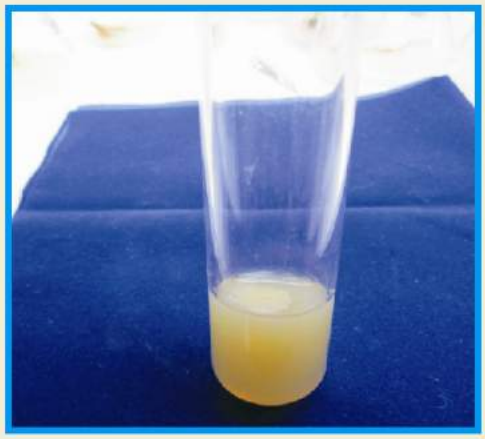
(A) Citrate Utilization Test (Control)



(B) Citrate Utilization Test (25% FA Treated)



(C) Hydrogen Sulphide Production Test (Control)



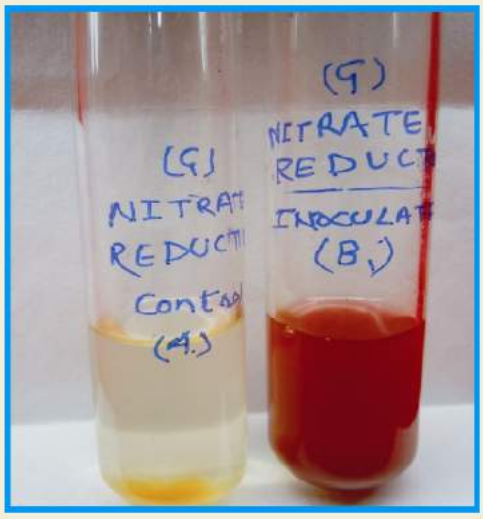
(D)Hydrogen Sulphide Production Test (25% FA Treated)

(E) Urease Production Test (Control)

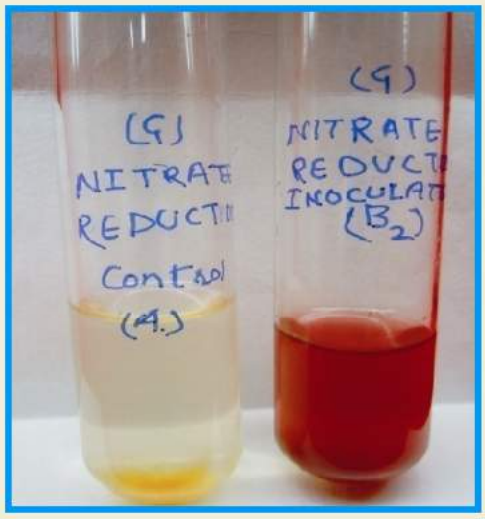
(F)Urease Production Test ( 25 % FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Glycine max* L**

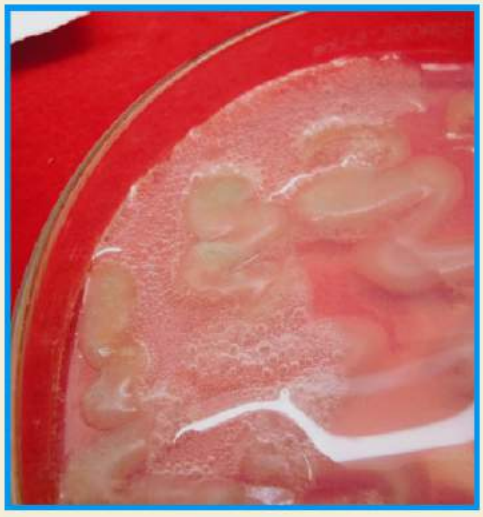
# PLATE - 25



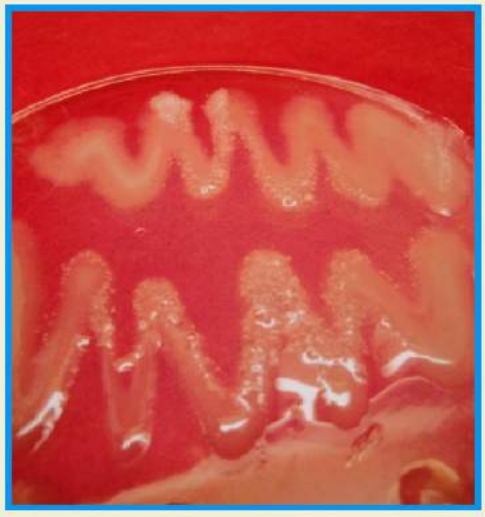

(A) Nitrate Reduction Test
(Control)




(B) Nitrate Reduction Test
(25% FA Treated)



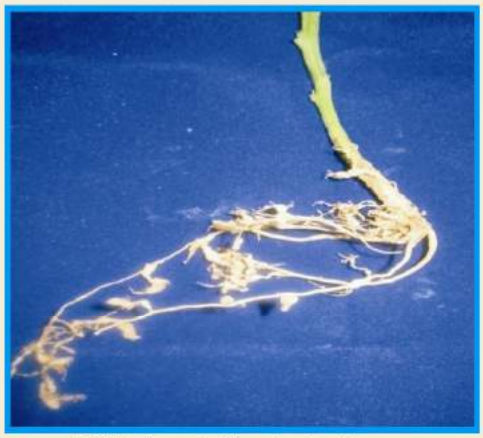
(C) Catalase Test
(Control)



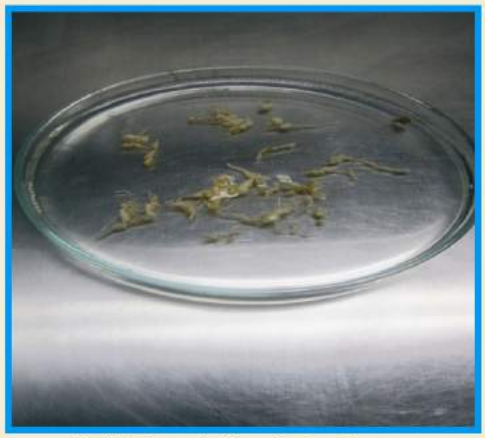
(D) Catalase Test
(25% FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Glycine max* L**

# PLATE - 26

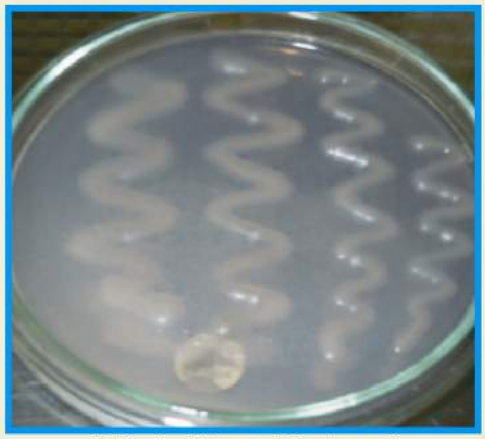


(A) Root Nodule from *Medicago sativa* (Control)

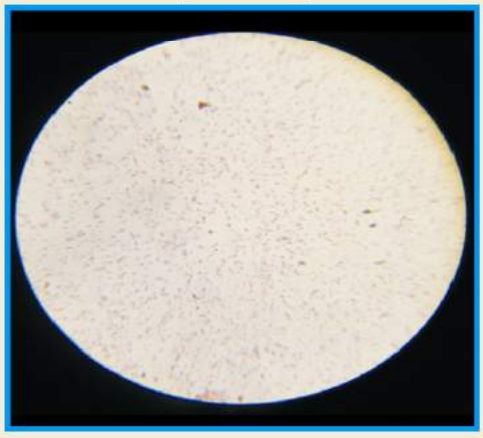


(B) Root Nodule from *Medicago sativa* (25% FA Treated)

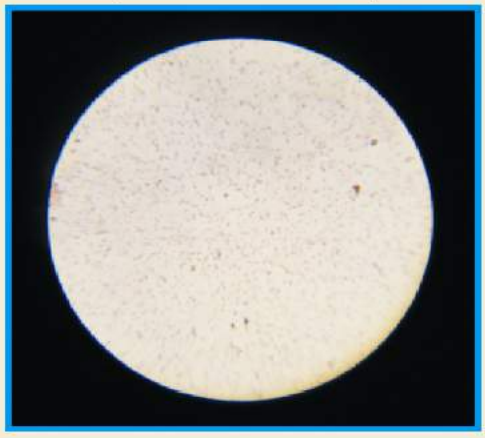
(C) Culture Plate of *Medicago sativa* (Control)



(D) Culture Plate of *Medicago sativa* (25% FA Treated)



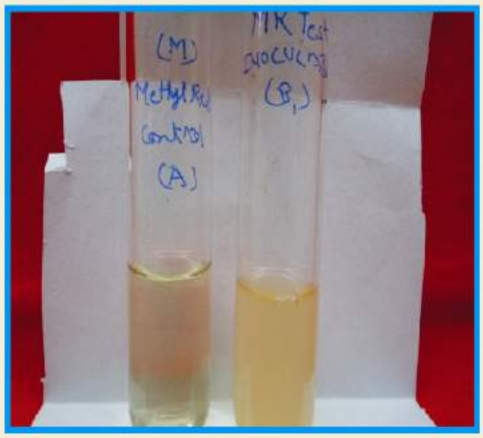
(E) Microscopic View of Bacteria Showing Gram Staining Reaction (Control)



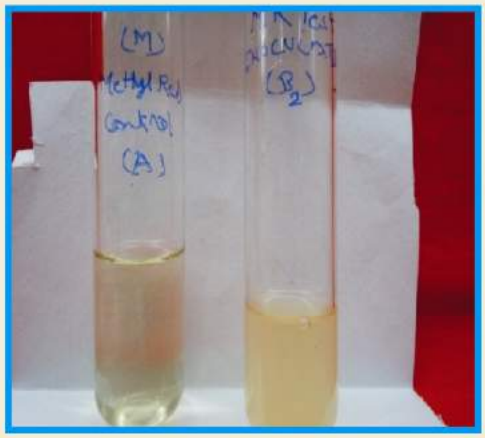
(F) Microscopic View of Bacteria Showing Gram Staining Reaction (25% FA Treated)

**Various Stages of Rhizobium Culture in Medicago sativa L**

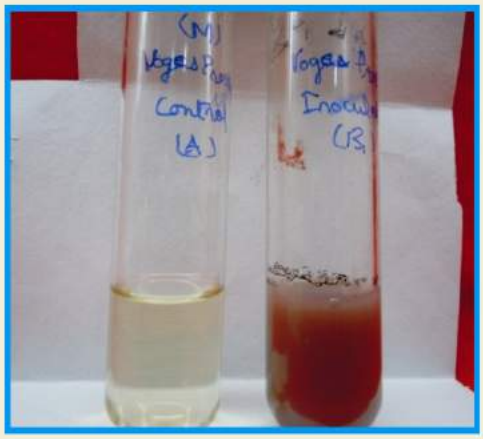
# PLATE - 27



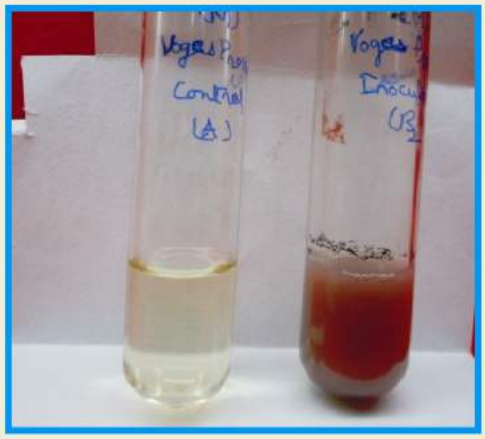
(A) Methyl Red Test
(Control)



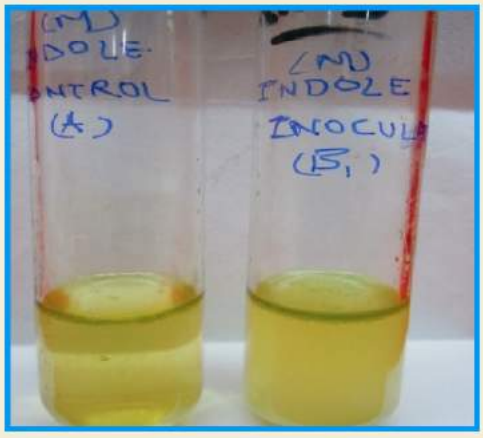
(B) Methyl Red
(25% FA Treated)



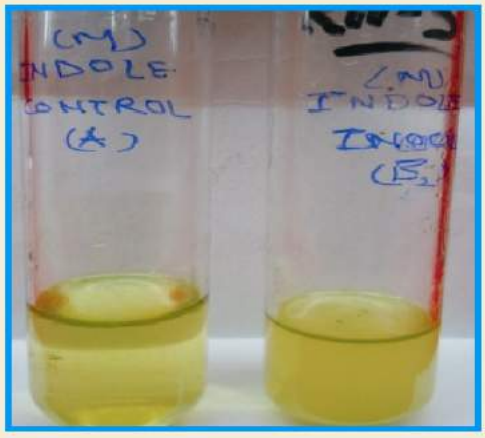
(C) Voges Proskauer Test
(Control)



(D) Voges Proskauer Test
(25% FA Treated)



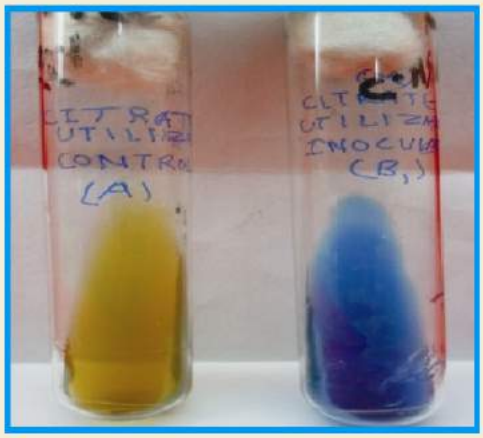
(E) Indole Test
(Control)



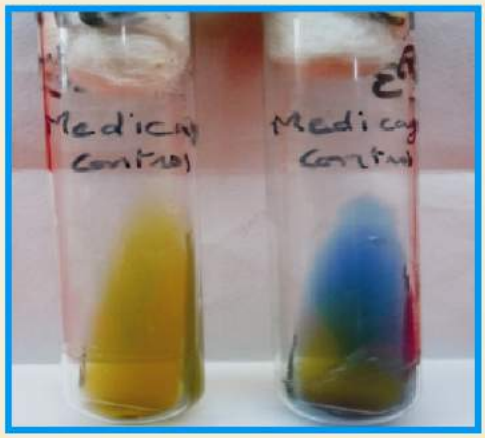
(F) Indole Test
(25% Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Medicago sativa* L**

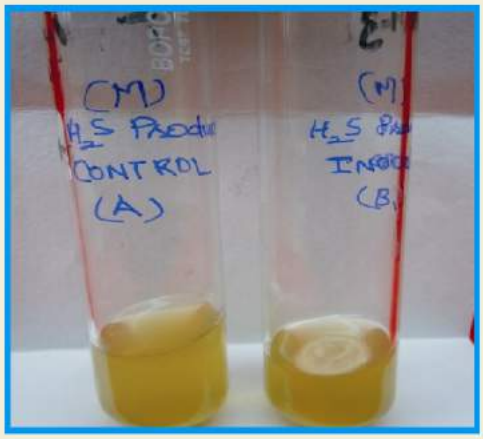
# PLATE - 28



(A) Citrate Utilization Test (Control)



(B) Citrate Utilization Test (25% FA Treated)



(C) Hydrogen Sulphide Production Test (Control)

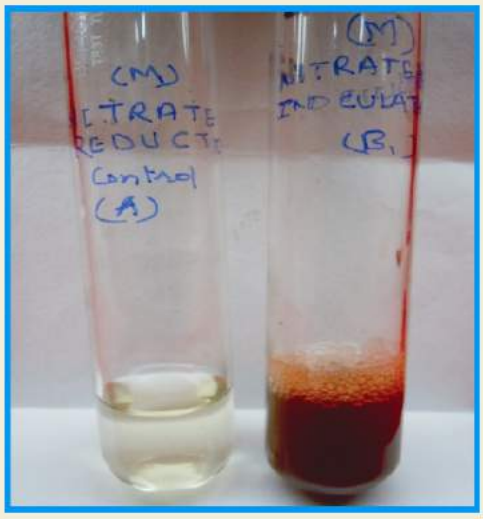
(D) Hydrogen Sulphide Production Test (25% FA Treated)

(E) Urease Production Test (Control)

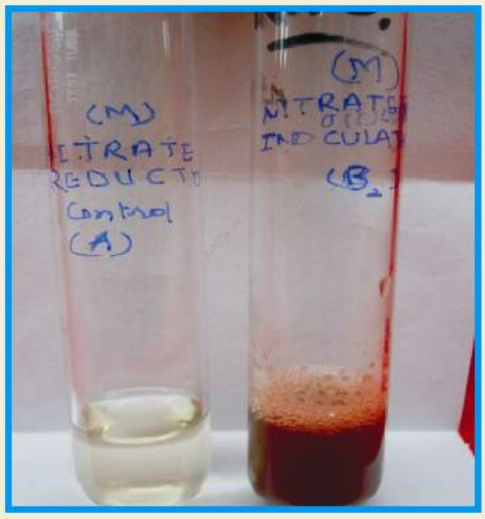
(F) Urease Production Test ( 25 % FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Medicago sativa* L**

# PLATE - 29




(A) Nitrate Reduction Test
(Control)




(B) Nitrate Reduction Test
(25% FA Treated)

(C) Catalase Test
(Control)

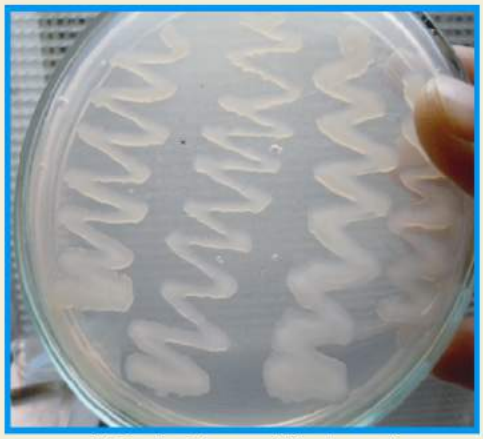
(D) Catalase Test
(25% FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Medicago sativa* L**

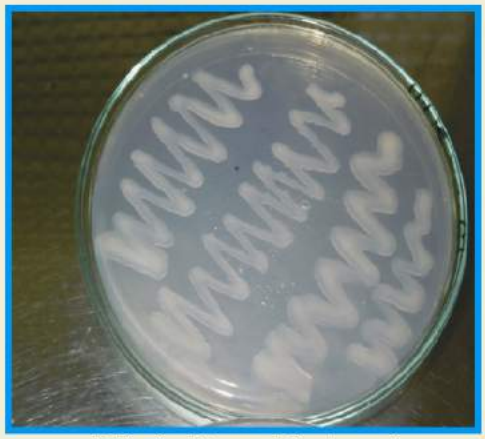
# PLATE - 30

(A) Root Nodule from *Trigonella foenum graecum* L (Control)

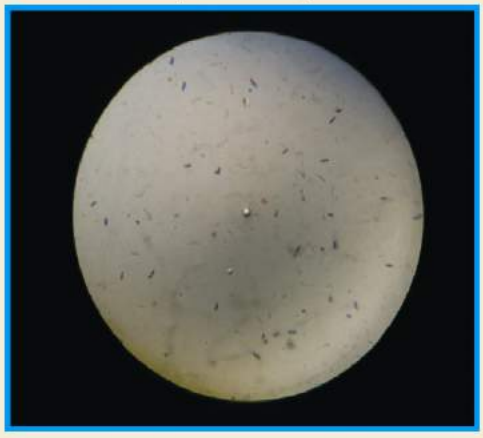
(B) Root Nodule from *Trigonella foenum graecum* L (25% FA Treated)



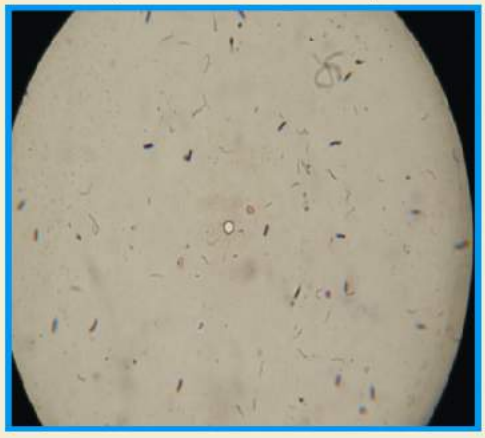
(C) Culture Plate of *Trigonella foenum graecum* L (Control)



(D) Culture Plate of *Trigonella foenum graecum* L (25% FA Treated)



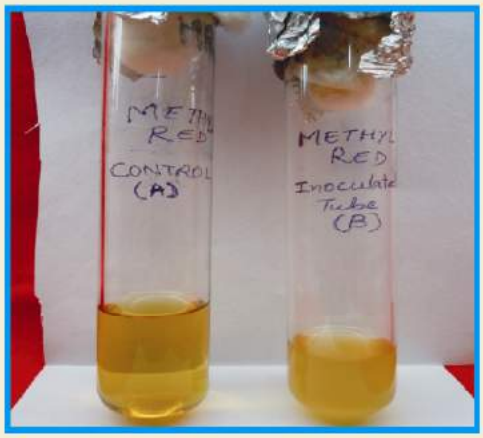
(E) Microscopic View of Bacteria Showing Gram Staining Reaction (Control)



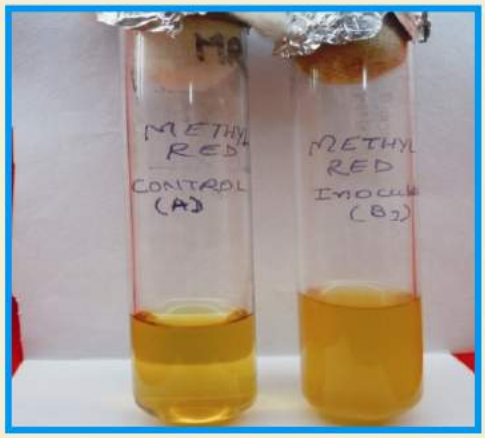
(F) Microscopic View of Bacteria Showing Gram Staining Reaction (25% FA Treated)

**Various Stages of Rhizobium Culture in *Trigonella foenum graecum* L**

# PLATE - 31

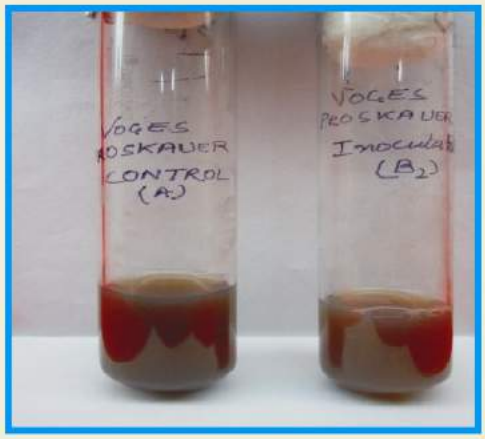



(A) Methyl Red Test
(Control)

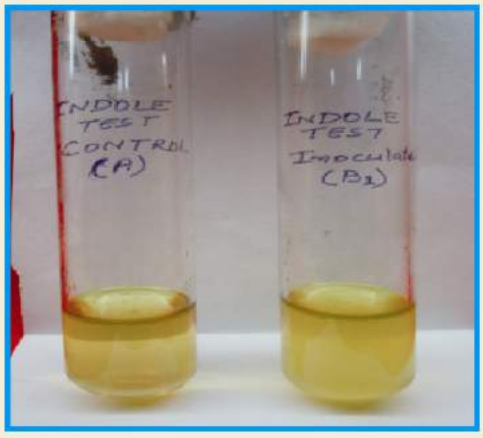



(B) Methyl Red
(25% FA Treated)

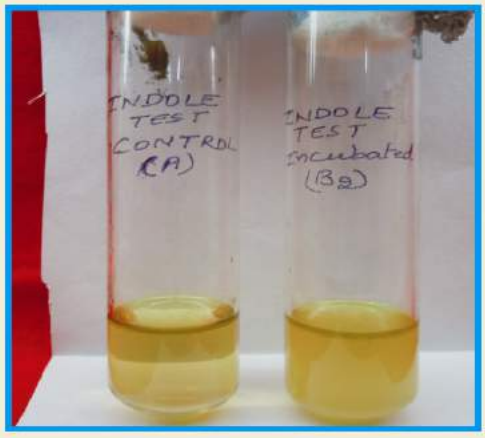

(C) Voges Proskauer Test
(Control)




(D) Voges Proskauer Test
(25% FA Treated)



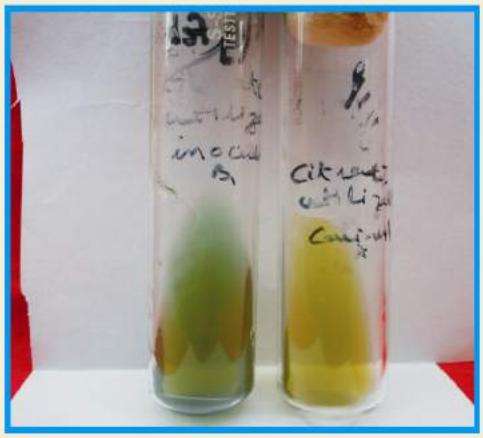

(E) Indole Test
(Control)



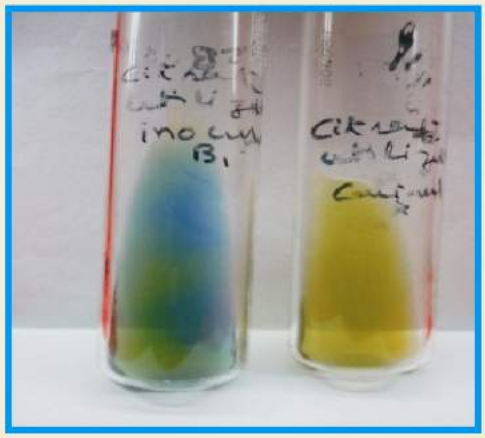

(F) Indole Test
(25% Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Trigonella foenum graecum* L**

# PLATE - 32

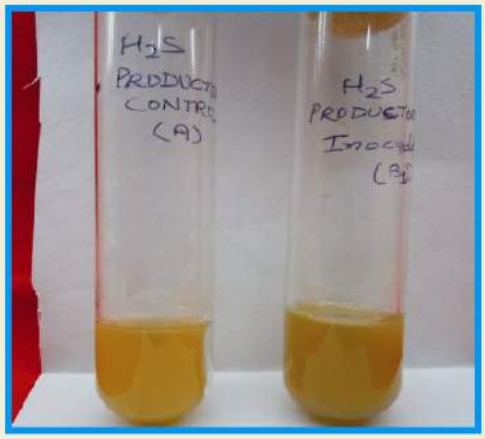


(A) Citrate Utilization Test (Control)

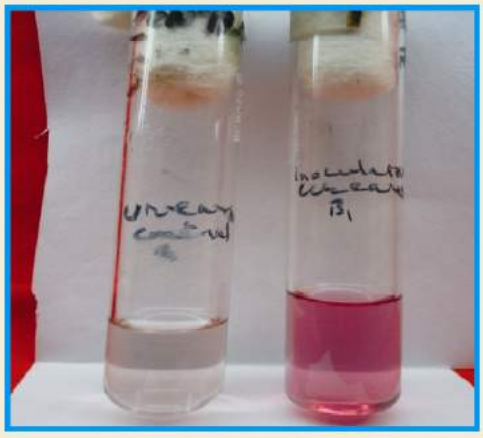


(B) Citrate Utilization Test (25% FA Treated)

(C) Hydrogen Sulphide Production Test (Control)



(D) Hydrogen Sulphide Production Test (25% FA Treated)

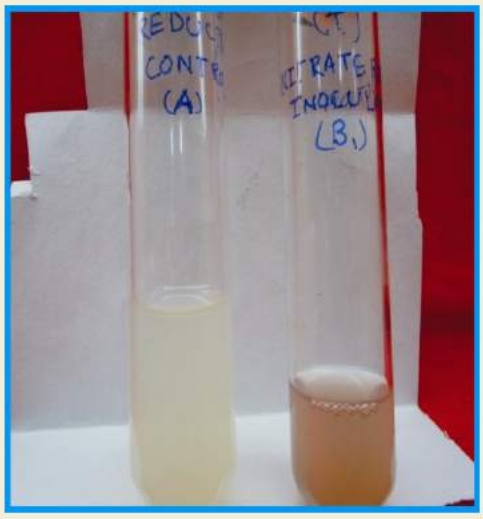


(E) Urease Production Test (Control)

(F) Urease Production Test ( 25 % FA Treated)

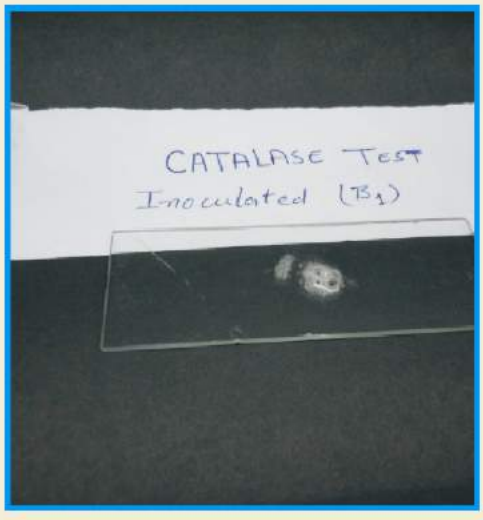
**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Trigonella foenum graecum* L**

# PLATE - 33

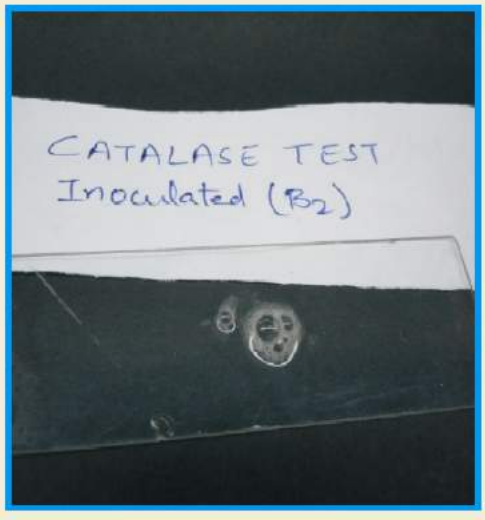



(A) Nitrate Reduction Test
(Control)


(B) Nitrate Reduction Test
(25% FA Treated)




(C) Catalase Test
(Control)




(D) Catalase Test
(25% FA Treated)

**Tubes Showing Biochemical Reaction by Rhizobial Strains of *Trigonella foenum graecum* L**

CHAPTER – 6

# DISCUSSION & ConClusion

# DISCUSSION & ConClusion

Soil is the essential natural resource, it developed below the continental land surface on the earth, also called as skin of earth. It is vital, biologically active, porous medium responsible for existence of many forms life. Rapid industrialization, soil erosion, deforestation cause degradation of soil result is leaching the vital nutrients from the top soil make the soil unproductive ultimately change the composition and complexion of soil, it also kill the soil bacteria these bacteria are necessary to fix nitrogen from the atmosphere and make it available to the plants. Problem of soil pollution differs from air and water pollution in the respect that the pollutants remain in direct contact with the soil for relatively longer period.

One objective of present study is aimed to find out the resistant species in polluted area, regular observation of few selective plots was under taken near thermal power station. The result indicates that ground vegetation i.e. herbs and the shrub seem to be more sensitive to pollution induced changes then the trees. Higher sensitivity of the herbs can be explained as they are very small plants and exposed entirely to the environmental stress, and the plant surface is tender which absorbs pollutants without resistance. Most of the herbs were annuals and during wet period they were in initial phase of their life cycle, and it is proved that plants are especially susceptible to environmental stress during the period of initial establishment (Thakre and Rao, 1985 and Verma and Sharma 2009). Trees around thermal power station were growing much before the power plant came in to existence, while in forest area trees are eliminated first by low dosages of pollutants, as duration of exposure increases, tall shrubs are eliminated followed in order by lower shrubs, herbs mosses and lichens. (Woodwell, 1970). Tolerant species may be used as bio-monitor for monitoring the quantitative and qualitative level of pollutants. Tree and shrub species act as pollution sink due to their higher ecological amplitude of tolerance, although the sensitive plants show morphological,

anatomical and biochemical changes and act as bio indicator of pollution levels. The herbaceous vegetation of an area depict the actual picture of pollution level caused by thermal power station, because only few tolerant species can survive there and the actually sensitive species are disappear in that particular area by the damaging effect of various types of pollutions caused by flyash. Herbs were less in number and diversity in comparatively last two decades. Table-3 show family wise distribution pattern of plants, changes were clearly observed during two decades, in previous studies 27 families out of total listed, were available while during our present study, 29 families were observed, although it is not the remarkable difference, but change in dominant families distribution pattern were found. According to review of literature of last two decades the most dominant families present there were *Compositeae*, *Fabaceae, Poaceae*. In place of these, present study indicated that, Amaranthaceae*, Caesalpiniaceae, Compositeae*, *Euphorbiaceae* were dominantly present in study area. The results presented can be compared to those of Thakre, (1983), Thakre and Agarwal, (1987), Agarwal and Agarwal, (1988), Dadhich and Kasat, (1988).

The review of literature indicates that the dominant tree species observed around KTPS were *Cassia siamea* L*, Cassia fistula* L, *Azadirachta indica,* and *Albizzia lebbeck L* among them *Mangifera indica* L and *Azadirachta indica Juss* show more resistance to emissions as compared to other tree species (Dadhich and Kasat, 1988). In present study 21 tree species were observed which include all the tree species observed by previous workers during last two decades, except *Lannea coromandelich, Poinciana regia*. In these 21 tree species (Table-2) *Cassia siamea* L*, Cassia fistula* L, *Azadirachta indica Juss, Delonix regia* L, *Bauhinia variegate L, Albizzia lebbeck L* were find near the campus while *Anoegissus spp* L*, Acacia nilotica* L and *Zizyphus spp W.A* were dominant far away from main plant and near to fly-ash dumping area. Although tree species show change vegetation profile but it doesn't indicates the actual picture of climate change due to emission during last two decades. Shrub and herbs were also compared, 13 shrub and 32 herb species were

observed by Dadhich and Kasat, (1988), while in this study 38 species of shrubs and herbs were observed in which 7 shrubs were dominant including *Ipomoea fistulosa, Lantana coromandelian Roxb, Thevetia peruviana pess, Ziziphus nummularia Wt &.Arn, Solanum xanthocarpum S.W Abutilion indicum L.* Herbs were less in number and diversity as compared to last two decades, dominant herbs were *Amaranthus spinosus L, Acyranthes aspera L, Chenopodium album L, Euphorbia hirta L, Phyllanthus fraternus W, Croton bonplandianum Baill, Alternanthera pungens Kunth, Tridex procombens L.* Similar results were found during the field survey by Kumari.V, (2009) near Parichha thermal plant station (U.P). It was concluded that trees such as *Acacia catechu, Acacia nilotica, Albizia procera, Azadiracta indica, Butea monosperma, Dalbergia sisoo, Ficus religiosa, Tamarindus indica, Ziziphus jujube, Eucalyptus globules, Cassia fistula, Dalonix regia and Mangifera indica* etc have high tolerance power than other species growing in that area this indicates that these plants can be grown in flyash affected areas. These trees can be grown in thermal power station and across the flyash transporting tract for development of green belt and pollution control. Similarly, some herbaceous species such as *Phaseolus aureus L, Phaseolus mungo L, Pisum sativa L, Cajanus cajan L, Agropyron and Melilotus* spp are thriving better than others these cultivated crops can also be recommended to farmer of KTPS area.

Observation of injury on sensitive plants has provided a means of monitoring air pollution, however under acute pollution defoliation takes place. According to Kumari.V,(2009), Flyash particles are very fine and thus tend to remain airborne for a long period. Flyash dust under certain conditions of humidity, stick to leaves or fruits and appear on the leaves of many vegetables. Higher amount on foliar deposition of flyash resulted in decreased transpiration rate due to barrier created by thicker layer. Similar findings were found by Agarwal *et al., (*2008).

During our research it is concluded that near Kota thermal power station, changes in dominant family's distribution pattern was clearly

observed during last two decades. Our result are supported by these studies, Pandey and Rao, (1979), Thakre, (1983), Thakre and Agarwal, (1987), Agarwal and Agarwal, (1988), Dadhich and Kasat, (1988), Singh and Siddiqui (2003), Khandelwal and Shrivastava, (2013). A comparative study of ecomophological characters of certain herb show less number, diversity and reduced growth with pollution burden symptoms around Kota thermal power station comparatively non polluted area by Khandelwal and Shrivastava, (2013). Different chemical as well as physical injuries like necrotic dark brown spots, chlorosis is observed in vegetation, layer of flyash interfere with the light required for photosynthesis and thus reduce the photosynthetic rate. Leaves laden with flyash absorb heat more effectively and consequently the increased leaf temperature results in increased transpiration rates. Our results resembles with Kumari, V (2009), Prem Kishor, (2010). Stunted growth and early etiolating of leaves were noticed which referred to toxic effect of specific flyash constituents in plants such as B,As,Se,Al and Cd. These elements are readily available to plants and accumulated in the tissues. Chaudhuri *et al.,* (2003), Kumari, V, (2009). The result can explain the facts that toxic compound reported to occur in flyash, which might have contributed towards the poor growth of vegetation due to continuous deposition of flyash in the crop lands around the thermal power station. It was also observed by Singh and Kolay, (2009), Lazar *et al.*,(2008), Khandelwal and Shrivastava, (2013,2014) that most of the vegetation at flyash disposal site showing suppressed growth which may be due to severe deficiency in nitrogen in the flyash rich soil.

India has suffered enormous pressure on its land resources, Kota is not the exception of this, as studied, soil of Kota is continuously degraded due to industrialization, urbanization and its coal based thermal power plant which is notorious for its byproducts like flyash along with atmospheric pollution and water pollution. Coal ash generated in bulk from thermal power plants. The impact of flyash on health and environmental consequences has been discussed extensively by Page *et al.,* (1979), Singh and Yunus, (2000), Ram(2008), Agarwal and Sinha,

(2001), Punjwani *et al.*,(2011), Krishna Rani and Sharma, (2010), Jala and Goyal (2006). Soil pollution caused by ash dumping draw the attention towards the proper management of coal ash and possibilities of its use in agriculture. In many developed countries numerous workers have demonstrated the role of flyash in improving soil physico- chemical properties and crop yield including Eiseewi, *et al.,*(1978), Adriano, *et al.,* (1980), Bhaisare, *et al.,* (2000), Tsadilas, *et al*.,(2002), Krishna Rani (2009,2010), Jala and Goyal, (2006), Panwar *et al.,(*1998). Management of flyash has attained an apparent scenario for scientific and strategic concern in India due to large scale dependence on coal based thermal power plants, waste utilization is the best option of pollution prevention and disaster risk strategy has been worked out during previous decades to open the door for flyash utilization. Now a day in India the use of flyash in agriculture has become of much concern. Various workers as Suwalka, (2003), Krishna Rani and Sharma, (2009, 2010), Agarwal, *et al.,* (2011), Katiyar, *et al.,*(2012), used the flyash as soil ameliorant.

In present study soil properties as influenced by fly ash addition in soil have been studied. Chemical analysis of flyash from KTPS Kota show that flyash is alkaline in nature and a potential source of macro and micro nutrients, similar result were obtained by Mehra, *et al*., (1986) by Shridhran and Pandian, (1998), Punjwani, *et al.,* (2011), Krishna Rani and Sharma (2009,2010). Our result are supported by several researchers as Sharma, *et al.,* (2002), Sikka and Kansai, (1994), Kalra, *et al.,* (1998), Sharma *et al.,*(1990), Deshmukh, (2000), Grewal, *et al.,* (2001), Garg, *et al.,*(2003), Page, *et al*.,(1979), Singh and Yunus, (2000), Agarwal and Sinha, (2001) Sharma and Kalra, (2006).

The present study conducted with loam soil amended with 0%, 5%, 10%, 15%, 20%, 25%, 40% (w/w) flyash indicates modifications in physiochemical characters of soil, indicate application of flyash in to soil has been reported to change the soil texture and structure in a way to improve the availability of soil water, air and nutrient by increasing porosity, water holding capacity, pH and slight increase in electrical

conductivity. A clear change in texture towards coarse to fine particle size along with change in colour i.e. darkest brown to greyish brown with higher percentages of flyash (Table-4). Decrease soil particle density pattern was observed highest density (1.49g/cc$^2$) was found in unamended soil followed by 0%, 5%, 10%, 15%, 20%, 25%, 40% minimum value of density found in 40% flyash amendment. (Table-4) (Figure-1,C). Water holding capacity increases with increased percentages of flyash application (range 40-58%) the maximum value was observed in 40% flyash amended soil, while minimum value observed in untreated soil. (Table-4) (Figure 1,A). Porosity of soil increased with greatly along with flyash application, ranges from 22-45% that is almost double in control to 40% flyash amendment. Increases in porosity in turn decrease the bulk density of soil. (Table-4) (Figure-1,B). The pH of soil was significantly influenced by flyash application. The table indicates that application of flyash to soil in increased percentages marked increases pH of amended garden loam soil from 6.9-7.2 (Table-4) (Figure-1,D). Electrical Conductivity also increased with percentage increase in flyash application due to increase the salt content of soil over control. (Table-4) (Figure 1,E). Availability of Zn,Mg,Fe,Cu also increased slightly in comparatively control to 40% flyash amendment. But in over all study patterns marked increase was only observed in Fe, Cu whereas Zn, Mg remained almost unchanged with fluctuating results. (Table-4) (Figure-2C-F) The result (Table-4), indicates the physical properties of soil and flyash amended soil, it was clear that the water holding capacity of soil increases when proportion of flyash increases up to maximum level i.e. in 40% flyash amended soil (58%). These results were supported by work of Agarwal, (1998), Adriono, *et al.,* (1980), Aitken, *et al.,* (1984), Ghodrati, *et al.,* (1995), Singh, *et al*., (1997), Agarwal,*et al.,*(2009), T.swamynarayan, (2010), thus flyash incorporation at a sufficient rate could exert a beneficial effect on soil WHC since fine textured substances can hold more water than coarse textured substances. Texture of soil changes simultaneously with increase proportion of flyash because soil is loam while flyash has silt texture so the amended soil was of fine textured

loamy soil. Our results were supported by work of Brady and Weil, (1996), Chang, *et al*., (1989), Chang, *et al.,* (1977). Percentage porosity of soil changes in positive side i.e. highest porosity find in 40% flyash amended soil (Table-4). The substantial increase in plant available water in flyash amended soil undoubtedly resulted from incorporation of the fine sized particles leading to increased total porosity and perhaps more importantly a shift in pore size distribution from primarily large macropores to more micropores. Similar results were obtained by Adriano and Weber, (2001), Aitken, *et al.,* (1984). Bulk density of soil show slight variation in all the amended percentages, and show reduction in higher percentages of flyash amended soils (1.18g/cc$^2$ in 40%FA). According to Campbell, *et al.,* (1983), Page, *et al.,* (1979) flyash amendment to a variety of agricultural soil tended to decrease the bulk density. This might be due to change in total porosity as well as modifications in pore size distribution as reported by Adriano, *et al.,* (1980), the large proportion of silt sized particles in flyash presumably result in this effect on soil bulk density. As present study show pH of flyash of KTPS Kota is very high (8.15) i.e. towards alkaline side, while soil of study site is less alkaline or almost neutral, when flyash mix with soil the overall pH change is of 7.16 and 7.2 in 25% and 40% flyash amended soils, i.e. application of flyash to agriculture soil increases the soil pH Results of various workers were coincide with our findings like Shridhran and Pandian, (1998), Phung, *et al.,(*1979), Mc Callister, *et al.,* (2002), Stevens and Dunn, (2004), KrishnaRani and Sharma, (2010). Contradictory result was found by Katiyar,*et al.,* (2012) where acidity increases with flyash amendments. E.C (0.3 in control to 0.4dSm-1in 40%FA) increased in accordance with the amount of ash added in the soil. Similar result was detected by Saxana and Ashokan, (1998). A significant increase in EC has also been reported with increase percentage of fly ash by Singh and Singh, (1986) (Table-4, Figure-1E).

Flyash contains a high concentration of toxic heavy metals concluded by Rautaray, *et al*., (2003), Lee, *et. al.,* (2006), Tiwari, *et. at., (*2007), Das Mohapatra, (2013), along with low nitrogen and phosphorous content and pH ranged from 4.5-12.0 depending on the S-content of

parental coal. Addition of alkaline flyash, which a pH over 9 (Cha, *et al.,* 1999, Haynes, 2009) can reduce soil acidity to a level, suitable for agriculture and can increases the availability of trace metals and other nutrients (Wong and Wong, 1989, Ko, 2000). Utilization of flyash to agricultural land would not always be beneficial for crops, as flyash did not affect the availability of nitrogen in soil (Druzina, *et al.,*1983),(Figure-1F) however, earlier reports suggested that small application of flyash in agricultural fields are suitable for better crop management (Singh, *et al.,* 2002). Flyash use as a fertilizer at commercial scale is uncommon in most of the countries as coal ash contains nonessential elements that adversely affect crops, soil and ground water quality. Use of Flyash in agriculture has to take into consideration about its possible toxic effect, if any, due to the presence of high concentrations of toxic heavy metals.

During present work more or less similar observation and result were found flyash composition show Major elements present in flyash (in the order of decreasing abundance) are Si,Al,Fe,Ca,Mg,K,Na,S,Ti,P and Mn all exist in their oxidized state, (Table-4). Results of present study were coincide with result of Singh and Yunus, (2000), Katiyar, *et al.,* (2012), Sharma and Kalra, (2006), Kumar, *et al.,* (2005). As our results indicates that lower concentration of flyash may be improved productivity due to the presence of elements like P,K who increases soil quality. The soils are composed mainly of $SiO_2$ where as the major matrix elements in flyash were oxides of Si, Al and Fe together with significant percentages of Ca, K,Na, P,Ti. In addition there was considerable variation in the ratio of these and other elements among the different source of flyash. Elemental analysis of soil with different concentrations of flyash reveal gradual increase of K level in soil, the P, N and Fe contents of soil increased steadily from control. (Table-4, Figure-2 A-F) Microelements Co, Cu has show progressive increase while Mn, and Zn show slow and steady increase with increasing flyash amendments Similar result were found by Saxana and Ashokan,(1998), Krishna Rani and Sharma, (2010). Whereas decreased phosphorous (P), potassium(K) and calcium(Ca) content was reported by Agarwal, (2009), T.swamynarayan, (2010), Jain,

*et al.,*(2009), Katiyar, *et al.,* 2012 with flyash addition. It is concluded from present study that concentration of all elements except N were higher in flyash than soil therefore flyash as an amendment for agricultural soil can improve the physical and chemical properties of soil and also improve soil fertility and crop productivity. These results are quite inspiring that flyash can be used as nutrient supplement for the plants. (Figure-1, 2)

As discussed earlier that flyash may affect vegetation directly through deposition on plant surface and indirectly through accumulation in soil media. Flyash contain almost all the essential plant nutrient but deficient in nitrogen & phosphorous (Rai,*et al.,* 2002). This deficiency may be overcome by adding various organic substrates and by growing leguminous crops because legumes are unique plants which have capacity to establish a symbiotic relationship with bacteria and convert atmospheric nitrogen in to usable ammonia. Several studies found that the addition of fly ash stimulated plant growth along with legumes Bisset, (1959), Agarwal and Gupta, (1993), Shridharan and Pandian, (1998), Pandey, *et al.,*(1994), Gowda, *et al.,*(2005) Haynes, (2009), Kumari, *et al.,*(2010), Newton, *et al.,* (2011) Shrabani Sen and V Kumar, (2012), Rai, *et al.,*(2004).As we know legumes are greatly used as food and are next to cereals. They constitute an important part of people's diet. They are popularly known as pulses. Leguminous crops are very commonly grown for crop rotation on the other hand they are excellent nitrogenous manures, when dead and ploughed under. They bring about an increase in nitrogen content of soil with the help of nitrogen fixing bacteria present in their root nodules.

During present research work our attempt is to use flyash from Kota Thermal Power Plant as soil ameliorant and to study the effect of various concentrations of flyash on four selected legumes that are *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L*, Glycine max* L*,* by conducting the germination experiments, by 'Roll towel method' by 'Germination tray experiments' and by 'Pot experiments' in natural conditions. To study the effect on physical,

biochemical and microbiological parameters data were collected and analyzed. Incorporation of flyash in soil in different compositions without adding any fertilizer, improve plant growth. Similar to this favorable growth response of few crops on flyash amended soils has been reported by many workers Wong and Wong, (1989), Pandey, *et al.,* (1994). Kumar, *et al.,* (2005), because micro and macro nutrients present in coal get concentrated in the ash. So the flyash contains sufficient concentrations of the nutrients essential for plant growth. Carbon and nitrogen are usually in small amounts and potassium and phosphorus are comparatively high, flyash used in study contained appreciable amount of nutrients and its application to soil increased the available N,P,K content, (because it is a lignite coal ash had relatively high P,K content, it is unweathered and fresh)(Table-4,Figure-1F,2A-F). Fly ash is highly alkaline in nature (pH- 9-11) rich in plant nutrients like Ca, Mg, K, P, S, Cu, Zn, Mn, Fe, B, and Mo suitable for improving important physical properties of both the mine spoil and normal sandy loam soil. (A joint project of Ministry of coal and Neyvelli Lignite Corporation, (N.L.C) and control fuel research institute,(C.A.R.D) Dhanbad (1998). Studies were conducted in mine spoil of Mine-1 and lateritic soil of C.A.R.D site. Crops like paddy, groundnut and maize were tested and positive results achieved during study period from 1996-2000.

Germination of *Cyamopsis tetragonoloba* L*, Medicago sativa L, Trigonella foenum graecum L,, Glycine max L,,* was performed in seed germinator by Roll towel method after 20%, 40%, 60%, 80%, and 100% of flyash treatment exhibit a promotive response only in lower concentrations of flyash to germination. (Table-5, Plate-9-13). *Trigonella foenum graecum L, Medicago sativa L,* decreasing trend in germination percentage in 20%, 40%, 60%, 80%, 100% flyash treatments was observed. (Table-5). In *Medicago sativa,* decrease in germination percentage was observed in flyash treatments over control, with highest percentage of germination was obtained in controls i.e. 100% germination (Table-5, Plate-11) The maximum germination rate was observed in *Trigonella foenum graecum L (90-100%)* among all four studied plant,

while minimum germination percentage was observed in *Glycine max L* that is only 70% which is lesser then germination percentage of *Cyamopsis tetragonoloba* L i.e.75%, by Roll towel method. The observation made during the present study are in agreement with work of Agarwal and Gupta, (1993), Agarwal, (1998) on *Vigna munga L, Glycine max L.* Selvakumari *et al.,(*2000), Shukla *et al*.,(2003) and Katiyar, *et al.,* (2012), conclude that application of flyash show significant effect on germination percentage of seeds of different plants. It is concluded that as regards germination, tolerance limit for flyash salinity for *Trigonella foenum graecum L* was more than that of all others (Figure-3 A-B). In overall study 100% flyash amended soil show minimum value of germination percentage in all plants. Drastic reduction in germination percentage was obtained after 40% flyash incorporation. *Glycine max L* is most sensitive to flyash treatment among four studied plants on the basis of germination percentage by Roll towel method (Table-5, Figure-3AB). Shrabani Sen and V.Kumar, (2012) present the promotive response on seed germination of *Vigna radiata* in different flyash/soil amendments.

Observation on the effect of flyash treatment on seedling growth of four studied plants, shows mix response, i.e. favorable seedling growth response in *Cyamopsis tetragonoloba L,* and *Trigonella foenum graecum L,* while opposite result obtain in *Glycine max L,* and *Medicago sativa L,* by 'Roll Towel method' (Table-5,Figure-3C-F). The present study showed that that increasing trend was found in *Cyamopsis tetragonoloba* L, with increased concentration of flyash treatments in terms of height. In other plants lower concentration of flyash show favorable results in comparative to higher (<40%). In *Cyamopsis tetragonoloba* L, root length was recorded highest in 80 % (7.1cm) followed by 100% flyash treatment while minimum was in control (3.78cm). But curling of root starts after 40% flyash treatment, maximum curling was observed in 60% flyash treatment (Plate-9).*Trigonella foenum graecum L,*show maximum curling symptoms with drastic increase in length of root in 20% (8cm) flyash treatment in comparative to control (2.09cm), (Table-5, Plate-12) *Glycine max L, s*how negative growth responses in terms of shoot length, (12.3 cm in control

and 7.8 cm in 100% amendment), root length (12.9 cm in control and 4.9 cm in 100% amendment) its show decrease with increase flyash treatments. (Plate-10). The flyash treatment in *Medicago sativa L,* show increase root length in 20 %(1.92 cm) flyash treatment after that reduction in root length was recorded minimum value was obtained in 80% (1.3 cm) flyash treatment. Similarly shoot length also show maximum value in 20 %( 5.18cm) treatment followed by 40%(5.01cm). Minimum value was obtained in 80% treatment (4.03). (Table-5, Plate-11). The overall results used in directing the further study at lower concentrations is more advantageous then the higher concentrations in all four selected legume plants.

The performance of selected plants i.e. *Cyamopsis tetragonoloba* L*, Medicago sativa L, Trigonella foenum graecum L, Glycine max L,* in soil amendments, revealed that flyash amendment to the soil improved the growth performance at initial stages with application of lower concentration, on contrary it was inhibitory at higher concentration. (Table-6, Plate-14, 15) (Figure-4,5,6) Highest germination percentage was obtained in *Trigonella foenum graecum L, (*99% in10%FA*),* followed by *Medicago sativa L,* (92.4% in 5%FA) similar finding were observed by Dubey, *et al.,* (1982**),** Kalra, *et al.,* (1997), Rai, *et al.,* (2003), and found that fly ash directly affect the seed germination if used as fertilizer by mixing with soil in very low concentration by seed germination experiments on wheat, gram, maize, sorghum and soybean. That minimum germination percentage was obtained in *Glycine max L, (*63.3% in 5, 10%*).* In present study it was found that alkaline stress in plant was due to flyash amendment. The results were identical with Singh, *et al.,* (2008) when studied the effects of flyash incorporation on heavy metal accumulation, growth and yield responses of *Beta vulgaris*. Agarwal, (1998), studied that germination of wheat and gram grown in flyash mixed soil exhibit a promotive response to germination. In contrary in acidic soil the inhibitory effect of flyash started from very early, it is evident from the results that flyash mixing was inhibitory to wheat at 20% while 30% in

gram. These observations indicate that in regarding to germination, tolerance limit for flyash salinity for gram was more than that for wheat.

In *Cyamopsis tetragonoloba* L root length show increased growth pattern in flyash amended soil highest root length recorded in 15% flyash amended soil (5.37cm) minimum root length was found in 40% flyash addition (3.75cm), In *Medicago sativa* L*,* almost similar values were obtained in control, 5%, 10%, 15%, 20% (3.5) flyash incorporation with slight increment in 25%flyash incorporated soil over all others (3.9cm). On the contrary minimum value was obtained from 40% flyash amendment (2.9cm). *Trigonella foenum graecum* L*,* a continuous increment was observed in root length of *Trigonella* with increased concentration of flyash (5.32-7.5cm) In *Glycine max* L*,* maximum value was obtained in 25% concentration (6.34) followed by 40% (6.25) and control (6.13). (Table-6, Plate-14,15) (Figure-5) In *Cyamopsis tetragonoloba* L show gradual decline in value of shoot length with higher concentrations of flyash, minimum value of shoot length obtained at 40% (24.4cm) flyash incorporation while maximum value was recorded in control (39.6cm). In *Medicago sativa L,* slight increment in 25% flyash incorporated soil over all others (3.9cm). On the contrary minimum value was obtained from 40% flyash amendment (2.9cm). In *Trigonella foenum graecum* L*,* similar to root length a continuous increment was observed in shoot length too with increased concentration of flyash, maximum value was obtained in 40% flyash incorporated soil(6.2cm). In *Glycine max* L*,* no clear trend was observed with increasing concentration of flyash, In *Cyamopsis tetragonoloba* L*, Medicago sativa* L *and Trigonella foenum graecum* L increase growth trend was observed in terms of fresh weight and dry weight*, Glycine max* L slight reduction was observed in plant fresh weight, minimum weight observed in pure flyash i.e. 40% (0.909mg/pt) (Table-6, Plate-14,15.Figure-6). Plants grown in mixture of flyash and soil, contained in pots, showed luxuriant growth at lower levels of flyash. Favorable seedling growth response in terms of plant height and dry weight at very low concentration of flyash was also reported by various researchers. Result of Agarwal and Gupta, (1993), Shridharan and

Pandian, (1998), Haynes, (2009), Katiyar, *et al.,* (2012), Singh, *et al*., (2008), Kumar, *et al*., (2009), are justified by present study where promotive growth response was found in terms of shoot length, root length, fresh weight, dry weight studied plants up to 25% flyash amendments, except in reduction in shoot length in *Cyamopsis tetragonoloba* L. Shrabani Sen and V. Kumar, (2012) present the promotive response on plant height, plant biomass, number of leaves and root nodulation compared to control in *Vigna radiate* in different flyash/soil amendments. Increasing concentrations of flyash and consequently reduced growth, biomass and yields, was observed by Singh, *et al.,* (2008), *Beta vulgaris* plants The study showed that the concentration of heavy metals reduce the growth in that flyash application to agricultural soil even at small scale. Plant growth and yield parameters were enhanced significantly of the varieties grown with 20 and 40% flyash level while 60% flyash onwards, the measured parameters tended to decline Drastic reduction was obtained at 40% flyash amendments. However, at 100% flyash, growth and yield were considerably less. Low dosages of flyash utilization show increase in plant height, in shoot length, in dry weight and pigment concentration. Similar observations were obtained by Shridharan and Pandian, (1998), Kalra, (1997,1998). Matte and Kene (1995), Agarwal *et al.,(*2004), Kumar, *et al.,* (2005), Haynes, (2009), Jala and Goyal, (2006) which justify our findings, Observation on the effect of flyash amended soil on seedling growth of two Varieties of *Glycine max* by Agarwal and Gupta (1993), show that in term of height the maximum was recorded at 10% flyash amendment and minimum was observed in pure flyash. Seedling growth in terms of dry matter in *Glycine max L was* observed maximum in 5% and 10% flyash and minimum in pure flyash. Favorable seedling growth response up to 10% flyash incorporation in soil as observed in two varieties of *Glycine max* can be attributed to increased availability of nutrients present in flyash. Flyash application increased the number of leaves, plant height, biomass and yield of three crop plants (palak, mung bean and chilli) and recorded maximum in flyash 25% treatment (25% FA-amended soil). Application of

more than 25% results in decline in growth and yields of plants by Katiyar, *et al.,* (2012), lower biomass of plant in different amendments of flyash was studied by Vajapyee, *et al.,*(2000), in legume plants, it may be due to accumulation of heavy metals of flyash from soil.

The pigment concentration of selected plants i.e. *Cyamopsis tetragonoloba* L*, Medicago sativa* L*, Trigonella foenum graecum* L*, Glycine max L,* in various soil amendments, reveal that in *Cyamopsis tetragonoloba* L the value of total chlorophyll was observed highest in 40 % and minimum in 5%. In *Medicago sativa* L the value of total chlorophyll was maximum in 15% and control and minimum was in 20%. In *Trigonella foenum graecum L* the value of total chlorophyll was maximum in 15% and 25% and minimum was in 5%. In *Glycine max* L the value of total chlorophyll was maximum in 25% and minimum was in 40 %.( Table-7, Figure-7A-D)) mixed response of flyash was obtain in all studied plants. These results were coinciding with the work of Agarwal and Gupta, (1993), Agarwal, (1998), Nagajyothi, *et al.,(*2009),T swaminarayan, *et al*., (2010), and found promotive responses in low concentrations of flyash in *Allium cepa*. It is may be due to the decrease in chlorophyll content in various plants is coupled with undesirable chemical properties of the flyash including low N and P contents by Singh, (2008), Mishra and Shukla, (1986), Wong and Wong, (1989), Singh, *et al., (*2010) studied morphological and biochemical responses of cow pea grown on flyash amended soil and reported plant growth, yield, pigment and protein content of cow pea were increased significantly at lower level(20-40%) but reverse was true at higher levels. These results supported the observations of our work (Table-7, Figure-7). Phytoremediation of flyash by assessing growth response of local tree species was studied by Agrawal, *et al., (*2011) and reported increment in physiochemical parameters as chlorophyll, protein and nitrate reduction.

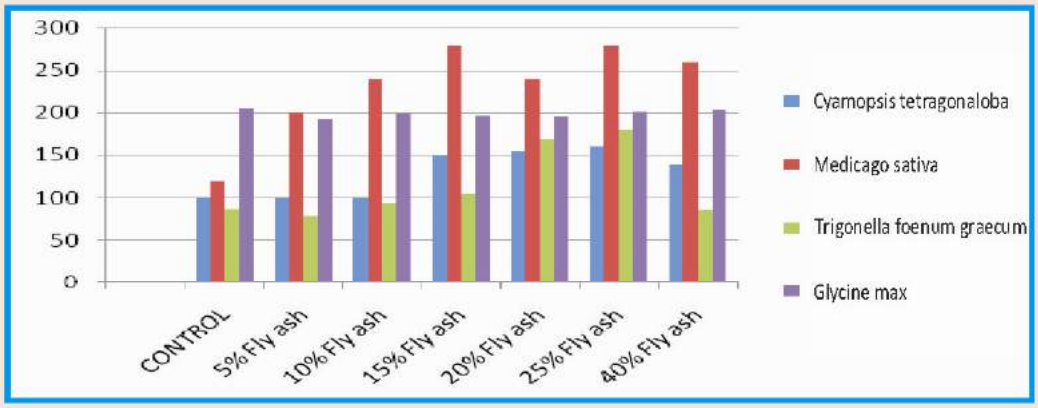
Variation in biochemical contents is also considered as an important vital event during growth and differentiation of cells in plants. To determine the effect of flyash on plants protein content, phenol content,

carbohydrate content, proline and ascorbic acid content was measured.(Table-8, Figure-8-11) Highest protein content was obtained in *Medicago sativa L* at 15 & 25% flyash treatment, followed by *Glycine max* L (control) while minimum was in *Trigonella foenum graecum L* at 5%. Variation in protein contents is also considered as biochemical event during growth of plant. Protein contents have been estimated in various plants as *Lens culinaris* L*, Vicia faba L, Cicer arietinum L, Phaseolus spp L, Pisum sativum L, Glycine max* L*, Solanum melongena* L*, Spinacia oleracea* L*, Vigna radiata* L reported by Audichya, (1999), Borhade, *et al.,* (1984), Chatrath, *et al.,* (1996) Ansari, *et al.*,(2011), Singh, *et al*., (2010). It is observed that protein content in leaves of in *B.vulgaris* decease significantly with increase concentration of flyash in soil at different duration of plantation by Singh, *et al.,* (2008). It is concluded that decrease in protein content is may be due to binding of sulpher containing amino acid with heavy metals to reduce their toxicity. Nitrgen content of leaves of cow pea were progressively decreased with increase in flyash level reported by Mishra and Shukla, (1986), Singh, *et al.,*(1994), Singh, *et al.,*(2010) with is directly related to protein content and root nodulation of plant (Table-8, Figure-8-11). Increasing trend of phenol with increasing flyash concentration was reported in *Cyamopsis tetragonoloba* L*, Medicago sativa* L *a*, while decreasing amount was found in *Trigonella foenum graecum* L *and Glycine max L.* Highest phenol content was obtained in *Trigonella foenum graecum* L in control while minimum was in *Glycine max L* in 25%, similar study was done Malik and Singh, (1980), Agarwal and Gupta, (1993), on different plants, and explain its production as a response for defending mechanism of injured plants. Amount of Ascorbic acid was increases with increase flyash concentrations. In *Trigonella foenum graecum* L highest ascorbic acid content was measured among all four plants. Maximum value was observed in 40 %, and minimum was in *Glycine max* L similar result were find by Agarwal and Gupta, (1993), in different varieties of soyabean (Table- 8 ). The present result were justified as higher levels of anti oxidants such as ascorbic acid and phenol was obtained in the plants grown in flyash mix

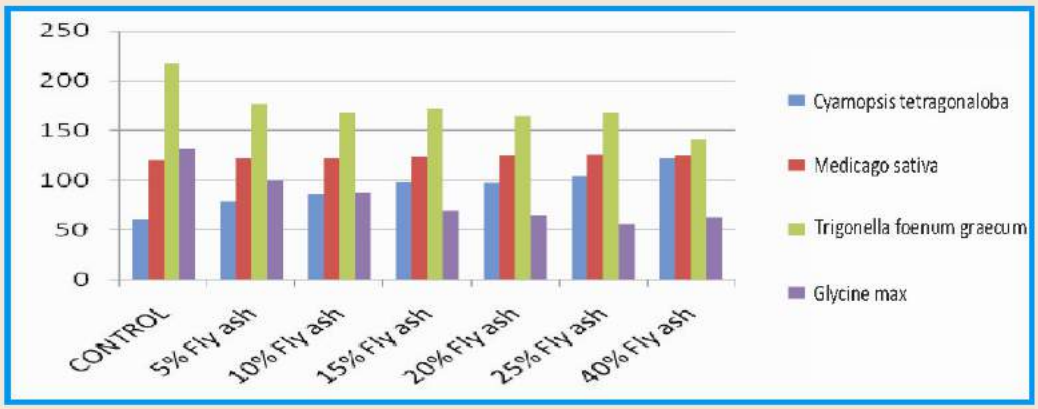
sludge amended soil, this is because heavy metal present in soil induced the formation of free radicals in plants which consequently increased ascorbic acid and phenol production to reduce the free radicals in plants (Singh, *et al.,* 2008). Marginally irregular patterns but significant differences in carbohydrate content were observed in *Cyamopsis tetragonoloba* L*, Medicago sativa* L and slight increase was found in *Trigonella foenum graecum* L. (Table-,8 Figure-8-11) Maximum value of carbohydrate content was in *Glycine max L (20.04mg/g)* at lower concentrations of flyash followed by *Trigonella foenum graecum* L and minimum was observed in *Medicago sativa* L*,* Similar work was done in different plant species like *Balanites aegyptica* by Vijayvergya and Vijay, (2006), *Cassia obtusifolia and Cassia siamea* by Sharma, *et al.,* (2006), two species of *Araucaria* by Unikrishnan. *et al.,* (2007), Sea weed by Sornalakshmi V and V Kumar, (2014)*,Terminalia catappa* by Nagesh, *et al.,*(2007) and finding are coincide with our results (Table-,8 Figure-8-11). In *Trigonella foenum graecum* L*,* proline content increase with increase concentration of flyash up to 15% which is highest among four, followed by *Cyamopsis tetragonoloba* L*,* in *Glycine max L* proline was observed in traces that could not be measured quantitatively. (Table-,8 Figure-8-11) Proline accumulation was studied by Ashraf and Harris, (2004), in root and leaf of plant tissue during abiotic plant stress in various plants. The over all effect of industrial waste flyash was studied on daily growth rate (DGR), chlorophyll, carotenoids, protein, carbohydrate, lipid and phycocolloids (agar and algin) content of four economically important seaweeds show promotive response studied Sornalakshmi V and V Kumar, (2014),

For nodulation studies plants were grown in control as well as 25%flyash treated soil (in pots) were uprooted from the pot after 45-60 days of plantation and their roots was washed to obtain root with intact nodules. Data on plant height, root length, number of lateral roots and nodules per plant were collected (Table-9, Plate-16,17). In present study it was observed that root system of plant was highly developed in all four plants. Results show that lower concentrations of flyash amendments

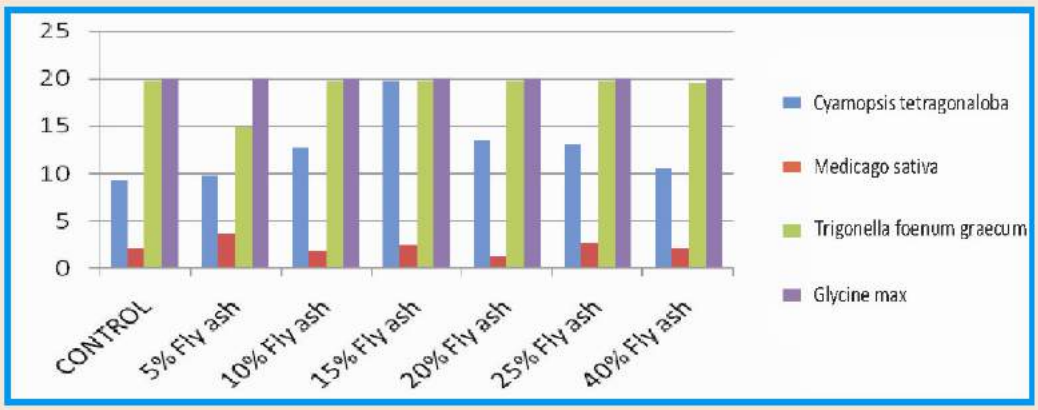
# FIGURE - 12




(A) Protien (µg/gm)



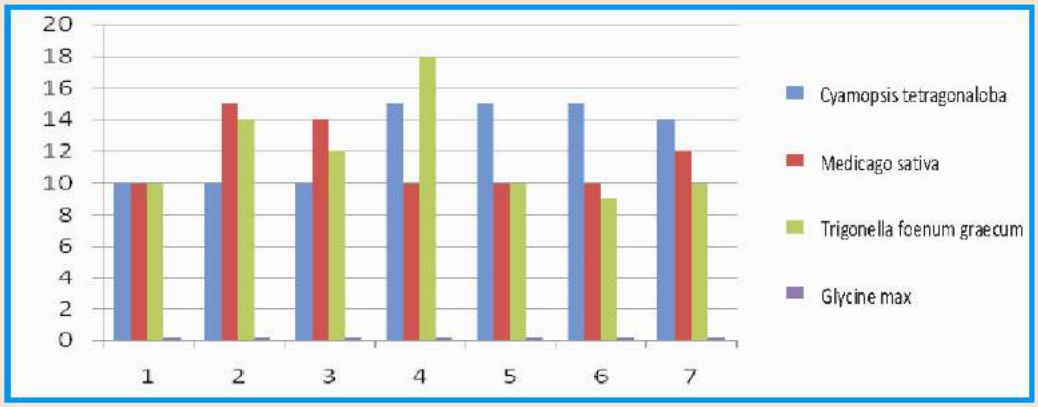

(B) Phenol (mg/ gm)



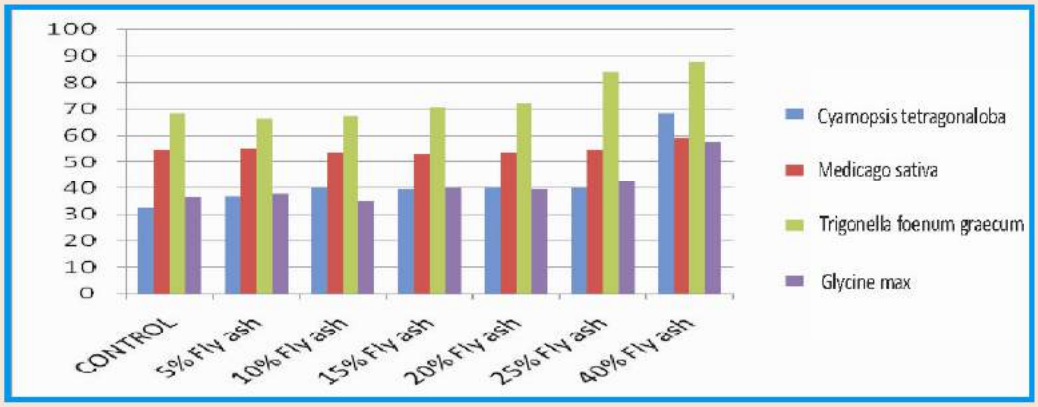

(C) Carbohydrate (mg/gm)480A

**Figure Shown Comparative Biochemical Parameter of Selected Legume Plant Grown in Various Flyash Concentrations**

# FIGURE - 13




(D) Proline (µg/gm)




(E) Ascorbic acid (mg/100gm)

**Figure Shown Comparative Biochemical Parameter of Selected Legume Plant Grown in Various Flyash Concentrations**

were beneficial for plant growth with special reference to root and nodule growth pattern. It is concluded that in all legumes 40% flyash amendments show negative response towards root growth pattern and nodule formation. Up to 25% flyash treatments plants show positive responses. In *Cyamopsis tetragonoloba* L maximum plant height was observed in 10% flyash amendments (59cm), followed by 5% and control (57cm). Maximum root length was observed in 10 %( 32cm), minimum plant length and root length was recorded in 40 % (46cm), (22cm) respectively. Number of lateral branches recorded maximum in 10 %( 17) and in others it ranges from (12-15). In contrary number of nodules found highest in control (14) followed by 5%, respectively. In *Medicago sativa L,* maximum plant height was observed in 5% and 10% (22cm), minimum was in 40 %( 11cm). Maximum root length found in control (12cm), minimum in 40 %( 6cm). Number of lateral branches (root), Number of nodules/plant were maximum in 15 %( 9) and (9) respectively, while lateral branches (root) were in all others (6-7). In *Trigonella foenum graecum L,* maximum plant height was observed in 10% (28cm) and minimum was in 40% (20cm). Length of roots ranges (7-9cm) in all amendments, maximum was in control and 10% (9cm). Number of lateral branches were maximum in 10 %( 13cm) followed by control (12cm), number of nodules per plant were (12) in 10% and 15%. In *Glycine max L* maximum plant height was in 10 %( 41cm), similarly root length was maximum in 10 %( 22cm) followed by control (21cm), minimum was in 40% (15cm). Number of lateral branches were maximum in 10% (24), and minimum in 40%(16).number of root nodules per plant found maximum in control(20) and reduction was observed in higher flyash concentrations.(Table-9,Plate-18,22,26,30). Gauri *et al.,* (2011), used the same procedure during study of characterization of rhizobium isolated from root nodules of *Trifolium alexandrium*. Similar result was found by Gochande and Khansole (2011). Choudhary, *et al.,* (2011), also studied the inoculation of rhizobium (VR-1 and VA-1) induces an increasing growth and metal accumulation potential in *Vigna radiata* and *Vigna angularis* L. growing under flyash, rhizobium inoculated plants grown on

100% flyash showed marked increase in relation to root–shoot length, biomass yield, photosynthetic pigment, protein content and nodulation frequency compared to uninoculated plant grown in control (100% flyash).

. In all the plant species studied, nodulation was initiated 3 weeks after seed germination and the number increased with age of the plant up to 70 days. It was observed that the number of nodules mainly increased during vegetative phase (30-70days) and decline during flowering and pod setting phase, studied result justified with observations of (Arya and Singh, 1996). In nodulation study of 45-60 days plants it was observed that legume nitrogen fixation starts with the formation of a nodule after one month, small nodules are visible with the naked eye. In the pots small nodules can be seen 2-3 weeks after planting, depending on legume species. They are usually white or gray inside when nodules are young and not fixing nitrogen. As nodules grow in size, they gradually turn pink or reddish in color, indicating nitrogen fixation has started. The pink or red color is caused by leg haemoglobin that controls oxygen flow to the bacteria.

The number of nodules increased with age of plants and highest number of nodules was observed at 45 days in *Trigonella foenum graecum L* and 50days in *Cyamopsis tetragonoloba* L and it was 60 days in *Medicago sativa* L and *Glycine max* L. Nodules in *Medicago sativa* L are finger like in shape. Mature nodules may actually resemble a hand with a center mass (palm) and protruding portions (fingers), although the entire nodule is less then ½ inch in diameter. Most of the nodules centered on the tap root. Among the plant studied the highest average number of nodules number per plant was observed in *Glycine max* L followed by *Cyamopsis tetragonoloba* L and lowest of number was observed in *Medicago sativa* L. Nodules of *Glycine max L* are round and can reach the size of a large pea. The root nodules of *Glycine max L* are round to oval type at the early stage but became elongated with increase in age. The nodules are pink in *Glycine max* L. While the nodules of *Trigonella foenum graecum* L and *Medicago sativa* L were cream and

aggregated. Much variation in nodule number was reported in *Glycine max* L Plant, similarly in Indigofera species highest of only 1-5 nodules per plant reported from Pakistan (Athar and Shabbir, 2008). In zollingeriana the highest number of 25 nodules per plants was reported (Anegbah, *et al.,* 2003). In the present study the size of the nodules ranged from 2.0-8.0mm, the maximum diameter observed was in *Glycine max* L, followed by *Cyamopsis tetragonoloba* L, *Trigonella foenum graecum* L, and *Medicago sativa* L. Increased number of root nodules also reported in very low concentration of fly ash in leguminous plants by Rao, *et al*., (1989) Chaudhary *et al.*,(2011). This is suggested that enhance growth may be due to extra nutrient available to plant from flyash so flyash can be used as fertilizers in low concentration in some nutrient deficient types of soils. Presence or absence of nodulating rhizobium in the nodules was also reported by Fred, *et al.,* (1932), Subba Rao, (1977), and Somasegaran and Hoben, (1994). Significant reduction in formation of nodule in leguminous plants was noticed may be attributed to flyash toxicity was concluded by Kumari,V (2009) during study of vegetation near parichha thermal power plant. The genus rhizobium was erected by Frank,(1890) based on its characters to form nodules on roots of legume plants (Table-10-13, 16).

As discussed earlier that each legume is nodulated by different species of rhizobium. It is concluded that *Cyamopsis tetragonoloba* L *is* nodulated *by Rhizobium leguminosarum, Glycine max* L *is* nodulated *by Bradyrhizobium japonicum, and Medicago sativa* L *is* nodulated *by Rhizobium meliloti (Sinorhizobium melitoti). Trigonella foenum-graecum* L *is nodulated by Rhizobium meliloti (Sinorhizobium melitoti)*. Different biochemical tests were proved as valid tests in identification of the organisms; Apart from it some diagnostic features of rhizobium could be conveniently use not only to determine and identify the organism but also delineate different species. From the prior study it is concluded that up to 25% flyash amendment did not show any negative response especially in nodule development and growth, so the comparative study of four legume plants, grown in control with plants grown in 25% flyash amended soil was

done. Comparative study includes isolation, culture and various microbiological tests. (Plate-18-33)(Table-16)

For the study of impact of flyash on *Rhizobium* bacteria, selected plants were grown in control soil and 25% flyash amended soil, nodule were collected, sterilized and rhizobium were inoculated on YEMA agar. These isolated bacteria from (control and 25% flyash amended) four different legumes were identified by their staining, morphology and cultural characters, all the results were similar to the standard results given by rhizobium as studied by Graham and Parker, (1964) Vincent, (1970), Gaur, (1975), Mahana, (1981) Gauri, *et al.,*(2011). (Plate-18-33)(Table-16) Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Cyamopsis tetragonoloba* L*, Medicago sativa L, Trigonella foenum graecum L, Glycine max L,* grown in control and 25% flyash amended soils and were examined through gram reaction (Plate- 18,22,26,30), Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Cyamopsis tetragonoloba* L grown in control and 25% flyash amended soils and were observed Gram negative, pink colour short rods, these rods were arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, these are fast growing bacteria with less than 6 hr generation time (Plate-18). *Glycine max L, grown in* control and 25% flyash amended soils and were examined through gram reaction (Plate-22), after staining Gram negative, pink colour short rods were observed, these rods were also arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Medicago sativa L and Trigonella foenum graecum L grown in* control and 25% flyash amended soils and were examined through gram reaction (Plate-26, 30) after staining Gram negative, pink colour short rods were observed, these rods were arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, Our research finding are supported by these workers as Graham and Parker, (1964)

Vincent, (1970), Gaur, (1975), Mahana, (1981), Mahana, *et al.,* (2000) found same result when studied various legume.

Two to three days old culture grown on YEMA plate examined for colony characters, colonies of *Cyamopsis tetragonoloba* L*, Medicago sativa L, Trigonella foenum graecum L, Glycine max L,* were circular, convex, whitish pink and glistering with entire margin. (Table-10,11,12,13, Plate-18,22,6,30). Fast growing rhizobia as *Cyamopsis tetragonoloba* L produce white, semi translucent, circular, mucilaginous colonies while slow growing strains *Glycine max L* produce white, opaque, circular, granular colonies, which do not exceed 1mm in diameter after prolonged incubation. Culture grown on YEMA plate examined for colony characters, the size of colony was 2-4mm in 3-5days incubation, colonies of *Cyamopsis tetragonoloba* L were circular, pin head like, convex, whitish pink and glistering with entire margin. 5-10 days old culture grown on YEMA plate examined for colony characters, colonies of *Glycine max,* were circular, convex, whitish pink and glistering with entire margin. These are slow growing bacteria having more than 12 hr generation time. The colony were not exceed more than 1mm in diameter in 5-7days incubation on YEMA. Three days old culture grown on YEMA plate examined for colony characters, colonies of *Medicago sativa L were* circular, convex, whitish pink and glistering with entire margin. Two to three days old culture grown on YEMA plate examined for colony characters, colonies of *Trigonella foenum graecum L* were circular, convex, whitish pink and glistering with entire margin.( Table-10-13,Plate-18,22,26,30).Similar result were obtain by Krichner and Buchanan,(1926), Allen and Allen, (1981), Bisset, (1959), Muthuswamy, *et al.,*(1973), Mahana,(1981), Garg, *et al.,*(1991), Oblisami,(1974), Yuang *et al.,*(2007) Gachande and Khansole,(2011) The bacterium showed well-marked growth on YEMA medium at PH 7.0.However poor growth was observed on Hofer's medium. Mahana, *et al.,* (2000), reported that the Rhizobium isolated from *Vigna mungo* L. showed marked variations in growth with respect to time period on YEMA while they do not show any growth on Hofer's alkaline medium at PH 10.0 with slight growth and evolution of gas

and acid production. According to Ditmer, (1930), those rhizobia, which produce acid are considered to be advanced type and those, which produce alkali, are ancient type. The tube contains agar at pH 7.3 were inoculated by isolated rhizobium from all four legume plants. The pattern of growth in the motility agar stab culture of rhizobium of *Glycine max L* was observed after 48-72 hr of incubation, and observed that bacteria move slowly from the stab line in to medium. The pattern of growth in the motility agar stab culture of rhizobium of *Cyamopsis tetragonoloba* L was observed after 48-72 hr of incubation, and observed that bacteria move fast comparative to other from the stab line in to medium. Rhizobium of *Medicago sativa L, and Trigonella foenum graecum L* move slow but comparative faster than rhizobium of *Glycine max.* Aneja, (2008*),* Basak and Goyal,(1980) (Plate-18-33)(Table-16)

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja, (1996) from *Cyamopsis tetragonoloba* L*, Medicago sativ L a, Trigonella foenum graecum L, Glycine max L*, (4 plants X 2(control/25%FA amended)=8) experimental sets. From (Table-10,11,12,13. Plate-18, 22, 26,30) it was clearly observed that Indole was not produced after incubation of isolated rhizobial inoculants in tryptophan broth. Similarly Methyl red and Voges-Proskauer reaction were examined in glucose phosphate broth by adding methyl red and α-napthol solution with KOH respectively report negative results. Citrate was utilized as a carbon source in Simon's citrate medium and represent as color change. Ammonia was produced by degradation of urea available in to the urea broth containing phenol red as an indicator by the bacterium inoculated. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by W/V). Nitrate was converted to nitrite by inoculant of Rhizobial strain. Production of hydrogen sulphide gas examined by SIM Agar method. The bacterium showed positive test for citrate, production of ammonia and Catalase activity. Nitrate is reduced to nitrite producing ammonia. (Plate-18-

33)(Table-16) Neal and Walker, (1935), suggested rapid nitrate utilization by slow growing root nodule bacteria justified our results as intensity of colour and duration of reaction time was different in all four maximum color was observed in *Glycine max L* while minimum was found in *Trigonella foenum graecum L*. Mahana, *et al.,* (2000) and Maheshwari *et al.,*(2012) reported Catalase activity in some isolates from *Vigna mungo* and Ecoli. The bacterium is negative for MR-VP and Indole reaction reported by Gachande and Khansole, (2011) Similarly, Graham and Parker, (1964), did not observe MR reduction in all the isolates of seven rhizobia groups. While Basak and Goyal, (1980), also reported that none of the rhizobial isolates of seven groups produces Indole. Bradyrhizobium in different carbon sources was observed by Padmanabhan, *et al.,* (1990). Elsheikh and Wood, (1989) also observed good growth of fast growing Rhizobium in the above carbohydrates. The isolates under study were confirmed as slow growing Bradyrhizobium since they showed an alkaline reaction turning the growth medium blue. *Rhizobium japonicum syn. Bradyrhizobium- japonicum* is associated with the root nodules of Soybean and fixes 100 kg nitrogen/ha/year, (Purohit and Kumar, 1998). Deka and Azad, (2006) studied the rhizobial isolates from 6 common pulses justify our results. Choudhary, *et al.,* (2011), Inoculation of flyash tolerant rhizobium increased the accumulation of Fe, Zn, Cu Cd and Cr in different tissues by enhanced translocation of metals to the aboveground part of plant. Although inoculation of flyash tolerant rhizobium strains (VR-1 and VA-1) enhanced the translocation of more Fe to shoot parts, nevertheless, the amount of rhizobium inoculants supplied to the plant was found to be very important since it has a positive role in increasing plant growth through increased $N_2$ supply via nitrogenous activity. Similar result was obtained by Gauri, *et al.,* (2011) for rhizobium collected from various agro climatic conditions. (Plate-18-33) (Table-16).

On the basis of morphological, cultural and biochemical characters and their comparison with standard pure culture strain from (MTCC) Chandigarh, Institute of microbial Technology, all of the characteristics were found similar to the standard strain. Results suggest that an

integrated approach employing bio technological means and inoculation of plants with host-specific fly-ash-tolerant rhizobium strain may prove a stimulus to a flyash management programme.

This study is helpful to analyze the impact of fly ash on leguminous plants and to study not only the ecomophological aspect but also the biochemical & microbiological aspect of the root nodule bacteria of leguminous plants. Result indicates some negative impact of higher concentration of fly ash on which we can do further research to minimize the environmental hazards and some safety measures can be suggested. Data generated in the present study may be useful to understand the positive impact on growth performance of *Cyamopsis tetragonoloba* L*, Glycine max L, Medicago sativa L, Trigonella foenum graecum L,* we can suggest that these plants can be grown after field trial in fly ash affected areas, Flyash can be used as fertilizer after some alteration, and used in agronomy. Our ultimate effort is to study the impact of fly ash on root nodules and study of microbiology and biochemical analysis of selected legume rhizobium by isolating inoculating and culturing the bacteria. In the light of above facts it is concluded that flyash up to 25% doesn't affect the rhizobium bacterial growth as well as its characters, so flyash can be used in future for soil amendments in different ways . We did not get any negative or mutational impacts on the rhizobium strains of *Cyamopsis tetragonoloba* L *(Rhizobium leguminosarum), Glycine max L (Bradyrhizobium japonicum), Medicago sativa L (Rhizobium meliloti)or (Sinorhizobium melitoti). Trigonella foenum-graecum L (Rhizobium meliloti) or (Sinorhizobium melitoti)*. It is concluded that these strains can be used for inoculation of other crops after further investigation and confirmation. It can enhance the nitrogen fixation leads to improve soil quality so floristic diversity of area will remain rich always. Our study surely beneficial in the direction of flyash utilization and enlightens the way to future research which must be beneficial for maintaining the biodiversity of the area.

# Table - 14 : Ecomophological Parameters of Selected Plants

| | *Cyamopsis tetragonoloba (RMG1002)* | | | | | | | *Glycine max (JS 335)* | | | | | | | *Medicago satava (t9)* | | | | | | | *Trigonella foenum graecum (SWATI)* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fly ash Concentration** | Control | 5% | 10% | 15% | 20% | 25% | 40% | Control | 5% | 10% | 15% | 20% | 25% | 40% | Control | 5% | 10% | 15% | 20% | 25% | 40% | Control | 5% | 10% | 15% | 20% | 25% | 40% |
| **Root Length (Avg.)** | 4.7 | 4.23 | 4.9 | 5.37 | 5 | 5 | 3.75 | 6.13 | 5.63 | 5.39 | 5.34 | 5.35 | 6.34 | 6.25 | 1.9 | 2.7 | 2.5 | 2 | 2.2 | 2.7 | 2.2 | 5.32 | 5.52 | 5.2 | 6.3 | 6.08 | 7.28 | 7.5 |
| **Shoot Length (Avg.)** | 39.6 | 37.17 | 31.6 | 25.5 | 27.4 | 26.6 | 24.4 | 16.83 | 18.12 | 15.87 | 18.5 | 15.7 | 17 | 18.75 | 3.7 | 3.5 | 3.2 | 3.4 | 3.5 | 3.9 | 2.9 | 4.54 | 4.37 | 4.37 | 4.49 | 5.06 | 5.40 | 6.2 |
| **Germination Percentage (%)** | 88.8 | 83.3 | 76.6 | 70 | 76.2 | 76.4 | 70 | 73 | 63.3 | 63.3 | 73.3 | 76.6 | 80 | 53.3 | 90 | 92.4 | 93.2 | 93.2 | 92.3 | 92.4 | 82 | 98.6 | 98 | 99 | 98.2 | 98 | 98.6 | 92.2 |
| **Fresh Weight (mg/pt)** | 1.27 | 1.45 | 1.51 | 1.66 | 1.74 | 1.72 | 1.72 | 0.984 | 1.001 | 0.919 | 0.914 | 0.912 | 0.912 | 0.909 | 0.872 | 0.9 | 0.904 | 0.914 | 0.913 | 0.918 | 0.923 | 0.338 | 0.367 | 0.380 | 0.405 | 0.0411 | 0.425 | 0.430 |
| **Dry Weight (mg/pt)** | 0.065 | 0.076 | 0.070 | 0.080 | 0.083 | 0.101 | 0.102 | 0.101 | 0.112 | 0.1 | 0.104 | 0.101 | 0.1 | 0.099 | 0.05 | 0.07 | 0.06 | 0.08 | 0.07 | 0.09 | 0.10 | 0.01 | 0.018 | 0.02 | 0.022 | 0.024 | 0.027 | 0.029 |
| **No of main latral branches (root)** | 12 | 14 | 17 | 12 | 15 | 13 | 12 | 23 | 22 | 24 | 19 | 17 | 20 | 16 | 7 | 6 | 7 | 9 | 6 | 7 | 6 | 12 | 9 | 13 | 8 | 8 | 9 | 8 |
| **No of nobules** | 14 | 12 | 11 | 11 | 9 | 11 | 7 | 20 | 14 | 13 | 14 | 12 | 11 | 10 | 8 | 5 | 7 | 9 | 9 | 8 | 8 | 10 | 9 | 12 | 12 | 11 | 10 | 9 |

# Table - 15 : Biochemical Parameters of Selected Plants

| | *Cyamopsis tetragonoloba (RMG1002)* | | | | | | | *Glycine max (JS 335)* | | | | | | | *Medicago satava (t9)* | | | | | | | *Trigonella foenum graecum (SWATI)* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fly ash Concentration | Control | 5% | 10% | 15% | 20% | 25% | 40% | control | 5% | 10% | 15% | 20% | 25% | 40% | control | 5% | 10% | 15% | 20% | 25% | 40% | control | 5% | 10% | 15% | 20% | 25% | 40% |
| **Protein (µg/gm)** | 100 | 100 | 100 | 150 | 155 | 160 | 140 | 205 | 192.5 | 199.7 | 197.2 | 195.8 | 201.4 | 204.0 | 120 | 200 | 240 | 280 | 240 | 280 | 260 | 86 | 80 | 95 | 105 | 170 | 180 | 85 |
| **Phenol Content (mg/gm)** | 60.9 | 78.7 | 86.4 | 98.5 | 97.4 | 105 | 122.3 | 131.3 | 100 | 87.5 | 69.99 | 64.65 | 55.88 | 62.88 | 120.2 | 121.9 | 121.7 | 123.7 | 125.4 | 125.8 | 124.7 | 217 | 176.7 | 168.5 | 171.9 | 164.4 | 167.6 | 140.8 |
| **Carbohy-drate Content 480A** | 9.29 | 9.81 | 12.8 | 19.8 | 13.6 | 13 | 10.9 | 20.05 | 20.06 | 20.06 | 20.06 | 20.03 | 20.05 | 20.04 | 2.08 | 3.73 | 1.86 | 2.44 | 1.33 | 2.73 | 2.17 | 19.82 | 14.9 | 19.8 | 19.82 | 19.81 | 19.82 | 19.48 |
| **Carbohy-drate Content 490A** | 7.95 | 8.27 | 12.3 | 16.9 | 11.3 | 10.8 | 9.26 | 16.83 | 16.80 | 16.85 | 16.83 | 16.83 | 16.84 | 16.83 | 1.84 | 3.29 | 1.67 | 2.22 | 1.22 | 2.52 | 1.93 | 16.9 | 11.5 | 16.9 | 16.9 | 16.62 | 16.9 | 16.9 |
| **Proline (µg/gm)** | 10 | 10 | 10 | 15 | 15 | 15 | 14 | Traces | Traces | Traces | Traces | Traces | Traces | Traces | 10 | 15 | 14 | 10 | 10 | 10 | 12 | 10 | 14 | 12 | 18 | 10 | 9 | 10 |
| **Ascorbic Acid (mg/100 gm)** | 32.4 | 36.6 | 39.6 | 39.5 | 40.1 | 39.3 | 68.2 | 36.4 | 37.77 | 34.8 | 39.8 | 39.4 | 42.2 | 57.7 | 54.5 | 54.8 | 53.5 | 52.9 | 53.7 | 54.4 | 59 | 68.2 | 66 .2 | 67.9 | 70.3 | 72.3 | 84 | 88 |
| **Carotenoid** | 0.42 | 0.23 | 0.42 | 0.37 | 0.38 | 0.37 | 1.13 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.42 | 0.43 | 0.49 | 0.49 | 0.53 | 0.49 | 0.519 | 0.52 | 0.55 | 0.53 | 0.27 | 0.39 | 0.52 | 0.49 | 0.53 | 0.30 |
| **Total Chlorophyll** | 1.28 | 0.94 | 1.29 | 0.99 | 1.02 | 0.44 | 1.32 | 2.38 | 2.39 | 2.39 | 2.39 | 2.38 | 2.4 | 2.33 | 1.49 | 1.46 | 1.43 | 1.5 | 1.42 | 1.47 | 1.47 | 1.3 | 0.86 | 1.2 | 1.38 | 1.34 | 1.38 | 1.07 |

## Table –16 : Cultural Morphological and Biochemical Character of *Rhizobium*

| Sr. No. | Characters | Cyamopsis tetragonoloba L (RMG1002) | Glycine max L (JS 335) | Medicago sativa L (T9) | Trigonella foenum graecumL (SWATI) |
|---|---|---|---|---|---|
| 1. | **Shape** | **Circular** | **Circular** | **Circular** | **Circular** |
| 2. | **Color** | **White creamish** | **White creamish** | **White creamish** | **White creamish** |
| 3. | **Opacity** | **Opaque/ Semitransparent** | **Opaque/ Semitransparent** | **Opaque/ Semitransparent** | **Opaque/ Semitransparent** |
| 4. | **Margin** | **Regular/entire** | **Regular/entire** | **Regular/entire** | **Regular/entire** |
| 5. | **Elevation** | **Convex/ Raised** | **Convex/ Raised** | **Convex/ Raised** | **Convex/ Raised** |
| 6. | **Shape** | **Rod shaped** | **Rod shaped** | **Rod shaped** | **Rod shaped** |
| 7. | **Oxygen demand** | **Aerobic** | **Aerobic** | **Aerobic** | **Aerobic** |
| Cont.... | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 8. | **Motility** | **Motile** | **Motile** | **Motile** | **Motile** |
| 9. | **Spore formation** | **Non spore forming** | **Non spore forming** | **Non spore forming** | **Non spore forming** |
| 10. | **Gram's nature** | **Gram Negative** | **Gram Negative** | **Gram Negative** | **Gram Negative** |
| 11. | **Production of Indole from tryptophan** | **- ve** | **- ve** | **- ve** | **- ve** |
| 12. | **Methyl red test** | **- ve** | **- ve** | **- ve** | **- ve** |
| 13. | **Voges-Proskauer test** | **- ve** | **- ve** | **- ve** | **- ve** |
| 14. | **Citrate utilization as source of carbon** | **+ve** | **+ve** | **+ve** | **+ve** |
| 15. | **Production of ammonia from urea** | **+ve** | **+ve** | **+ve** | **+ve** |
| 16. | **Production of Hydrogen peroxide** | **-ve** | **-ve** | **-ve** | **-ve** |
| 17. | **Nitrate Reduction** | **+ve** | **+ve** | **+ve** | **+ve** |
| 18. | **Catalase test** | **+ve** | **+ve** | **+ve** | **+ve** |

CHAPTER – 7

# FUTURISTIC APPROACH

# FUTURISTIC APPROACH

Thermal fly ash' notorious waste of thermal power plants is the point of interest of present study, because it creates various problem to the environment and affects the plant growth. Land filling is the traditional method of flyash disposal, but the dual factors of increasing pollution and stricter legislation have prompted research in to alternative methods of disposal and utilization of this waste material, over the years numerous studies on the flyash use as soil amendment have been conducted. Simultaneously the use of flyash as a soil amender was long been studied, and found reasonable to few extents, so the present study was a attempt to find out the impact of flyash on growth performance of plants and root nodule bacteria.

During present research we have concluded that fly ash is hydrophilic in nature, the water retention capacity of soil can be increased by the addition of fly ash without affecting the porosity of soil, In fly ash mixed layer, water retained in soil, dilutes the soil solution and this in turn enhances the rate of cation exchange phenomenon. Other soil reactions which are hindered because of high concentration of soil components may also be accelerated by the dilution of soil solution. Water retention prevents soil from cracking which is harmful to plant growth. Thus, fly ash acts as a soil conditioner. Such conditioning practice can be employed in semiarid regions where rainfall in meager. During present study analysis of soil amended with fly ash indicate that, application of fly ash in to soil has change the soil texture and structure in a way to improve the availability of soil water, air and nutrient.

It was observed in our study that due to thermal power plant, the flora of Kota city was affected and it seems that loss of plant diversity is may be due to loss of fertility of soil. It was also found that soil

pollutants affects different soil components, which play important role in maintaining soil fertility. In the present study by seasonal survey of study area i.e. Kota thermal power plant and comparative floristic data analysis (two decades) it was found that the result of cumulative effects of all factors, the biodiversity of study area is comparatively less and confined to limited species. Plants show morphological, and biochemical changes in last few decades in comparative to non polluted sites. In this study we have found out the resistant species in flyash affected area, Trees were less affected comparatively shrubs and herbs, It is concluded that herbaceous are very sensitive to pollution, The herb plant belong to family Amaranthaceae, Caesalpiniaceae, Euphorbiaceae and Compositae show maximum growth near KTPS,. *Acyranthus aspera L, Euphorbia hirta L, Tridex procumbance* L show marked difference in all ecomorphological criteria near KTPS. These species may be recommended as bio indicator or bio-monitor plants for monitoring the pollution. Similarly Tree species dominantly present in this area are *Azadiracta indica Juss, Cassia siamea L, Delonix regia L, Acacia nilotica L and Ziziphus* spp Wt.Arn and shrubs were *Ipomoea fistulosa, Lantana camera L, Solanum xanthcarpum SW etc show maximum* tolerance.

The vegetation of that area reflects the original image of pollution level caused by thermal power plant. Plant community show symptoms, or there can be substantial losses in production and in biodiversity. Although we are aware of threats of pollution caused by thermal power plants but we don't have alternate of coal (energy source) as it is the cheapest and maximum available fossil fuel in country like India. It is concluded that one of major cause of the soil pollution problems in Kota city is land disposal and deposition of excessive amount of solid wastes that is flyash.

Our basic aim was to found out the best suitable concentration of fly ash as soil amenders for improving the ecomophological

characters and productivity of studied leguminous plant and ultimate effect of flyash on root nodule bacteria that certainly important to maintain the fertility of soil in future, we have got success up to few extend.

From an environmental point of view the Rhizobium-leguminous symbiosis is preferable compared with use of industrial N fertilizers, that require much energy to produce, transport and spread, our results showed that flyash in lower concentrations not cause any detrimental effects on studied legumes although few stress symptom were there, and in the case of rhizobial strains ie *Rhizobium leguminosarum* of *Cyamopsis tetragonoloba L*, *Rhizobium meliloti of Trigonella foenum-graecum L and Medicago sativa L, Bradyrhizobium japonicum* of *Glycine max L not* show any mutational effect up to the 25% flyash treatments.

On the basis of present study following conclusion with recommendation are made------

- Through individual study of these plant, different concentrations of flyash amendments were beneficial in different plants as result show that *Glycine max L* was most sensitive to direct flyash treatments in Roll Towel Germination experiments, while other three were comparatively tolerant for it, as the result of this experiment it is not recommended, the direct use of flyash on seed or even any exposed part of the plant (by dry/wet spray) because it causes stress symptoms as observed in form of curling of roots in initial plant establishment.

- In Pot and Germination tray experiments this is clearly observe that flyash was show beneficial effects in some parameters in all four crop plants while mixed result were observe in others up to 25% amendments, after that some

detrimental effects were observe in growth parameters of plants. Individually best recommended percentage for flyash amendments are- 10-15% in *Cyamopsis tetragonoloba* L, 15-20% in *Medicago sativa* L, 25% in *Trigonella foenum graecum* L, and 15-20% in *Glycine max* L.

- As Bacteria's is a tiny and lower most component of any food chain. But these tiny members have their own importance, without bacteria we can't imagine any food chain. But these important members can survive in good quality of soil, in place of it they return fertility of soil by maintaining NPK contain of soil, So the management of bacteria for pollutant free soil is the demand of today. As our result support that combination of Flyash with legume plant having different strains of rhizobia is showing positive growth responses and even not show any change in morphological, cultural and in biochemical characters in any rhizobial strains in four studied plants.

Few general recommendations about flyash utilization in direction of generation of wealth from the waste are -

- Flyash optimizes pH of soil and improve aeration. it may work as substitute of gypsum and dolomite

- Flyash is like the parental coal, contain almost every naturally occurring elements. Plant nutrition with this complex material is not straight forward, as demonstrated by contrasting results from the study. Although containing neutralization value this is beneficial may be in reclamation efforts.

- Flyash improves fertility status of soil. Coal residue applied on cropland are not practical sources of essential

plant nutrients N,P,K , but they can however efficiently serve as a supplementary source of Ca, Mn, Mo, B, Se to soil .

- Flyash can be used to solve various soil health problems such as acidity, texture, compaction and permeability up to few extend depends on basic soil quality.

- It can improve growth, maturity and biochemical status of crop plants. It improves nutritional quality of food crops. Specially legume plants.

- Crop grown in flyash amended soil are safe for human consumption, and soil and ground water quality is not affected.

- Flyash can be used as land filler barren land, mine fills and low lying areas to ensure that there is a good ground cover of tolerant grasses, shrubs and trees to build organic matter through practices such as green manure crops as legumes mulching, strategic grazing which minimize the impact of fly-ash. These measures have multiple benefits and will also support healthy populations of soil bacteria.

- Poor aeration and drainage encourages undesirable anaerobic bacteria. Reducing compaction by flyash and building soil organic matter by legume plants will improve water infiltration without compromising moisture storage and will discourage anaerobic bacteria. Healthy populations of soil bacteria are encouraged by groundcover of legumes.

- Coal ash (Raakh) was used as sanitizer for insect and pest control from ancient time, so flyash(coal residue) can

use as base for preparing bio- pesticide for crop plant and eor applying in agricultural land, it now come as an aid for farming community because of its great availability and low cost.

**Control**

The results of present study gives some good indication regarding the positive effect of flyash on growth performance of soil bacteria and on the physico-chemical characters of soil, than it is very useful to us, that the various pollutant can be utilize for improving soil health.

The data regarding present research will be useful for mitigating problem of soil pollutants (flyash) in Kota district. Present investigation explores complex links between environment, pollutant, vegetation and soil bacteria. It explores the ways in which different pollutant can affect the soil bacteria. Present research work is a one step towards, to improve the information regarding impact of flyash on soil, vegetation and bacteria. The study also improves our fundamental understanding of legume- bacteria interactions under flyash affected condition.

CHAPTER – 8

# SUMMARY

# SUMMARY

Country like India has suffered enormous pressures on its land resources as these are world's highest populated regions, and have suffered serious land degradation. Soil is an integral part of land resources but it is an irreplaceable and non-renewable asset. The widespread industrialization and increasing consumption have changed the complexion of soil. Thus the soil is getting heavily polluted day by day by toxic materials and dangerous microorganisms which enter the air, water and the food chain. For all this, man is the original and basic pollutant responsible for pollution hazards and toxic effects. The pollutants are directly discharged in soils of surrounding areas and these effluents affect the quality of soil. Contamination of the soil causes to lose of its useful properties such as fertility, water-holding capacity, permeability and binding capacity. Thermal Power Stations are spread all over the country. The impact of coal residue generated from Thermal Power Stations especially flyash on health of plants, animals and environmental consequences has been reviewed extensively all over the country. Every year Indian Thermal Power Station produce more than 100 million tons of fly ash, which is expected to, reaches 175 million tons in near future. Agriculture and wasteland management have emerged as prime bulk utilization area for fly ash in country. Fly ash is comprised primarily of fine sand and silt sized particles. Addition of fly ash to the soil of poor buffering capacity increases soil pH due to presence of basic metal oxides and alters the availability of some nutrients. Fly ash can be used for reclaiming the problem soil and enhance the crop productivity depending upon the nature of soil and fly ash. It affects physical, chemical and biological properties of soil and has impact on the available macro and micronutrients of plants.

Kota Thermal Power Station (KTPS) is one of thermal power station of Rajasthan situated in Kota and it fulfils the requirement of

energy. In the race of industrial and economic growth soil of Kota region has also pay a lot and the soil quality of Kota region is continuously degrading due to different flyash and soil pollutants. The main causes of soil pollution are deforestation, bad agricultural practices, water logging and different industrial activities which take place in Kota city including 'Kota Thermal Power Station' (KTPS).Flyash is a by-product from thermal power station causing pollution to the local area of power plant as well as fly ash dumping station and transporting tract.

Kota Thermal Power Station is based on coal, lead to a number of impacts on environment via, atmospheric pollution, by gaseous emission, solid waste pollution by way of ash dumping, water pollution by way of waste effluents and ash discharge in the nearby water ways. But large quantities of ash generated, leads to increasing scarcity of water and land for disposal of ash. The present ash disposal systems which is in use are causing serious disposal and ecological problems on account of its potential for contamination of surface and ground water and surrounding air due to escape in atmosphere, causing serious problems to various biotic life forms. It is observed that during last 20 years there is decrease in productivity of leguminous plant and soil degradation is also recorded, it indicates that flyash has negative impact on plants and soil quality. Soil bacteria and bacteria present in nodules of legumes plays an important role in maintain soil fertility and soil quality and affects plant production these bacteria also play an important role in a food chain.

In the light of above facts present research work taken to study the impact analysis of flyash on growth performance of selected legumes with special reference to root nodule bacteria. Present study is summaries in to four parts -

- The first part deals with floristic survey of study site that is Kota thermal power plant, collection of flyash and soil sample and selection of four legumes for further study.

- The second part deals with seed germination test of flyash treated seeds (selected legumes) to find out appropriate concentrations for pot experiments.
- Third part includes eco-morphological and biochemical study of selected legumes grown in different soil amended with flyash.
- Fourth part deals with isolation purification and culture of *Rhizobium* bacteria from control and 25% flyash amendments, along with biochemical testing to analyze impact of flyash on various *Rhizobium* strains.

Present research is performed to know the impact of fly ash on growth performance of following four selected legumes that are well known and highly consumed in this region as food or as fodder. Selected legumes are-Cyamopsis tetragonoloba L, Glycine max L, Medicago sativa L, Trigonella foenum-graecum L. Our attempt is to find out how fly ash has altered the character of soil and impact of such alters soil character on growth performance of selected plants as well as on root nodule bacteria. The brief introduction of selected plants –

**Cyamopsis tetragonoloba L *(RMG1002)*** also known as Cluster bean occupies very important place among commercially utilized crops in India. It is a rich source of high quality galactomanan gum and Protein rich guar meal which is in great demand in the world market. Cluster bean is compatible for nodulation with both fast growing *Rhizobium* and slow growing *Bradyrhizobium* species.

**Glycine max L (JS 335)** also known as Soya bean. The bean pods and seeds are a source of oil and protein and are good source of vitamin B. Fermented pods are used to make soya sauce and other sauces and soya milk. Inoculation with nitrogen-fixing bacteria is desirable, the strain *Rhizobium japonicum* being specific to soya bean.

**Medicago sativa L *(T9)*** also known as alfalfa It is one of the highest yielding forage legumes. It is grow as a cover crop to reduce

erosion. It is a nitrogen fixer. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. It is compatible with non-aggressive grasses. It is inoculated with an effective strain of *Rhizobium meliloti*.

**Trigonella foenum-graecum L (SWATI1)** also known as Fenugreek, It is a useful agro forestry species. The plant and seeds have a characteristic strong odour. The plant seeds are edible and used as condiments and as flavouring agent. Oil can be extracted from the. This oil has potential in the perfume and cosmetic industries. The seeds also contain the drug diospenin, used in the synthesis of hormones.

There is no single book which contains all the information so a lot of information got from different book and articles. The first from 1950 to till date. In the past years more investigation were conducted on the effects of gaseous pollutants fly ash one of them. More detailed reviews of the literature of vegetation injury from the pollutant released from industrial & power plant may be found in the publication by Thomas and Hendricks, (1956), Brandt and Heck, (1968), Wentzel, (1956), Scurfield, (1960). The damaging effects of air pollutant on vegetation have long been recognized by Das Gupta, (1957), Woodwell, (1970), Majernik and Mansfield, (1972), Bleasdale, (1973), Mansfield, (1976), Furukawa, *et al.*, (1980), Kasat and Agrawal, (1981), Koziol and Whatley, (1984), Agrawal (1985), Singh and Rao, (1983), Gupta, *et al.,* (1988), Sirohi and Singh, (1989).

Several studied with higher plant exposed to industrial exhaust show decrease in chlorophyll contents. Pandey and Rao (1979), Chapekar (1982), Rabe and Kreeb (1979), Varshney and Varshney (1979), Mishra (1986), Sharma (1986), Prasad and Rao (1982), Dubey, *et al.,* (1982), Agrawal and Sharma (1984), Shrivastava *et al.*,(2002) reported the effect of automobile air pollution on growth of some plants at Kota region. Farmers of Punjab, Haryana and Maharashtra blame thermal power plants for sudden decrease in production and found highly contaminated with heavy metal and not fit for consumption. (Art. India

beyond coal. 2013) Flyash particles deposits on standing crops smoke from chimneys pollutes the air that plants use for photosynthesis and no farming technique can avoid contamination from these sources.

Agrawal and Gupta (1993) studied impact of fly ash amended soils on pigment characteristic of seedlings of maize, sorghum, wheat, gram and soyabean. Pandy *et al.,* (2009, 2010) reveals the fly ash could be efficiently used in barren or sterile soil for improving quality and enhancing fertility, the purpose of this study is to explore the possibility of fly ash addition in to degraded soil for improving nutritional and physico-chemical properties. According to Gupta *et al.,* (2002), fly ash deposition effect physiochemical properties of soil, practical value of flyash in agriculture as an effective and safe fertilizer on soil amendment can be established after repeated field experiments, Rai *et al.,* (2003) also observed performance of seed germination and growth of *Vicia faba* L. in soil amended by different concentrations of fly ash result revealed that flyash amendment to the soil improved the growth performance at initial stages with application of lower concentrations, it was inhibitory at higher exposure concentration flyash delayed the nodulation as lesser number of nodules was recorded at higher amendments.

A revegetation trial was conducted to evaluate the feasibility of growing a legume species on flyash ameliorated with combination of various organic amendments, blue green algae bio fertilizers and rhizobium inoculation, a significant enhancement in plant biomass photosynthetic pigments, protein content and in vivo nitrate reductase activity were found in the plants grown in ameliorated flyash in comparison to plant growing in unamended flyash or garden soil (Rai *et al.*,2004). Researches on the utilization of thermal flyash and its impact on different agricultural crops and plant species were carried out through different projects by Cheng and Chu (2007), Ram (2008), Bhdoria (2009), Verma and Sharma (2009), Singh (2010). Effect of certain heavy metals on seed germination and seedling growth performance of legume plant *Cyamopsis tetragonoloba* observed by Jain *et al.*,(2009), Vijaylaxmi *et al.*,

(2006) working on influence of pollutants on soil bacteria reported no significant relation between physiochemical character and bacterial densities in polluted sites.. Singh and Agrawal, (2010), with *Vigna radiata* have found that both growth and yield shows positive responses when soil amended with different concentration of fly ash in a field experiment. Premkishor, *et al*., (2010) explained the use of fly ash in agriculture to improve soil fertility and its productivity. Effects of fly ash on microbial $co_2$ evolution from agricultural soil have been reported by Arthur, *et al.,* (1984). Many researchers discussed utilization of fly ash by crops under green house condition in ecology and resource development. It has been shown that fly ash has a vast potential for use in agriculture. (Krishna Rani and Sharma, 2009 and Punjwani, *et al*., 2011). For agricultural related studies a large number of demonstrative trials executed by different technological institute and laboratories at various sites in dispersed locations the country under varied agro-climatic conditions on a spread of crops forestry and horticulture species had brought in to focus fly ash as an important resource material.

In this over all studies, the study is concentrated on the pollutant released from thermal power plant especially fly ash and their impact on the vegetation. Though a number of studies have been carried out on different plants in various part of world, but review of studied literature indicate that not much work is reported on the impact of fly ash on legumes ie Cyamopsis tetragonoloba L*,* Glycine max L*,* Medicago sativa L ,Trigonella foenum-graecum L. and their root nodule bacteria in Kota region, so our study may be a small start.

Our study was limited to Kota District (South Eastern Rajasthan or Hadoti Plateau) which is situated at the edge of Malwa plateau at 23˚45’ Feastern corner of Rajasthan state. Its total area is 24156.6 square kilometres and from administrative point of view it is known as Kota division. The average surface elevation of land surface is 300 meter above M.S.L. Previously Kota is known as “Industrial city” of Rajasthan. It has Thermal Power Station, Which fulfil the energy requirement of

Rajasthan. Now a days Kota city Known as “Education Hub” of India. Both name given “Kota” a unique identification and lot off economic growth. The texture of soil of Kota region comes under loamy to sandy loam type. The western parts of the region possess sandy-loam in preponderance while loamy-sand soil is characteristic of the eastern part of this region. The soil series dominant in the Kota region and adjoining areas are found to be mainly Chambal soil series and Kota variant soil series. Climate of Kota region is sub humid to semiarid with three season’s namely rainy, winter and summer seasons.

Survey has been done at surrounding areas of Kota Thermal Power Station. Collection fly ash has been done from Kota Thermal Power Plant. Listing and collection of plants from study site and comparative data analysis was performed between last two decades. After above survey leguminous plants were selected for the further study. Certified seeds of selected legume plants were collected from Rajasthan State Seed Corporation Limited. Pure culture of rhizobium strain were collected from MTCC Chandigarh. Fly ash analysis has been done and suggests that it is a potential source of many macro and micro element to the plants including some toxic metals. Fly ash generated from Kota thermal power plant is alkaline and hydrophilic in nature. Air dry samples of soil and flyash were mixed accordingly i.e. control (0% FA), 5%,10%, 15%,20%, 25% and 40% FA by weight, Flyash was mixed with soil with a tumbler to provide a homogeneous mixture Physico-Chemical analysis of soil and fly ash mixture has been done. To analyze the impact of fly ash on growth performance and biochemistry and laboratory study was done by following steps– (i) Germination Experiment – using ‘Roll towel method’ following parameters were studied i.e. Length of shoots, Length of root, Time of germination, Germination percentage etc of selected legumes in 0%,10%,20%,30%,40%,50%60%,80%100% flyash concentrations (by 24 hr pre-soak treatment)(Table-1)(ii) Pot Experiment –Seeds of selected leguminous plants were planted in pot (normal and treated soil with revised percentage of fly ash / soil i.e. 0%, 5%, 10%, 15%, 20%, 25%, 40%), Root length, Shoot length, germination

percentages, fresh weight, Dry weight were studied.(Table-2) (iii) Biochemical Analysis– Protein contents, (spectrophotometer method of Folin-Lowry's method), phenol content (spectrophotometer method of Malik and Singh) Proline contents, (colorimetric determination), Carbohydrate contents ((spectrophotometer method of phenol sulphuric acid reagent method), Chlorophyll content (spectrophotometer method of Arnon), Ascorbic acid content (titramatrically) were calculated in selected legume plants i.e. Cyamopsis tetragonoloba L*,* Glycine max L*,* Medicago sativa L*,* Trigonella foenum-graecum L. in 0,5,10,15,20,25,40% concentrations of flyash (iv) Nodulation studied- plants grown at normal as well as 25% fly ash treated soil (in pots) are uprooted from the pot after 45-60 days of plantation and their roots will be washed to obtain root with intact nodules. Data on plant height, root length, dry biomass, number of lateral roots and nodules per plant were collected. (v) Isolation and purification - Isolation and purification of *Rhizobium* will be done as method given by Subba Rao (1989). Morphological, cultural and physiological characteristics of *Rhizobia*l strains will be tested according to the methods given by Vincent (1970) and Aneja (2008). On the basis of staining (Gram staining), morphology (cell shape, cell arrangement, motility), cultural (growth, colour, elevation and margins) characteristics bacterial strains were identified and after biochemical test confirmed results were obtained. With biochemical test (such as, MR, VP, Indole Test, Nitrate Reduction, Urease production, Catalase Test, Citrate utilization, $H_2S$ Production,) In order to test the significance of data, the statistical analysis of recorded data was made with standard procedures. The method of standard deviation is used to find out the deviation between various parameters of present study. In order to establish interrelationship between various parameters graphical presentations were made which are presented in thesis.

To analyse the impact of flyash on root nodule bacteria of Kota district (Raj) result of various experiments (field, pot, laboratory) are presented in tables, objective set were developed on observation that concluded some results in form of table specifying different comparative

studies. All these comparative studies are included in the thesis. A comparative account is displayed to indicate the detail.

Chemical analysis of flyash from KTPS Kota show that flyash is alkaline in nature and a potential source of macro and micro nutrients, similar result were obtained by Mehra, *et al.,* (1986) by Shridhran and Pandian, (1998), Saxana and Chauhan (1998), Krishna Rani and sharma (2010). In present study soil properties as influenced by fly ash addition in soil have been studied. Similar work was done by several researchers as Sharma*, et al.,* (2002), Sikka and Kansai (1994), Kalra, *et al*.,(1997), Sharma (1998), Deshmukh (2000), Grewal, *et al*., (2001), Garg *et al*., (2003), Page *et al.,* (1979), Singh and Yunus (2000), Agarwal and Sinha (2001). The result indicates the physical properties of soil and flyash amended soil, it was clear that the water holding capacity of soil increases. In the previous studies conducted with loam soil amended with flyash indicated modifications in physiochemical characters of soil, by many workers as Tarkalson, *et al.,* (2010), Punjwani, *et al.,* (2011), Yeledhalli, *et al.,* (2007), Kalara, *et al.,* (2003), Prem kishor, *et al.,* (2010).

On the other hand result from survey near Kota thermal power station (KTPS) changes in dominant family's distribution pattern is clearly observable during last two decades. The results presented can be compared to those of Thakre, (1983), Thakre and Agarwal, (1987), Agarwal and Agarwal, (1988), Dadhich and Kasat, (1988), Khandelwal and Shrivastava, (2013), Khandelwal and Shrivastava, ( 2014).

Results of preliminary seed germination experiments conducted during present study show marked effect on germination. Germination of Cyamopsis tetragonoloba L, Glycine max L, Medicago sativa L, *Trigonella foenum graecum* L was performed in seed germinator by roll towel method after 0%,20%,40%,60%,80%,100% fly ash treatment exhibit a promotive response only in lower concentrations of flyash to germination in all four plants. The maximum germination percentage was observed in *Trigonella foenum graecum* while minimum germination percentage was observed in *Glycine max.* Similar result were obtained by

Agarwal and Gupta, (1993), Haynes, ( 2009), Singh, e*t al.,* (2008), Kumar, *et al*.,(2009), Katiyar, *et al.,* (2012, 2013). Effect of flyash treatment on seedling growth*,* shows favourable seedling growth response in 20 and 40% flyash except in Cyamopsis *tetragonoloba,.* Drastic reduction in germination percentage was obtained after 40% flyash incorporation*.* Similar result found by by Singh,*et al.,* (2008), Agarwal *et al.,* (2004), Panwar *et al.,* (1998), Singh *et al.,* (1997), Jala (2006), Kalara (1997). The overall results used in directing the further study at lower concentrations is more advantageous then the higher concentrations in all four selected legume plants. Ecomophological study of selected legumes under revised concentrations of flyash show that the performance of selected plants i.e. Cyamopsis tetragonoloba L, Glycine max L, Medicago sativa L, *Trigonella foenum graecum* L*,* in soil amended by 0%, 5%, 10%, 15%, 20%, 25%, 40% of fly ash has been studied through pot experiments, overall result revealed that flyash amendment to the soil improved the growth performance at up to 25% except in Trigonella show best result in 40%,similar observation were reported by Lal *et al.,* (1996), Ghuman *et al.,* (1994), Shukla *et al.,* (2003), L Selvakumari *et al.,*(2000), Khan (2001), Khan and Khan (1996), Pandey *et al.,* (1994), Singh *et al.,* (1994) on contrary it was inhibitory at higher concentration. Percentage germination reduced in 40% in all four plants. Our results were similar to Matte and Kene (1995), Kalara *et al.,* (1997, 1998), Kumar *et al.,* (1998), Bhaisare *et al.,* (2000).

In biochemical analysis the value of *chl a, chl b*, total chlorophyll and carotenoids were expressed in terms of mg/1 mg of fresh weight. In *Cyamopsis tetragonoloba* the value of total chlorophyll was observed highest in 40 % and minimum in 5%. In *Medicago sativa* the value of total chlorophyll was maximum in 15% and control and minimum was in 20%. In *Trigonella foenum graecum* the value of total chlorophyll was maximum in 15% and 25% and minimum was in5%. In *Glycine max* the value of total chlorophyll was maximum in 25% and minimum was in 40 %.( Table-3). Similar observation was taken on maize, sorghum and soyabean by Agarwal and Gupta (1993), Agarwal (1998), Aberg (1953). Variation in

biochemical contents is also considered as an important vital event during growth and differentiation of cells in plants. To determine the effect of flyash on plants protein content, phenol content, carbohydrate content, proline and ascorbic acid content was measured. Highest protein content was obtained in *Medicago sativa* at 15 & 25% flyash treatment, followed by *Glycine max* (control) while minimum was in *Trigonella foenum graecum* at 5%. Variation in protein contents is also consider as biochemical event during growth of plant. Protein contents have been estimated in various plants as *Lens culinaris, Vicia faba, Cicer arietinum, Phaseolus spp, Pisum sativum and Glycine max* reported by Audichya (1999), Borhade *et al.,* (1984), Chatrath *et al.,* (1996). Highest phenol content was obtained in *Trigonella foenum graecum* in control while minimum was in *Glycine max* in 25%, similar study was done Malik and Singh (1980), Agarwal and Gupta (1993) on different plants, and explain its production as a response for defending mechanism of injured plants. Maximum value of carbohydrate content was in *Glycine max* at lower concentrations of flyash followed by *Trigonella foenum graecum* and minimum was observed in *Medicago sativa.* Carbohydrate contents have been studied in different plant species like *Balanites aegyptica* by Vijayvergya and Vijay (2006), *Cassia obtusifolia and Cassia siamea* by Sharma *et al.,* (2006), two species of *Araucaria* by Unikrishnan *et al.,* (2007). In *Trigonella foenum graecum, L* proline content increase with increase concentration of flyash up to 15% which is highest among four, followed by *Cyamopsis tetragonoloba, in Glycine max* proline was observed in traces that could not be measured quantitatively. Proline accumulation was studied by Ashraf and Harris (2004) in root and leaf of plant tissue during abiotic plant stress in various plants. In *Trigonella foenum graecum* highest ascorbic acid content was measured among all four plants. Maximum value was observed in 40 %, and minimum was in *Glycine max* similar result were find by Agarwal and Gupta (1993) in different varieties of soyabean .(Table-3)

'Nodulation studies' were performed for all selected plants (Nodules of 45-60 days plants). Results indicate that lower concentrations

of flyash amendments were beneficial for plant growth with special reference to root and nodule growth pattern. Similar result were found by Singh (1997), Pandey *et al.,* (1994) in different rhizobial strains in legume plants, It is concluded that in all four legumes 40% flyash amendments show negative response towards root growth pattern and nodule formation. Data on plant height, root length, number of lateral roots and nodules per plant were collected. Variation in nodule number was reported in 0% and 25% fly ash treated plants ie *Cyamopsis tetragonoloba, Glycine max,* L *Medicago sativa*, L *Trigonella foenum graecum*, L. Similarly in *Indigofera* species highest of only 1-5 nodules per plant reported by Athar and Shabbir (2008). The highest number of 25 nodules per plants was reported by Anegbah *et al.,* (2003). In the present study the size of the nodules ranged from 2.0-8.0mm, the maximum diameter observed was in *Glycine max,* followed by *Cyamopsis tetragonoloba, Trigonella foenum graecum and Medicago sativa.*

The overall best results were obtained in 25% flyash treatment. These results inspired to choose plants grown in control soil and 25% flyash amended soil for further study. For biochemical analysis of bacteria which are obtained from root nodules of legumes are streak on already prepared Yeast Extract Mannital Agar (YEMA). Similar work was done, Mahana *et al.,* (2000) on *Vigna munga*, After 24-48 hour inoculation bacterial colonies were appeared, marked their location and re-streak again to avoid contamination. These isolated bacteria from (control and 25% flyash amended) four different legumes were identified by their staining, morphology and cultural. All the results were similar to the standard results given by Rhizobium. To study the fast or slow growing nature of the isolates they were grown on freshly prepared YEMA plates containing bromo-thymol blue adjusting the pH to 6.8. *Rhizobium leguminosarum* of Cyamopsis tetragonoloba L was found fast growing followed by *Rhizobium meliloti of Trigonella faenum-graecum*L *and Medicago sativa,*L *while Bradyrhizobium japonicum* of Glycine max L *was* slow growing strain, as describe by Somasegaran and Hoben (1994). The bacterium of *Cyamopsis tetragonoloba (Rhizobium leguminosarum),*

*Glycine max (Bradyrhizobium japonicum), Medicago sativa (Rhizobium meliloti) or (Sinorhizobium melitoti). Trigonella foenum-graecum (Rhizobium meliloti) or (Sinorhizobium melitoti)* showed well-marked growth on YEMA medium at pH 7.0 although it show marked variations in growth with respect to time period on YEMA, similar result on different strains isolated in various species of plants were obtained by various workers. However poor growth was observed on Hofer's medium Purohit & Kumar (1998). Mahana *et al.,* (2000). reported that the Rhizobium isolated from *Vigna mungo L.* showed marked variations in growth with respect to time period on YEMA while they do not show any growth on Hofer's alkaline medium at pH 10.0. Rhizobial strain of 25% *Trigonella foenum-graecum and Medicago sativa* show slight reduction in colony density, similar result justify our results that various pollutants like fly ash and automobile pollutants shows negative impact on bacterial diversity. Gianfreada and Bollag (1996), Pell *et al.,* (1998), Sannino and Gianfreda (2001), Yuang *et al*., (2007) who concluded that addition of synthetic polymers has caused the shift of microbial population and effect the growth of microbial population. Fly ash interfere with the microbe mediated processes operating in soil thus unbalancing the ecobalance (Babich and Stozky, 1974 and Babich *et al*., 1983). In favour of this researchers found that on addition of fly ash in silt loam soil (Arthur *et al*., 1984), in sandy soil (Wong and Wong, 1986), decreased microbial respiration and nitrification activity in soil (Cerevelli *et al*., 1986).

Different biochemical tests were proved as valid tests in identification of the organisms; Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test (MRVP), Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide, Strains were isolated from 0% and25% flyash treated legume plants i.e. Cyamopsis tetragonoloba L nodulated by *Rhizobium leguminosarum,* Glycine max L nodulated by *Bradyrhizobium japonicum,* Medicago sativa L nodulated by *Rhizobium meliloti. (Sinorhizobium melitoti).* Trigonella foenum-graecum L nodulated by *Rhizobium meliloti. (Sinorhizobium*

*melitoti)*as described by Aneja (1996). The biochemical tests were carried out in growth medium at 28˚C for 48 hours incubation in (03 replicates). Catalase test, Urease test Nitrate reduction test Citrate utilization, find positive in all four rhizobial strains. While Indole test, MR,VP, Hydrogen sulphide find negative in *Rhizobium leguminosarum, of* Cyamopsis tetragonoloba L, *Bradyrhizobium japonicum, of* Glycine max L *Rhizobium meliloti on Medicago sativa and* Trigonella foenum-graecum L. Similar work was done by Maheshwari (2012) on different bacteria's as Ecoli. (Table-4) The bacterium showed positive test for citrate utilization, production of ammonia and Catalase activity. Nitrate is reduced to nitrite producing ammonia. Neal and Walker, (1935) suggested rapid nitrate utilization by slow growing root nodule bacteria. Mahana, *et al*., (2000) reported Catalase activity in some isolates from *Vigna mungo L*. The bacterium is negative for MR-VP and Indole reaction. Similarly, Graham and Parker (1964) did not observe MR reduction in all the isolates of seven rhizobia groups. While Basak and Goyal (1980) also reported that none of the rhizobial isolates of seven groups produces Indole.

This study is helpful to analyze the impact of fly ash on leguminous plants and to study not only the ecomophological aspect but also the biochemical & microbiological aspect of the root nodule bacteria of leguminous plants. Result indicates some negative impact of higher concentration of fly ash on which we can do further research to minimize the environmental hazards and some safety measures can be suggested. Data generated in the present study may be useful to understand the positive impact on growth performance of Cyamopsis tetragonoloba L*,* Glycine max L*,* Medicago sativa L*, Trigonella foenum graecum L,* we can suggest that these plants can be grown after field trial in fly ash affected areas, can be used as fertilizer after some alteration, and used in agronomy. Our ultimate effort is to study the impact of fly ash on root nodules and study of microbiology and biochemical analysis of selected legume rhizobium by isolating inoculating and culturing the bacteria. In the light of above facts it is concluded that flyash up to 25% doesn't affect the rhizobium bacterial growth as well as its characters, so flyash can be

used in future for soil amendments in different ways . We did not get any negative or mutational impacts on the rhizobium strains of *Cyamopsis tetragonoloba (Rhizobium leguminosarum), Glycine max (Bradyrhizobium japonicum), Medicago sativa (Rhizobium meliloti)or (Sinorhizobium melitoti). Trigonella foenum-graecum (Rhizobium meliloti) or (Sinorhizobium melitoti)*. It will be suggested that these strains can be used for inoculation of other crops after further investigation and confirmation. It can enhance the nitrogen fixation leads to improve soil quality so floristic diversity of area will remain rich always. Our study may be a small start in this direction.

CHAPTER – 9

# REFERENCES

# REFERENCES

**Aberg, B.** 1958. Ascorbic acid, Hdb.Pflz.physiol., 6: pp.479-499.

**Abubakar, S.Z.,** M. A. Suleiman, and G. B. Murtala, 2004. Targeted bench mark survey report on farmers practices for special mass food production programmed for Kano State, submitted to Kano State Agricultural & Rural Development Authority (KNARDA), pp. 37-41.

**Adholeya, A.,** N.P. Bhatia, S. Kanwar, S. Kumar, 1998. Flyash source and substrate for growth of sustainable agro forestry system, proceedings of Regional Workshop cum symposium on flyash disposal and utilization.pp44

**Adriano,D.C.,** A.L.Page, A.A.Elseewi, A.C.Chang, I.Strughan 1980. Utilization and disposal of flyash and other coal residue in terrestrial eco-system, review. J. Environ. Quality vol.9: pp. 333-334.

**Adriano,D.C.** and J.T.Weber, 2001. Influence of flyash on soil physical properties and turf grass establishment. J. Environ. Qual. 30: pp. 596-601.

**Agarwal,Divya.,**M.K.Agarwal,M.YunusandS.K.Gautam,2011.Phytoremediation of flyash by assessing growth responses of local tree species, J. of Pune & Applied science & Technology Vol. (12) pp 123-134.

**Agarwal, M.** and S.B. Agarwal, 1988. Phytomonitoring of air pollution around a thermal power plant. Proceedings Internation conference on Trop. Micro Meteorology and air poll; pp. 215-218.

**Agarwal, S.K.** and A.P. Sharma, 1984. Air pollution induced the ranged physiology in plants. Changes pertaining to chlorophyll, carotenoid and phenol contents in *Cassia fistula*, L. *Dalbergia sissoo*. Acta. Ecol. 6:2:37, pp. 43

**Agarwal, S.K.** and R. Gupta, 1993. Impact of fly ash amended soils on pigment characteristic of seedlings of two varieties of *Glycine max. Acta, Ecol.* 15(2), pp. 73-78.

**Agarwal, S.K.,** 1985. Foliar biochemical changes in response to air pollution, Acta. Ecol.7:2, pp. 39-42.

**Agarwal, S.K.,** 2007. Environmental biotechnology, biofertilizers, chapter12, pp 337-349.

**Agarwal, S.K.,**1998. Flyash utilization and mitigation strategies, 3rd Regional workshop cum symposium flyash disposal and utilization, KTPS, Kota. pp 52-63.

**Agarwal, T.P.** and A.N. Sinha, 2001. Fly ash uses and abuses and proceeding of the IAEM National Conference on Recent Advances in waste management. Brzark Information System Pvt. Ltd. Varanasi.pp.51-53.

**Agarwal. S.,** G.R.Singh, and B.R. Yadav, 2009. Utilization of flyash for crop production. Effect on the growth of wheat and sorghum crops and soil properties. Journal of agriculture physics, vol.9. pp. 20-23.

**Agrawal,S.B.,** A. Singh, M. M. Bhatt, 2004. Impact of fly ash amendment on seed germination, growth and yield of *Vigna mungo* L. Asia J. Microbiol. Biotech. Environ. Sci.6 (3).pp. 421-426.

**Aitken, R.L.,** D.C Champbell, and L.C.Bell, 1984. Properties of Australian flyashes relevant to their agronomic utilization. Aust. J. soil Res.22, pp. 443-453.

**Allen, O. N.** and E. K. Allen, 1981. The leguminosae. A source book of characteristics, uses and nodulation. The University of Wisconsin Press, Madison, Wisc. U.S.A. pp. 341-351.

**Alloway, B. J.,** 1990. Heavy metals in soils. John Wiley and Sons, Inc New York.

**Anegbah, P.,** Z.Tachoundjeu. and M. Amakiri, 2003. Characteristics of nodules of agro forestry tree legumes for sustainable production in Niger Delta Region, Nigeria. Improvement and culture of nitrogen fixing trees 3(1), pp. 2-3.

**Aneja, K.R.,** 1996. Experiments for microbiology, plant pathology and tissue culture. wishwa prakashan. New Age International (P) Limited, New Delhi, pp. 190-217.

**Aneja, K.R.,** 2008. Experiments in microbiology, plant pathology and biotechnology. FourthEdition, New Age International Publishers Limited.

**Ankita,** Naval., Ganesh. Gajre, Seema. Mishra, A.D.Dixit, 2014. Fly Ash a suitable material for solid waste management through vermicomposting. In *flyash utilization for sustainable environment management, waste to resource material*, SIES, Indian Institute of Environment Management, Navi Mumbai. ISBN- 13: pp. 124-136.

**Ansari, F.A.,** A.K.Gupta, and M.Yunus, 2011, Flyash from coal fed Thermal Power Plants. Bulk utilization in Horticultures, A long term risk management option, Int. J. Environ., Rev. 5(1) pp. 101-108.

**Apoorva, Worlikar**.Nikita. Parab, and Seema. Mishra, 2014. Potential of fly ash in the management of early growth of grape root stocks under field conditions. In fly ash utilization for sustainable environment management: waste to resource material, SIES, Indian Institute of Environment Management, Navi Mumbai. ISBN- 13: pp. 99-112.

**Arnon, D.J.,** 1949. Copper enzyme isolated chloroplast, Polyphenol oxidase in *Beta vulgaris* plant Physio, 130, pp 267-272.

**Arthur, M.A.,** T.C.Zwick, D.A.Tolle, and P.VanVoris, 1984. Effects of flyash on microbial $co_2$ evolution from agricultural soil have been reported by water air soil pollute, 22, pp 209.

**Article 14 June**, 2013. Rajasthan patrika positive impact of flyash on different crops, Project NTPC, Kota.

**Article June,** 2013. A journey from food bowl to ash dykes, Lesson from Punjab; India beyond coal.org. Accessed 22-5-2013.

**Arya, M.P.S.** and R.Singh, 1996. Effect of N, P and K on the nodulation, growth and yield characters of horse gram (Macrotyloma uniflorum) (Lam) verdc. Legume research 19, pp.65-69.

**Ashrof, M.,** and P. Horris, 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science, 166. pp. 3-16

**Athar, M.** and S. M. Shabbir, 2008. Nodulating leguminous weeds of some major crops of Pakistan, Phytologia 90(2), pp. 246-251.

**Audichya, M.** 1999. Regeneration studies on oil containing seeds through tissue culture. PhD thesis University of Rajasthan, Jaipur.

**Aziz, A.,** Martin. J. Tanguy, F. Larher,1999. Salt stress induced proline accumulation and changes in tyramine and polyamine level and linked to ionic adjustment in tomato leaf discs, plant science 145, pp. 85-91.

**Babich, H.** and G.Stotzky, 1974. Air pollution and microbial ecology. Crit. Rev. Env. Cont. 4, pp. 353-420.

**Babich, H**., R. J. F. Bewely, and G. Stotzky, 1983. Application of the “Ecological Dose” concept to the impact of heavy metals on some microbe mediated ecologic processes in soil. Environment Contamination and Toxicology. 12, pp. 421-426.

**Banerjee, Saikat.,** A. K. Singh, S.K. Banerjee, 2003. Impact of fly ash on foliar chemical and biochemical composition of naturally occurring ground flora and its possible utilization for growing tree crops. Indian Forester, 129(8), pp. 964-977.

**Basak, M. K.** and S. K. Goyal, 1980. Studies on tree legumes 3rd characterization of the symbionts and direct and reciprocal cross inoculation studies with tree legumes and cultivated legumes. Plant and Soil 56, pp. 39-51.

**Basak, M. K.** and S.K.Goyal, 1980. Studies on tree legumes *Rhizobium* symbiosis, Nodulation pattern and cross inoculation trials with tree legumes and cultivated legumes. Annals of arid zone. 19, pp. 427-431.

**Bhadoria,** 2009. Potential of flyash utilization in agriculture at global review.

**Bhaisare, B.,** D.B. Matte, W.P. Badole, A. Deshmukh, 2000. Effect of flyash on yield uptake of nutrient quality of green gram grown on verticol'J soil crops, vol.10, no.1, pp.122-124.

**Bisset, K. A.,**1959. Some Characters of Rhizobium Strains from Tropical Legumes. *J . gen. Microbio*l. 20, pp. 89-90

**Bleasdale, J.K.A.,**1973. Effect of coal smoke pollutants gases on growth of Rye grass. Environ. Pollut. 5, pp. 275-285.

**Bogorad, D.,** 1966. *In chlorophylls. E*ds.L.P.Vernon and G.R.Seely Academic Press, New York.pp.435-479.

**Borhede, V.P.,** S.S. Kadam, D.L. Sulunkhe, 1984. Change in phytate phosphours and mineral, during germination and cooking of horsr gram and moth bean , Qual. Plant , 34,pp. 151-157.

**Brady,N.C.** and R.R.Weil,1996. Architecture and physical properties. In M.Carnis (ed). The nature and properties of soils Prentice Hall, Upper Saddle River,N.J. pp 99-141.

**Brandt, C.S.** and W. W. Heck, 1968. Effects of air pollution on Vegetation. In "Air pollution, IInd. Vol. I. Ed. A.C. Stern Academic press, New York, pp. 401-443.

**Buck, J.K**., R.J. Honston, and W.A. Beimborn, 1990. Direct seedling of anthracite refuge using coal flyash as a major soil amendment. Proceedings of the Mining and Reclamation Conference and Exhibition, April 23-26, Charleston, West Virginia, pp. 213-219.

**Campbell, D.J.,** W.E.Fox, R.L.Atiken and L.C.Bell, 1983. Physical characteristics of sand amended with flyash.Aust.J.Soil.Res.21pp.147-154.

**Capp, J.P.,** 1978. Power plant flyash utilization for land reclamation in the Eastern United States. In reclamation of drastically disturbed lands, Schaller, F.W. and P. Sutton (Eds.). Madison, WI, ASA., pp. 339-353.

**Cerevelli, S. G.,** A. Petruzzeli, A. Perna and R. Menicagli, 1986. Soil nitrogen and ash utilization, A laboratory Investigation. *Agrochimica* 30, pp. 27-35.

**Cetin, S**., and Pehlivan, 2007. The use of fly ash as a low cost, environmentally friendly alternative to activated carbon for the removal of heavy metals from aqueous solutions. Colloids Surfaces Physicochem. Eng. Aspects, 298, pp. 83-87.

**Cha,** D.W.,H.S.Lee, J.H.Jung,1999. Production and composition of power plant coal ash in Koria . in proc. Agricultral utilization of flyash symposium Gyeongsang National University Chin j, pp. 1-23.

**Chang, A. C.,** L. J. Lund, A. L. Page, and J. E. Warneke , 1977. Physical properties of flyash amended soils*. J. Envi. Qual*. 6, pp.267-270.

**Chang,A.C.,** L.J. Lund, A.L. Page, and J. E. Warneke, and C.O.Nelson, 1989. Municipal sluges and utility ashes in California and their effects on soil,in BarYosef, B,Barrow NJand Goldshmid, J(Eds) *Inorganic contaminants in Vadosa zone Ecological studies vol. 74 Springer, Verlag,* Berlin, pp. 125-139.

**Chapekar,S. B.,**1981. Significance of growth changes in plants in pollution indication. WHO workshop on biological indicators and indices of environmental pollution. Cont.Bd.prev. cont. Water poll. Osm Univ, Hyderabad. pp. 27-32.

**Chapherkar, S.B.,** 1982. Air pollution and plants. Indian Rev. Life. Sci. 2, pp. 41-46

**Chatrath**, A., R.Chandra, S. Khetrpal, and R. Polisetty, 1996. Changes in nitrate, amino acid and sugar content during growth and differentiation of chickpea explants (*Cicer arietinum*). Indian J. Plant Physiol. 41, pp. 80-83.

**Chaturvedi, R.** and Jyoti. Shah, 2007. Salicytic acid and plant disease resistance. spinger , pp.335-370

**Chaudhari, D.S.,** H.Tripathy, M.A.Veeresh, Powell and B.R. Hart, 2003. Mobility and bio availability of selected heavy metals in coal ash and Sewage Sludge amended acid, Environmental Geology 44:419-432.

**Chaudhary,S.K.,** M.Inouhe, U.N. Rai, K. Mishra, D.K. Gupta, 2011. Inoculation of *Rhizobium* (VR-1 and VA-1) induces an increasing growth and metal accumulation potential in *Vigna radiata* and *Vigna angularis* L. growing under flyash Ecological Engineering Volume 37, Issue 8, August 2011, pp. 1254–1257

**Cheng, C.Y.** and L. M. Chu, 2007. Phytotoxicity data safe guard the performance of recipient in leachate irrigation Chinese University, Hongkong.

**Cheung,K.K.C.,** J.P.K. Wong, Z.Q.Zhang, M.H.Wong,2000. Revegetation of lagoon ash using the legume species *Acacia auriculiformis and Leucaena leucocephala. Env. Pollut*. 109. pp. 75–82.

**Ciravolo, T. G.,** and D. C. Adriano,1979. Utilization of Coal fly ash by crops under green house condition.M. Wali (ed). *Ecology and Coal Resources Development Pergamon,* Oxford pp. 958-966.

**Dadhich, L. K.** and M.L. Kasat.1988. Studies on the effects of thermal power emissions 230 on plant community in and around Kota. Trends in pollution and toxicology, 30, pp. 229-230.

**Das Gupta, S.N.,** 1957. Air pollution in relation to plant diseases. Presidential Address. Section of Botany 45th Session Proc. Indian Sci.Cong. Assoc. Madras pp88-107.

**Das Mohpatra, P.,** S.M. Mustakin, and B.B. Kar, 2013, Studies on plants developed in flyash medium and their heavy metal absorption capacity IJIRSET. Vol. 2 issue 8, pp. 3657-3663,

**Deka, A.K.** and P. Azad, 2006. Isolation of Rhizabium Strains , cultural and Biochemical characteristics Legume Rev., 29(3), pp. 209-212.

**Deepti,S.,** Seema Mishra,2014. Exploitation and assessment of flyash as a carrier for biofertilizers. Presented in: National Conference on Fly Ash Utilization for Sustainable Environment Management. Organized by SIES IIEM, Nerul; pp. 74.

**Deshmukh A,** D. B. Maiti, and B. Bharti, 2000. Soil properties as influenced by flyash application, *J. Soil Crops,10*, pp 69-71.

**Ditmer E. E.,** 1930. The origin of cultivated Phoseolus. Burtt Applied Bot Genet Plant Breed 23, pp. 309-406.

**Druzina,V.D,** E.D, Miroshrachenko, O.D Chertov,1983. Effect if industrial pollution on nitrogen and ash contents in meadow phytocoenotic plants. Bot.Zh.68, pp.1583-1591.

**Dubey, P.S.,** L.Trivedi, and S.K. Shringi, 1982. Pollution studies of betul forest area to satpura Thermal Power Station aerial discharge. Final Report D.O. En. Project No. 19/27/78, Env. pp. 53.

**Dubey, R. C.** and D. K. Maheshwari, 2002. Practical Microbiology. S. Chand and Co. Ltd., New Delhi. pp.352

**Dubois,M.,**K.M.Griller,J.K.,Hamiton,J.K.Robers,P.A. and F.Smith. 1956. Method in microbiology.5 Academic Press, Londen.

**Dwivedi, S.,** R. D. Tripathi, U.N. Rai, S. Srivastava, M. K. Shukla, K. K. Gupta, K. K. Tiwari, and C. P. Dwivedi, 2005. *Abst. Third International Conf. On plants and Environmental Pollution.* pp108

**Eisenberg,S.H.,** M.E. Tittlebaun, H.C. Eaton, M.M. Soroczal,1986. Chemical characterstics of selected flyash feachales, J.*Envir. Sci. Health,21* pp. 383-402.

**Elliott, L.F.,** D. Tittemore, R.L. Papendick, V.L. Cochran, and D.F.Bezidicek, 1982. The effect of Mount St. Helen's ash on soil microbial respiration and numbers. J. Environ. Qual., 11, pp. 164-166.

**El-mogazi, D.,** D. J. Lisk, L.H. Weinstein, 1988. A review of physical, chemical and biological properties of fly ash and effects on agriculture ecosystem. *The science of the Total Environment* 74, pp. 1-37.

**Elseewi, A.A.,** F.T. Bingham, and A.L. Page, 1978. Growth and mineral composition of lettuce and Swiss chard grown in flyash amended soil. *Environmental chemistry and cycling processes*. Conferance, Springfield,VA .pp. 568-581.

**Elsheikh, E.A.E.** and M. Wood, 1989. Soil Biol. Biochem., 21, pp. 883-887.

**Faizan, S.** and A. A. Khan, 2004. Effect of Fly ash on the growth and productivity of wheat *(Triticum aestivum)*. *Indian Journal of applied and pure biology.* 19(2), pp. 153-158.

**Finkelman, R.B.**, H.E. Belkin, B.S. Zhang, and J.A. Centeno, 2000. Arsenic poisioning caused by residual coal combustion in proc 31st Congr, Guizhau Province, China.

**Frank, B.,** 1890. Uber die pilzsymbiose der leguminosen. Landw Jb 19, pp. 523-640.

**Fred,E.B.,** I. L. Baldwin, and E. I. L. McCoy,1932. Root nodule bacteria and leguminous plants. Studies Science University of Wisconsin 5 Madison.

**Furr, A.K.,** T.F. Parkinson, R.A. Hinrichs, D.R. Van Campen and C.A. Bache, 1977. National survey of elements and radioactivity in fly ashes.

Absorption of elements by cabbage grown in fly ash soil mixtures. Environ. Sci. Technol., 11, pp. 1194-1201.

**Furukawa, A.,** C. Isoda, H. Iwaki, and T. Totsuka, 1980. Interspecific differences in resistant to sulphur dioxide. Studies on the effects of air pollution on the plant, and mechanism of phytotoxicity. Res. Rep. Natal. Environ. Stud. Japan. 11, pp.113-126

**Gaind, S.** and A.C. Gaur, 2004. Evaluation of flyash as a carrier diazotraphs and Phasphobacteria, Bioresour , Technol, 95, pp. 187-190.

**Gangloff, W. J.,** M.Ghodrati, J.T. Sims, and B.L. Vasilas, 2000. Impact of fly ash amendment and incorporation method on hydraulic properties of a sandy soil. *Water Air Soil Pollut.* 119, pp. 231-245.

**Garampalli, R.H.,** S. Deene and C. Narayana. Reddy, 2005. Infectivity and efficacy of Glomus aggregatum and growth response of *Cajanus cajan (L.)* Millsp. in fly ash amended sterile soil. J. Environ. Biol., 26, pp 705-708.

**Garg, R. N.,** N. Kalra, R. C. Harit, and S. K. Sharma, 2003. Flyash incorporation effect on soil environment of texturally variant soils, *Asian J. Environ. Develop,10* pp. 59-63.

**Garg, R.,** 1991. Induced mutations in legumes with special reference to nitrogen metabolism. Ph.D. Thesis. University of Ajmer, Rajasthan, India.

**Gaur, Y. D.,** 1975. Studies on root nodule bacteria of *Cicer arietinum* L. (Chickpea). Ph. D., Thesis, University of Rajasthan, Jaipur, lndia.

**Gauri.**, A.K. Singh, R.P. Bhatt, S. Pant, M.K. Bedi, and A. Naglot, 2011. Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. Journal of Agriculture Technology7(6) :1705-1723.

**Ghodrati,M.,** J.T. Sims, and B.L.Vasilas,1995. Evaluation of flyash as a soil amendment for the Atlantic coastal plain. Soil hydraulic properties and elemental leaching. Water Air Soil pollut. 81,pp. 349-361.

**Gianfreada, L.** and J.M. Bollag, 1996. Influence of natural and anthropogenic factor on enzyme activity in soil, Soil Biochemistry, Marcel Dekker New York. 9, pp.123-193.

**Gochande, B.D.** and G.S. Khansole, 2011. Marphological, cultural and Biochemical charactraizion of *Rhizabium Japonicum* sys. and *Bradyrhizabium Japenicum* of soyabean, Bioscience Discovery Vol. 02 No-1 pp. 1-4.

**Gowda,CLL.,** R. P. S. Pundir, H.D. Upadhyaya, M. Seymour, Masood. Ali, M.M. Rehman, X. Zong, S. Khalil, A.M. Waqqani, S.H. Sabaghpour, A. Ungmaythan and J.K. Mligo, 2005. Impact of research and development in food legume on production and productivity in the last two decades, proceeding (IFLRC-iv), pp.18-22.

**Graham, P. H.** and C. A. Parker,1964. Diagnostic features in the characterization of the root nodule bacteria of legumes. Plant and Soil. 20, pp.283-395.

**Grewal K S.,** P. S. Yadav, S. C. Mehta, and M.C. Oswal, 2001. Direct and residual effect of fly ash application to soil on crop yield and soil properties, *Crop Res, 21* pp. 60-65.

**Guman,G.S.,** 1994. Water,Air & Soil poluution 72 ; pp.285-295

**Gupta, A.K.,** 2001. Flyash Utilization from Waste to Wealth- A case Study. CSIR Pool ship project- Annual Technical Report 2001, Award No. B- 7542/ 19-5-99 at BBAU, for council of Scientific and Industrial Research, Govt. of India, at BBA Central University, Lucknow. India.

**Gupta, A.K.,** S. Sinha, 2009. Growth and metal accumulation response of *Vigna radiate* L. var. PDM 54 (mung been) grown on flyash amended soil, effect on dietary intake, Vol. 31, issue 4.pp. 463-473.

**Gupta, D.K.,** U.N.Rai, R.D.Tripathi,M. Inouhe, 2002. Impact of fly ash on soil and plant responses. Journal of plant research. Springer, Japan. Volume-115 issue 6, pp.401-409.

**Gupta, M.C.,** M. Solanki, and M.P. Singh, 1988. Anatomical response of *Mangifera indica* (L). to coal smoke pollution. Acta. Ecol. 10, 1, pp.29-32.

**Gupta, G.S.,** G Prasad and V.H. Singh, 1990. Removal of chrome dye from aqueous solutions by mixed adsorbents; Flyash and coal. Water Res.,24 pp 45-50.

**Gupta,A.K.,** S.Dwivedi,S Sinha,R.D.Tripathi, U.N.Rai and SN.Singh 2007. Me tal accumulation and growth performance of *Phaseolus vulgaris* grown in flyash soil. Bioresour.Technol., 98;3404-3407.

**Haynes,R.J.,** 2009. Reclamation and revegetation of fly ash disposal sites. Challenges and research needs. Journal of environmental management, vol.90 (1), pp. 43-53.

**Hodgson,D.R**. and R. Holliday,1966. The agronomic properties of pulverized fuel ash, Chem.Ind, 20, pp.785-790.

**Jain.,** Usha, S. Bhansali, and Monalisa, 2009. Effect of certain heavy metal on seed germination & seedling growth on legume plant *Cyamopsis tetragonoloba.*

**Jala.**, Sudha and Dinesh. Goyal, 2006. Fly ash as a soil ameliorant for improving crop production–a review. *Bioresource Technology* 97(9), pp1136-1147.

**Kalara, N.,** H.C. Joshi, A. Chaudhary, S.Kumar, R. Chaudhary, K.C.Joshi, and S.K. Sharma, 1998. Flyash effects on germination of crop, *Effect of Fly Ash Incorporation on Soil Properties and Crop Productivity*. Division of Environmental Sciences, I.A.R.I., New Delhi, pp. 20-28.

**Kalra, N.,** M.C. Jain, H.C. Joshi, R. Choudhary, R.C. Harit, B.K.Vatsa, S.K. Sharma, and V. Kumar, 1998. Fly ash a soil conditioner and fertilizer. *Bioresour. Technol*. 64, pp.163–167.

**Kalra, N.H.,** C**.** Joshi, A. Chaudhary, R. Chaudary, S. K. Sharma, 1997. Impact of flyash incorporation in soil on germination of crops, *Bioresour Technol,61,* pp.39-41.

**Kalra.N.,** M.C.Jain, R.Choudhary, R.C.Harit, B.K.Vatsa, S.K.Sharma, and V.Kumar 2003. Soil properties and crop productivity as influenced by flyash in soil. Environment monitoring Assessment,87, pp. 93-109.

**Kasat, M.L.** and S.K. Agarwal,1981. Impect of air pollution on epidermal structure and epidermal features. Acta. Ecol. 3,1, pp. 24-26.

**Katiyar D.**, A.Singh, P.Malaviya, D. Pant, P.singh, G.Abrahum, S.K.Singh, 2013. Impact of flyash amended soil on growth and yield of crop plants. Int. J. Environment and Waste Management, Vol10, Nos2/3,pp.150-162.

**Katiyar. D.,** Anoop. Singh, Piyush. Malaviya, Deepak. Pant,Pratibha. Singh, Gerard. Abraham , S.K.Singh, 2012. Impact of flyash amended soil on growth and yield of crop plants. Int. J. environment and waste managedecades ment, vol.10 Nos . 2/3, pp.150-162.

**Kaur,S.,** B. Verma, V. Kant,1997. Plant obtained from the khair tree (*Acacia Catechu*) using nodal segment , spinger, 17; pp.427-429

**Kay.,** 1979. Food legume TPI crop and product digest pp.435.

**Khan, M.R.,** 2001.Use of flyash in the cultivation of ornamental plants for domastic purpose.Proceeding of IAEM, National conference on resent advances in waste management, Brazark information system Pvt Ltd.Varanasi, pp.85-89.

**Khan, M.R.,** M.W. Khan, and K. Singh, 1997. Management of root-knot disease of tomato by the application of fly ash in soil. Plant Pathol., 46, pp.33-43.

**Khan,M.R.** and M.Khan.1996. The effect of flyash on plant growth and yield of tomato. Environment pollution,92, pp.105-111.

**Khandelwal.M** and N.Shrivatava 2013. Status of plant diversity during last two at KTPS Campus IJES 17(2) pp 119-124.

**Khandelwal. M** and N.Shrivatava 2014. Comparative study on growth performance of some perennial terrestrial angiosperms, growing in non polluted and polluted area around the Kota thermal power plant .IJABR vol 4(2)168-171.

**Khandelwal.M** and N.Shrivatava 2013. Impact of pollutant emission of KTPS on growth performance of selected herbaceous flora of Kota District. Ad plant sci 26(ii) pp469-472.

**Kishor, P.,** A.K. Ghosh, and D. Kumar, 2010. Use of fly ash in Agriculture. A way to improve soil fertility and its productivity. *Asian Journal of Agricultural Research.* 4 (1), pp. 1-14.

**Ko,B.G.,** 2000. Effects of flyash and gypsum application on soil improvement and rice cultivation. Ph.D.Thesis.Gyeongsang National University,Chinju.

**Koziol,K.J.** and F.R. Whately 1984. Gaseous air pollutants and plant metabolism Butterwarths, London.

**Krichner**, and Buchanan,1926. Rhizobium joponicum syn. Brodyrhizobium joponicum.Int. J. Syst. Bocteriol. 3O, pp. 335 742.

**Krishna ,Rani.** and K. Sharma, 2010. Utilization in agricultural and related fields, a better alternative for eco-friendly maintenance of Coal FlyAsh, Journal of chemical and pharmaceutical reseach2 5, pp.365-372.

**Krishna ,Rani.** and K.Sharma, 2009. Application of coal flyash, cow dung and grass cutting mix in cultivation of Chilli, Enviromental pollution control journal (EPS),13(1)48-51.

**Krishnamoorthy, R.,** 2000. Ash utilization in India- Prospects and problems , In Barrier and utilization of flyash in India. Central Board of Irrigation and Power, New Delhi. Pp.15-27.

**Kumar, A.,** A.K. Sahare, R.P.Singh, V.N. Sharma, and A. Kumar, 1999. Effect of fly ash and fertilizer Levels on yield and trace metal uptake by soyabean and wheat crops. J. Ind.Soc.soil sci. 47(4) pp. 744-747.

**Kumar, K.V.,** Subhi. Srivastava, N.Singh and H.M. Behl, 2009. Role of metal resistant plant growth promoting bacteria in ameliorating flyash to the growth of *Brassica juncea*. *Journal of Hazardous Materials*. 170 (1), pp. 51-57.

**Kumar, V.,** 2008. Cultivation of legumes is being recommended and is a commonly adapted practice, Isolation of *Rhizobium* from pea plant in fly ash formulation. Indian Journal.com.

**Kumar, V.,** G. Singh, R. Rai, 2005. Flyash, A material for another green revolution, Field demonstration project by flyash mission (FAM)/(FAUP) Flyash utilization programme, TIFAC,Dst ,NewDelhi xii 2.1-2.3.

**Kumari, B.S.,** M. R. Ram, and K. V. Mallaiah, 2010. Studies on nodulation Biochemical analysis and Protein Profiles of Rhizobium isolated from Indigofera species. Malaysian journal of Microbiology, vol.6 (2), pp.133-139.

**Kumari,Vimlesh.,** 2009. Physicochemical properties of flyash from thermal power station and effect on vegetation. Global journal of environmental research 3(2), pp.102-105.

**Lal, J.K.,** B. Mishra, A.K. Sarkar, and S.Lal, 1996. Effect of flyash on growth and nutrition of soyabean, *journal of the Indian society of soil science*, 44, pp.310-313.

**Lazar,G.**, C.Capatina, and C.M.Simonescu, 2008. Evaluation of the heavy metals content in soil around a thermal station. Revista de Chimie.,59(8),pp.939-943.

**Lee, H.,** H.S. Ha, C.S. Lee, Y.B. Lee, and P.J. Kim, 2006. Fly ash effect on improving soil properties and rice productivity in Korean paddy soil. Bioresour. Technol., 97,pp. 1490-1497.

**Lindemann, W.C.,** 2008, Nitogen fixation by legumes, cooperative Extension Service College of Agriculture and Home Economics, New Mixico State University, Guide A-129.

**Lindsay, W.L.,** and W.A.Norvell, 1978. Development of DTPA soil test for zinc , Manganese, and copper , Soil, Sci. SOC AM J. 42 ,pp. 421-428.

**Lowry,O.H.,** N.J.Rosebrough, A.L.Farr, and R.J.Randall,1951. Protein measurement with the folin phenol reagent. J.Biol.Chem.,193.,pp.265-275.

**Machulla,G.,** S. Zikeli, M. Kaster, and R. Jahn, 2004. Microbial biomass and respiration in soils derived from lignite ashes, A profile study. J. Plant Nutr. Soil Sci., 167,pp. 449-456

**Mahana, V.K.,** 1981. Studies on causal organism, origin, development and biology of root nodules of tree legumes. Ph.D., Thesis, University of Rajasthan, Jaipur, India.

**Maheshwari Neha**.,2012. Influence of different pollutants on soil bactiria o kota district phD thesis, university of Kota.

**Mahna,S. K.,** Rekha. Garg and M. Parvateesam, 2000. Cultural and Biochemical Characteristics of root nodule bacteria from induced mutants of Vigno mungo L. Seed Pathology, Printwell publ, Jaipur. pp. 417-421.

**Maiti, S.S.,** M. Mukhopadhyay, S.K. Gupta, and S.K. Benerjee, 1990. Evalution of Fly ash as a useful material in agriculture, *J. Indian Soc .Soil. Sci.38* pp.342-344.

**Majernik, O.** and T.A. Mansfield, 1972. Stomatal response to raised atmospheric carbon dioxide concentration during exposure of plant to sulphur dioxide pollution Envirn. Pollut. 3,pp.1-7

**Malik, E.P.** and M.B.Singh, 1980.'Plant Enzymology and Hittoenzymology'(1st Edn). Kalyani Publications.NewDelhi,pp286.

**Mansfield, T.A.,** 1976. Effect of air pollutants on plants. Cambridge University press. New York.

**Matsi, T.** and V.Z. Keramidas, 1999. Flyash application on two acid soils and its effect on soil salinity, pH, B, P and on ryegrass growth and composition. Environ. Pollut., 104,pp. 107-112.

**Matte, D.B.** and D.R. Kene 1995. In; hoc. National seminar on use of lignite flyash in agriculture, Annamalai; pp44-48

**Mc Callisler, D.L.,** K.D. Frank, W.B. Stevens , G.W. Hergest, R.K. Renken , and D.B. Marx 2002. Coal flyash as an acid reducing soil amendment an its side effects, soil sci; 167 , pp. 811-820

**Mehra, A.,** M. E. Farag, and D.K. Baneerje, 1986. Impact of fly ash from coal fired power stations in Delhi, with particular reference to metal contaminations. *Environ. Monitor. Assess*. 50, pp.15-35.

**Mishra, L.C.** and K.N. Shukla, 1986. Effects of fly ash deposition on growth, metabolism and dry matter production of maize and Soyabean. Environ. Poll Res 42,pp.1-14

**Mishra, Seema.,** V.Ramachandhran, 2014. Fly Ash Utilization for Sustainable Environment Management. Waste to Resource Material, Published by, *SIES- Indian Institute of Environment Management, Nerul, Navi Mumbai.* ISBN- 13:978-81-929052-0-4, pp. 225.

**Moliner, A.M.** and J.J.Street, 1982.Effect of flyash and lime on growth and composition of corn (*Zea mays* L) on acid sandy soils. Crop Sci.Soc.Fla.Proc.41, pp.217-220.

**Muthusamy, S., P**. Vidhyasekaran and C.V.Govindsamy, 1973. Studies on Rhizobium strains of groundnut. Indian Journal of Microbiology 13,pp.143-148.

**Nagajyothi, P.C.,** N. Dinakar, S. Suresh, Y. Udaykiran, C. Suresh and T. Damodharam, 2009. Effect of industrial effluent on the morphological

parameters and chlorophyll content of green gram (*Phaseolus aureus* Roxb). *J. Environ. Biol.* 30,pp. 385-388.

**NageshD.,**RSwarne,P.Shrinivas,S.Sriram,S.RajanSundara,T.S.Nagamani ,2007. Biochemical studies in leaves of *Terminalia catappa*,proc 94 Indian Science Congress part II (Abstract) ,Annamalinagar, pp129

**Naik.** and Tyson, 2000. Environmental benefit from the use of coal consumption products, Proc. 12th ICFDU pp. 40-43

**Nason, A.** and W.D. McElory,1963. In plant physiology Ed.P.C. Stewart. Academic Press, New York.

**Neal, O. R.** and R.H.Walker,1935. Physiological studies on Rhizobium. Utilization of Carbonaceous materials. J. Bacterial 30,pp.173-187.

**Neelima, M.R.,** S. Khandual, A. Tripathy and R.K. Sahu, 1995. Proceedings of workshop on fly ash management in the State of Orissa, April 11. RRL, Bhubhaneshwar, India, pp.76-89.

**Newton, Z.,** Lupwayi, Ann. C. Kennedy and R. M. Chirwa, 2011, Grain Legume impacts on soil biological process in sub Saharan Africa, African Journal of plant science Vol.5(1) pp. 1-7.

**Nowakowa. J.** 1981. Influence of precipitation water on growing properties of NMWP ash sub soils, *Ocher Ter ,Gorniczych*, 51pp.48-52.

**Oblisami,G.,**1974. Studies on Rhizobium and nodulation pattern in a forage legume, *Clitoria ternate* isolated from paddy field. Indian National Science Academy Proceeding B 40,pp. 618-623.

**Oichtel, J.R.,** 1990. Micobial respiration in flyash / sewage sludge amended soil. Environ. Pollute. 63,pp.225-237.

**Olson.** and Hodges, 1987. Recommended dietary intakes (RDI) of vit. c in humans Ann J. Clin, Nutri 45,pp.693.

**Padmanabhan, S.,** *et al.,* 1990. Soil Biol. Biochem., 22,pp. 23-28.

**Page A.L.,** A. A. Elseewi and A. R. Straughan, 1979. Physical and chemical properties of flyash from coal fired power plants with reference to environmental impacts, *Residue.Rev 71*pp.83-120.

**Pandey, I.P.,** P.Sadhana, M.P. Tiwari and A. Seth, 2005. Abst. Third International conference on plant and environmental pollution.pp 101.

**Pandey, S.N.,** and D.N. Rao, 1979, Effects of coal smoke sulphur dioxide pollution on the accumulation of certain minerals and chlorophyll contents of wheat plants. Trop. Ecol. 19, pp.155-162

**Pandey, V.C.,** P.C. Abhilash, Nandita. Singh, 2009. The Indian prospective of utilizing flyash in phytoremediation, phytomanagement and biomass production. Journal of environment management vol. 90.

**Pandey,V.,** J.Mishra, S.N.Singh, N.Singh, M.Yunus, and K.J.Ahamad, 1994, Growth response of *Helianthusannus L* growth on flyash amended soil. *Ind. J. Env. Biol. 15,*pp.117-125*.*

**Pandey,V.C.,** Nandita.Singh,2010. Impact of flyash incorporation in soil systems, Agriculture, Ecosystem and Environment vol136,issue1-2, pp.16-27.

**Panwar, K.,** P.S.Dubey, and G.P.Verma,1998. Germination behavior of some important crop species in fly ash incorporated soils. *Advancement of crops and Monitoring of Environment – Progress in Ecology.* 10: 295-305.

**Pati, S.S.,** and S.K. Sahu, 2004. $CO_2$ evaluation and enzyme activities ( dehydrogegense, protease and amylase) of flyash amended soil in presence and absence of earthworms (Under laboratory condition). Geo derma , 118,pp.289-301

**Pell, M.,** B. Stenberg, and L.Torstenson, 1998. Ambio, 27, pp.24-28

**Phung, H.T.,** L.J. Lund, A.L. Page and G.R. Brandford,1979. Trace elements in fly ash and their release in water and treated soil *J Environ Qual,8* pp.175-177.

**Pichtel, J. R.** and J.M. Hayes, 1990. Influence of fly ash on soil microbial activity and populations. *J. Env. Pollut*. 19,pp. 593-597.

**Pohlman, G. G.,** 1931. Changes produced in nitrogenous compounds by Rhizobium metilotiond Rhizobium jo po nicu m. Soit Sci. 31, pp. 385-406.

**Power, W.L.,** 1930. The role of sulphur in plant nutrition. Jam.Soc.Agron. 22;pp. 371-374

**Prabakar, J.,** N. Dendorkar and R.K. Morchhale, 2004. Influence of flyash on strength behavior of typical soils. Constr. Build. Mater, 18;pp.263-267.

**Prasad, B.J.** and D.N. Rao, 1982. Relative sensitivity of leguminous and a cereal crop to sulphur dioxide pollution. Environ. Pollut. Ser. A. 29; pp.57-70.

**Prem Kishor.,** A.K. Ghosh and Dileep. Kumar, 2010. Use of Flyash in Agriculture: A Way to Improve Soil Fertility and its Productivity.*Asian, Journal of Agricultural Research,* pp.1- *14.*

**Pribac,C.,** and A. Ardelean,2008. In vitro culture of *Trigonella foenum-graecum* plantules and their anatomic characterization. Proceedings of the EMC 14th European Microscopy Congress, sept1-5,Springer,Berlin,Heidelberg,pp.181-182.

**Punjwani, J.,** K.Rani, K.Sharma, K.K.Gupta,2011. Application impact of coal flyash and water hyacianth on cultivation of tomato, Int. J. of res. in chem.and env. Vol-1 Issue 1 pp.71-76.

**Purohit S. S.,** and A.Kumar, 1998. Plant Physiology, Agro Botanical Publishers (lncual/Agro Botonica Bikone.289.

**Rabe, R.,** and K.H.Keeb, 1979. Enzyme activities and Chorophyll and protein contents in plants as indicators of air pollution, Environ. Pollut. 19; pp.119-137.

**Rai, U. N.,** K.Pandey, S.Sinha, A.Singh, R.Saxana, D.K Gupta 2004. Revegetating flyash landfills with *Prosopis juliflora* L; impact of different amendments and *Rhizobium* inoculation, Environment international vol 30,issue 3,pp.293-300.

**Rai, U. N.,** R.D. Tripathi and N. Singh, 2002. Integrated biotechnological approach for phytoremediation of fly ash dykes. Pp.35. *Abstract: Second International Conference on Plants and Environmental Pollution (ICPEP – 2)*. NBRI, Lucknow.

**Rai,U.N.,** *2003.* Performance of seed germination and growth of *Vicia faba* L in flyash amended soil. N.B.R.I. Lucknow.

**Rajkumar, G.R.,** 2000. Effect of flyash on growth and yield of crop, nutrient uptake and soil properties with special emphasis on toxic elements and radio nuclides, *ph.D thesis,* University of Agricultural Sciences, Dharwad, india.

**Ram, L.C.,** K. Sangeet, Ramesh Jha, C. Tripathi, 2008. Remediation of fly ash Land fills through plantation. Remediation: - journal of Environmental clean up costs Technology & Techniques.

**Rao,D.N.,**1989.Plants as pollution monitoring device fertilizer news 24; pp.25-28.

**Rautaray,S.K.,** B.C.Ghosh, B.N. Mitra, 2003.Effect of flyash, organic waste and chemical fertilizers on yield, nutrient uptake and heavy metal content and residual fertility in rice-mustard cropping sequence under acid lateritic soils.Bioresour.Technol.90,pp.275-283.

**Ravi, kumar.T.N.** N.A.Yeledhalli, M.V. Ravi and K. Narayan Rao,2008. Phhysical Physio-Chemical and Enzyme Activities of Vermiash Compost. *Karnataka J. Agric. Sci.* 21(2);pp. 222-226.

**Ray, P.,** and A. Adholeya, 2008. Correlation between organic acid exudation and metal uptake by ectomycorrhizal fungi grown on pond ashin vitro. BioMetals, 22;pp.275-281.

**Robinson,T.,** G. McMullan, R. Marchant and P. Nigam, 2001. Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. Bioresour. Technol., 77;pp. 247-255.

**Sahu K.C.,** 1998. Impact of residual soil on heavy metal leachate from ash pond at Korba-an experimental approach. Ind.J.Environ. Prot.19, pp.498-504.

**Sannino, F.** and L. Gianfreda, 2001. Chemosphere 45; pp. 417-425.

**Sarangi, P.K.,** D. Mahakur, and P.C. Mishra, 2001. Soil Biochemical activity and Growth response of Oryza sativa in flyash amended soil, Bioresour Technol., 76; pp.199-205.

**Sarkar, A.** and R. Rano, 2007. Water holding capacities of flyashes, Effect of size fractionation. Energy Sources, Part A: Recovery, Utilization Environ. Effects, 29;pp. 471-482.

**Saxana M** and R Chauhan, 1998.Flyash vermin compost an enricher to waste land soil Regional workshop cum symposium- Flyash Disposal and Utilization KTPS, Kota. pp.13-19.

**Saxana,M.** and P. Ashokan, 1998. Converting wasteland to agriculturally productive land- A coal ash management, Reginal workshop cum symposium- Flyash Disposal and Utilization KTPS, Kota. pp.45-51.

**Saxena, L.M.,** 1987. Analysis of de arranged physiology in plants induced by cement,dust pollution. PhD. thesis, Sukhadia University, Udaipur.

**Schutter, M.E.,** J.J. Fuhrmann, 2001. Soil microbial community responses to flyash amendment as revealed by analyses of whole Soil and bacterialisolates. Soil Biol. Biochem., 33;pp.1947-1958

**Scurfield, G.,** 1960. Air pollution and tree growth. For Abstr. 21;pp.339-347, 517-528.

**Selvakumari, G.,** M. Baskar, Jayanthi, K.K. Mathan, 2000. Effect of integration of flyash with fertilizers an organic manures on nutrient availability, yield an nutrient uplake of rice in Alfisols, J. Indian Soc. Soil Sci., Vol. 48 no-2 pp. 268-278.

**Shankar Ganesh, K.,** A.L.A. Chidambrum, P. Sundaramoorthi, L. Bhaskaran and M. Selvaraju, 2006. Influence of chromium and cadmium on germination, seedling growth and photosynthetic pigment of soybean *(Glycine Max)*. 12; pp.291- 296.

**Sharma S.K.,** N.Kalra and G.R.Singh, 2001. Impct of coal-burnt ash on environment and crop productivity. J. sci.Ind.Res.,60; pp.580-585.

**Sharma, B. M.,** R.K. Agrawal and P. Kumar,1990. Water retention and nutrient availability in a fly ash amended desert sandy soil. A study in vitro and soil Res. Rehab. 4; pp. 53-58.

**Sharma, K.**, Sudhir and Naveen Kalra, 2006. Effect of fly ash incorporation on soil properties and productivity of crops: A Review. *J. Sci. Ind. Res.* 65; pp. 383-390.

**Sharma, S.,**1986. Fly ash power plant pollutants. science today 20;pp.21-22

**Sharma, S.K.,** N. Kalra and G. R. Singh, 2002.Soil physical and chemical properties as influenced by flyash addition in soil and yield of wheat, *J. Scien Ind Res,61,*pp 617-620.

**Sharma,S.K.,**1998. Effect of fly ash incorporation on soil properties and crop productivity, PhD Thesis, CCSU, Meerut/IARI, New Delhi. pp.60.

**Shetty,B.V**. and V.Singh,1991. 'Flora of Rajasthan', Botanical survey of India. Kolkata.

**Shrabani Sen,.** and Vipin Kumar, 2012. The effect of different soil amendments on growth of mung been (vigna radiate L) in coal mine overburden dumps , IJETAE vol. 2 issue 12, pp. 264-267

**Shridharan,A.** and N.S.Pandian,1998. Flyash Characterization. Regional Workshop cum symposium on Fly ash Disposal and Utilization at K.T.P.S. Kota pp-25-38.

**Shrivastava, N.,** and S. Joshi, 2002. Effect of particulate pollutants from Automobile air pollution at Kota on the growth performances of some plants. Geobios.30.

**Shukla,M.K.,** R.D.Tripathi,U.N.Rao,S.Dwivedi,S.Chandra,S.Mishra,S.Rai and S.Dwivedi, 2003. Studies on bio utilization of flyash using leguminous crops; Abstract,National Symposium on Cyanobacteria and Plants Under Environmental Stress Responses, Defense Strategies and Biotechnological Prospects,BHU.,Varanasi,pp.49.

**Sikka, R.** and B. D. Kansai, 1994. Characterization of thermal power plant flyash for agronomic purposes and to identify pollution hazards, *Bioresour Technol,50* pp.269-273.

**Sims,J.T.,** B.L.Vasilas and M. Ghodrati, 1995. Development and evaluation of management strategies for the use of coal fly ash as a soil amendments. Proceedings of the 11th International Symposium of the American Coal Ash Association, (ISACAA'95), Orlando, Florida, pp. 8.1-8.18.

**Singh H,P.** and K.Kolay,2009. Analysis of coal for trace elements and their geoenvirnmental implications. Water Air nd Soil Pollution,198 (1-4);pp.87-94.

**Singh L.P.**, Z.A. Siddiqui and M.W.Khan, 2002.Effect of flyash amended soil on plant growth, yield and biochemical characteristics of Wheat.Sec.Inter.Conference on Plant and Envion. Pollu. pp.65-66.Feb Lucknow.

**Singh**, Anupama and S.B. Agrawal, 2010. Response of Mung bean cultivars to fly ash: growth and yield. *Ecotoxicology and Environmental safety*. 73 (8);1950-1955.

**Singh**, Anurag, R.K. Sharma, and S.B. Agrawal,2008. Effects of fly ash incorporation on heavy metal accumulation, growth and yield responses of *Beta vulgaris*. *Bioresourcse Technology*. 99(15): 7200-7207.

**Singh, D. K.,** P.K.Gupta, Nikita Parab, Seema Mishra, 2014. Response of onion (*Allium cepa L.*) grown in fly ash amended soil during kharif season. Presented in: National Conference on Fly Ash Utilization for Sustainable Environment Management. Organized by SIES IIEM, Nerul. pp. 73.

**Singh, L. P.** and Z.A. Siddiqui, 2003. Effects of fly ash and *Helminthosporium oryzae* on growth and yield of three cultivars of rice. *Bioresour. Technol.* 86;pp. 73–78.

**Singh, N.** and M. Yunus, 2000. Environmental impacts of fly ash. In: M. Iqbql, P. S. Srivastava, and T.O. Siddiqui, (Eds). Environmental Hazards-Plants and people. C. B. S. Publishers and distributors, New Delhi. pp.60-79

**Singh, N. B.** and M. Singh,1986. Effect of fly ash application on saline soil and on yield components, yield and uptake of N, P, K of rice and wheat at varying fertility levels. *Ann. Agri. Res*,pp. 245-257.

**Singh, Nandita.,** 2010. Impact of fly ash on crops the vicinity of Kahalgaom Bihar. STPT, NTPC

**Singh, NB.** and M. Singh, 1986. Effect of fly ash application on saline soil and on yield components, yield and uptake of NPK of rice and wheat at varying fertility levels, Ann,Agric,Res 7,14 pp.245-257.

**Singh, S. N.,** K.Kulshrestha, K.J.Ahmed, 1997. Impact of fly ash soil amendment on seed germination, seedling growth and metal composition of *Vicia faba* L. *Ecol. Eng*. 9: 203-208.

**Singh, S.K.** and D.N. Rao, 1985. Evaluation of plants for their tolerance to air pollution. Indian Association for Air Pollution Control, New Delhi Vol. 1;pp.218-224.

**Singh,K.,** M.W. Khan and M.R. Khan, 1994. Growth and Root knot disease of Soyabean under the stress of flyash, In- Emerging technology in Environmental conservation, Nation symposium, New Delhi

**Singh,N.,** S.N.Singh, M.Yunus and K.J. Ahamad, 1994. Growth response and elemental accumulation in *Beta vulgaris* L raised in flyash amended soils, Ecotoxicology 3;pp.287-298

**Singh,Yogita,** D.V.Rao and Amla Batra, 2011. Biochemical changes in Brassica juncea (L), International Journal of Pharmaceutical Sciences Review and Research Vol 7, Issue 1, pp74.

**Sirohi, A.** and D. Singh, 1989. Anatomical response on *Salvadora persica* L. to coal smoke pollution. Acta ecol. 11;2 pp.83-85.

**Somasegaran, P.** and H.J. Hoben, 1994. Handbook for Rhizobia. Springer- Verlag, New York, pp.1-438.

**Sornalakshmi, V.** and Venkataraman Kumar, 2014. Effect of fly ash on the growth and biochemicals of some Seaweed Bioscience Discovery, 5(1):01-05, Jan. 2014 ISSN: 2229-3469 (Print)

**Stevens, G.** and D. Dunn, 2004. Flyash as liming material for cotton, J. environ. Qual . 33: pp. 343-348

**Subba Rao, N.S.,** 1977. Soil Microbiology. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 166-228.

**Subba Rao, N.S.,** 1989. Soil micro organisms and plant growth. Oxford and IBH publishing House New Delhi, India.

**Suwalka, R.L.,** 2003.Utility of flyash in agriculture – A Review, Agric Rev., 24(3);pp 197-203

**T. SwamyNararayan.,** Nilakantha Dash, Gayatri Nahak, Bandita Deo, and R.K.Sahu, 2010.Effect of coal flyash on growth, Biochemistry, Cytology and Hevy metal content of ***Allium cepa L***, Newyork science journal 3(5) :pp.10-16.

**Tarkalson, D.,** C.A. Shapino, J.L. Petersen, 2010. Use of flyash as liming material of corn and Soyabean production on an acidic sandy soil, Online : Crop. Management doi 10 : 199/CM 2010- 436.

**Taylor ,E.M.** and G.E.Schuman,1988. Flyash and lime amendment of acidic coal soil to acid revegetation J. Environ. Qual., 17;pp.120-124.

**Thakre, R.** and A.L. Aggarwal, 1987. Plant community responses to thermal power station emissions ENVIS no; 06/005. Vol. 3;pp..21-31.

**Thakre, R.** and M.V.Rao, 1985. Physico- chemical analysis of soils amended with fly ash and their effect on germination of Wheat, Cotton, and Tomato .*Geobios.*pp.41-47.

**Thakre,R.** and A.L.Aggarwal, 1987. Plant community responses to thermal power station emissions ENVIS no; 06/005. Vol. 3;1,pp..21-31.

**Thakre,R.,**1983. Effect of thermal power plant emissions on near by vegetation .J.I.P.H.E; pp.9-20.

**Thomas, M.D.** and R.H. Hendricks,1956. Effect of air pollutants on plants. In P.L. Magil (Ed.), Air pollution Handbook : 1-44:new York McGraw-Hill.

**Tiwari,S.,**B.Kumari,S.N.Singh,2007.Evaluationof metal mobitity/immobility in flyash induced by bacterial strains isolated from the rhizospheric zone of *Typha* growing on flyash dumps.j Biortech.

**Tsadilas, C.D.,** V. Samaras, P. Kazari and J. Sgauras, 2002. Flyash and Sewage sludge application on an acid soil and their influence on same soil properties and wheat biomass production national conference 12 th ISCO conference N.A.R.F. pp. 118-123.

**Unnikrishnan V.,** M.Khaja, S. Reddy, K.S.Kumar, S.S.Rajan, F.M.Sharon, 2007. Biochemical studies in leaves of Araucaria species. In: Proc 94th Indian Science Congress Part II (Abstracts). Annamalainagar.pp. 149-50.

**Vajpayee P.,** U.N. Rai, R.D. Choudhary, R.D. Tripathi and S.N. Singh, 2000. Management of flyash landfills with *Cassia surattensis* Burn, a case study Bull. Environ. Contain. Toxical, 65:675-682.

**Vallini,G.,** F.Vaccari, A.Pera, M.Angolucci,S.Scatenaand, G.Varallo,1999. Evalution of composted coal flyash on dynamics of microbial populations and heavy metal uptake*. Compost Science and Utilization*,7;pp. 81-90.

**Vershney,C.K.,** K.K. Garg and S.R.K. Varshney, 1979. Effects of sulphur dioxide on Pea seedlings Indian J. Air. Pollut. Cont. 2;pp.47-49.

**Verma, S.** and K.C. Sharma, 2009. Air pollution tolerance index of some plant species of Ajmer, Rajasthan. Abstract 29 All india botanical conference Udaipur.

**Vijayakumar, N.** and P.Narayan Swamy, 1995. Use of flyash as dust insecticide, flyash in Agriculture Proc, Nation Seminar on use of lignite FA an agriculture pp. 31-33.

**Vijayalakshmi, D.C.,** Krishnakant and Singara, Charya., 2006. Influence of pollutant on soil bacteria, Ind. J. Env. Ecopla. 12 ;pp.361-366.

**Vijayavergiya, R.** and P.Vijay, 2006. Quantitative estimation of primary metabolites of Balanites aegyptiaca L. Abstract Vol. of XXIX. All India Botanical Conference, Udaipur; pp. 16.

**Vincent, J.M.,** 1970. A manual for the practical study of the root nodule bacteria, I.B.P. hand Book Blackwell scientific publications Oxford.

**Vitekari, H.N.,** Ashish, P. Talele, R.G. Mane, V.S. Gaik, J.V. Shah, 2012. Flyash based biopesticides . A Comprehensive Review, IJPBS, Vol-2 , Issue 1 , pp. 76.

**Weinstein,L.H.,** J,F.Osmeloski, M.Rutzke, A.O. Beers, J.B. Mc Cahan, C.A. Bache, D.J.Lisk, 1989. Elemental analysis of grasses and legumes growing on soil covering coal fly ash landfill sites J. Food Safety 9),pp 291-300,

**Wentzel, K.F.,** 1956. The part played by industrial and domestic smoke in damage to forest by flumes and in atmospheric pollution. Forstarchiv 27;pp.84-89

**Wong, M. H.** and J. W. C. Wong,1989. Germination and seedling growth of vegetable crops in fly ash amended soil. *Agr. Ecos. Env*. 26:pp. 23–35.

**Wong, M. H.** and W.C.Wong,1986. Effect of fly ash on soil microbial activity. *Env. Pollut. Ser A*, 40; pp.127-144.

**Woodwell, G.M.,**1970. Effect of pollution on the structure and physiology of ecosystem. Science, 168;pp 429-433.

**Yeledhalli,N.A.,** S.S.Prakash, S.B.Gurumurthy and M.V.Ravi, 2007. Coal flyash as modifier of Physico-chemical and Biological properties of soil karnataka j. Agric.Sci.,20(3) pp.531-534.

**Yuang,D.F**andChiou,L.Y.,2007.Microbioal population structures inactivated sludge before and after the application of synthetic polymer. Intle.j.Env.Sci.Tech.4(1).

CHAPTER – 10

# publications

# PUBLICATIONS

*Indian Journal of Environmental Sciences 17(2) 2013, pp.119-124*
*Green Earth Foundation*

## STATUS OF PLANT DIVERSITY DURING LAST TWO DECADES AT KTPS CAMPUS

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**ABSTRACT**

Emission from thermal power plant in Kota adversely affects plant species growing near the source. In polluted air changes in plant communities can be expected to occur both in species composition and in community productivity. Plants respond to atmospheric pollution in many ways. Plant communities react to the introduction of a pollution stress by selectively eliminating the susceptible species. The present study deals with the plant community responses to thermal power emission during last 20 years, A comparative data of vegetation found around KTPS has been collected through previous studies and by random survey during years 2011-12. Plant communities dominantly present there during 1988-1992 (phase I) were compared with plant communities found recently i.e. during 2011-2012 (phase II). Result show family wise distribution pattern of plants during two decades, changes were clearly observable, however during phase I, 27 families out of 42 were available, while during phase II, 29 families were observed, which is not markable difference in number of species present there but the actual changes were observed in species composition i.e. in phase II dominant families were different from that of phase I.

### INTRODUCTION

Air pollutants contaminate air, water, soil, and affect plants, wildlife as well as human health too. Pollutants known to affect the structure and functions of all natural ecosystems. The burning of fossil fuel i.e. coal release huge amounts of carbon dioxide content in the atmosphere and also produces large amounts of $SO_x$, $NO_x$ and particulates, all of which are hazardous to plant communities. The concentration of $SO_2$ in the vicinity of emission sources can be high enough to damage plants. It effects morphological features, physiological and biochemical mechanism of plants (Rao 1989). Coal dust forms colloidal gels which after crystallization and solidification develop in to a crust. Such crust makes plant surfaces compact and render them more or less impervious to water and gasses which upsets their physiochemical properties and alters their biological activity. Emission from thermal power plant adversely affects plant species growing near the source. In polluted air, changes in plant communities can be expected to occur both in species composition and in community productivity (Agarwal et al. 2004, Dadhich and Kasat 1988). There are certain plants which may be used as indicators to the air pollutants. Plants respond to atmospheric pollution in many ways. Plant communities react to the introduction of a pollution stress by selectively eliminating the susceptible species. While at lower dose plant community may not show any visible symptoms, still there can be substantial production losses (Dadhich 1981).

The present study deals with the plant community responses to thermal power emission during last 20 years at Kota city (Map 1, 2). A comparative data of vegetation found around Kota Thermal Power Station (KTPS) has been collected by previous studies and by random survey during years 2011-12. Plant communities dominantly present there during 1988-1992 (phase I) were compared with planf communities found recently i.e. during 2011-2012 (phase II). This comparative study shows the actual picture of effect of pollution emission from KTPS. Only terrestrial vegetation cover is included for study purpose. During phase I study, only four units of KTPS were working, with the installation capacity of 110, 110, 210, 210mw, but all six units 210, 195mw (along with previous four) were working in phase II study. During phase I the coal consumption capacity was lesser than phase II.

The major objective of the present study was to record change in plant community structure in response to pollution by thermal power plant.

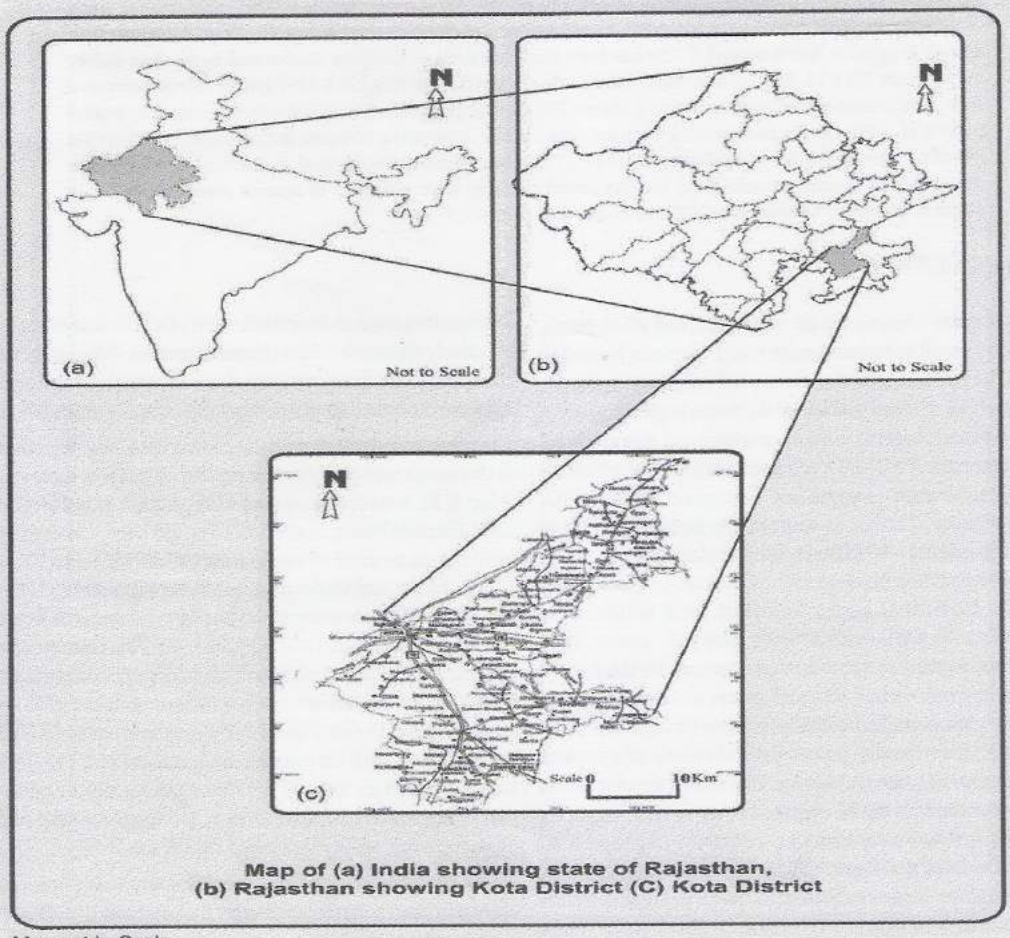
## STUDY AREA

The study area viz. Kota city located in the south-east at 25'10" N latitude and 75'52" longitude of Rajasthan state is on the bank of river Chambal. Kota thermal power station is Rajasthan's first major coal based power station operating since 1982. Presently it is in operation with installed capacity of 1045 mw. This plant was envisaged to create the State's own thermal generating capacity and to meet the growing power demand. Kota thermal power station is located on the left bank of river Chambal in principal industrial city, Kota.

## MATERIALS AND METHODS

Survey of selected sites has been done periodically; five different plots were established for the study of ecosystem at each site. The land selected was not previously in use for agriculture purposes and hence the impact of human activity was eliminated. The dominant plant species in the site were marked and recorded (Shetty and Singh 1991). Foliar damage was the morphological criterion for knowing about

MAP-1




Map of (a) India showing state of Rajasthan, (b) Rajasthan showing Kota District (C) Kota District

Map not to Scale

the impact of emissions on plants. Species richness referred to the number of species per plot ranging from herbs to tall trees. The area experienced the conditions of drought except for a wet spell of two or three months. Herbaceous annuals had luxuriant growth during the rainy period. Therefore ground layer vegetation was also included to determine species richness

## RESULTS AND DISCUSSION

During present study, herbs and shrubs were found to be more sensitive to pollution induced changes than the tree. The results presented can be compared to those of (Thakre 1983, Thakre and Agarwal 1987, Agarwal and Agarwal 1988, Dadhich and Kasat 1988).While in forested area trees are eliminated first by low dosages of pollutants. As duration of exposure increases, tall shrubs are eliminated followed in order by lower shrubs, herbs, mosses and lichens (Woodwell 1970). Higher sensitivity of the herbs can be explained as they are very small plants and exposed entirely to the environmental stress, and the plant surface is tender which absorbs pollutants without resistance. Furthermore, most of the herbs were annuals and during wet period they were in initial phase of their life cycle, and it is proved that plants are especially susceptible to environmental stress during the period of initial establishment (Thakre and Rao 1985).Tress around thermal power station were growing much before the power plant came in to existence. In phase I, 11 tree species were observed in which most dominant were *Cassia siamea, Cassia fistula, Azadirachta indica,* and *Albizzia*

Table 1. Flora of KTPS during phase II (2011-2012)

| Sr. No. | Name of Plants | Family |
|---|---|---|
| 1 | *Abutilion indicum* | *Malvaceae* |
| 2 | *Acacia nilotica* | *Mimosaceae* |
| 3 | *Acyranthes aspera* | *Amaranthaceae* |
| 4 | *Acalypha spp* | *Euphorbiaceae* |
| 5 | *Ageratum conyzoides* | *Compositae* |
| 6 | *Albizzia lebbek* | *Mimosaceae* |
| 7 | *Alianthus excelsa* | *Simaraubaceae* |
| 8 | *Alternanthera pungens* | *Amaranthaceae* |
| 9 | *Amaranthus spinosus* | *Amaranthaceae* |
| 10 | *Amaranthus hybridus* | *Amaranthaceae* |
| 11 | *Anogeissus acuminata* | *Combretaceae* |
| 12 | *Azadiracta indica* | *Meliaceae* |
| 13 | *Bauhinia varigata* | *Caesalpiniaceae* |
| 14 | *Callistemon citrinus* | *Myrtaceae* |
| 15 | *Cassia siamea* | *Caesalpiniaceae* |
| 16 | *Casuarina equisetiflolia* | *Casuarinaceae* |
| 17 | *Celosia argentea* | *Amaranthaceae* |
| 18 | *Chenopodium album* | *Chenopodiaceae* |
| 19 | *Coccinia cordifolia* | *Cucurbitaceae* |
| 20 | *Commelina attenuata* | *Commelinaceae* |
| 21 | *Convolvulus microphyllus* | *Convolvulaceae* |
| 22 | *Corchorus spp* | *Tiliaceae* |
| 23 | *Croton bonplandianum* | *Euphorbiaceae* |
| 24 | *Dalbergia siso* | *Fabaceae* |
| 25 | *Delonix regia* | *Caesalpiniaceae* |
| 26 | *Eclipta prostrata* | *Compositae* |
| 27 | *Eukalyptus rudis* | *Myrtaceae* |
| 28 | *Euphorbia hirta* | *Euphorbiaceae* |
| 29 | *Ficus benghalensis* | *Moraceae* |
| 30 | *Ficus religiosa* | *Moraceae* |
| 31 | *Gomphrena celosoides* | *Amaranthaceae* |
| 32 | *Holoptelea integrifolia* | *Ulmaceae* |
| 33 | *Ipomoea fistulosa* | *Convolvulaceae* |
| 34 | *Ipomoea spp* | *Convolvulaceae* |
| 35 | *Indigofera spp* | *Papilionaceae* |
| 36 | *Lantana camera* | *Verbenaceae* |
| 37 | *Lantana indica* | *Verbenaceae* |
| 38 | *Launea asplenifolia* | *Compositae* |
| 39 | *Mangifera indica* | *Anacardiaceae* |
| 40 | *Malvastrum coromandelianum* | *Malvaceae* |
| 41 | *Mitragyna parvifolia* | *Rubiaceae* |
| 42 | *Oxalis corymbosa* | *Oxalidaceae* |
| 43 | *Parkinsonia aculeata* | *Caesalpiniaceae* |
| 44 | *Peristophe paniculata* | *Acanthaceae* |
| 45 | *Phoenix sylvestris* | *Arecaceae* |
| 46 | *Phyllanthus fraternus* | *Euphorbiaceae* |
| 47 | *Polygonum barbatum* | *Polygonaceae* |
| 48 | *Rumax dentalis* | *Polygonaceae* |
| 49 | *Saraca indica* | *Caesalpiniaceae* |
| 50 | *Sida cordifolia* | *Malvaceae* |
| 51 | *Solanum xanthocarpum* | *Solanaceae* |
| 52 | *Sonchus asper* | *Compositae* |
| 53 | *Syzygiun qumini* | *Myrtaceae* |
| 54 | *Thevetia peruviana* | *Apocynaceae* |
| 55 | *Tridex procombens* | *Compositae* |
| 56 | *Tectona grandis* | *Verbenaceae* |
| 57 | *Taphrosia hamiltonii* | *Papilionaceae* |
| 58 | *Vernonia albicans* | *Compositae* |
| 59 | *Zizyphus spp* | *Rhamnaceae* |

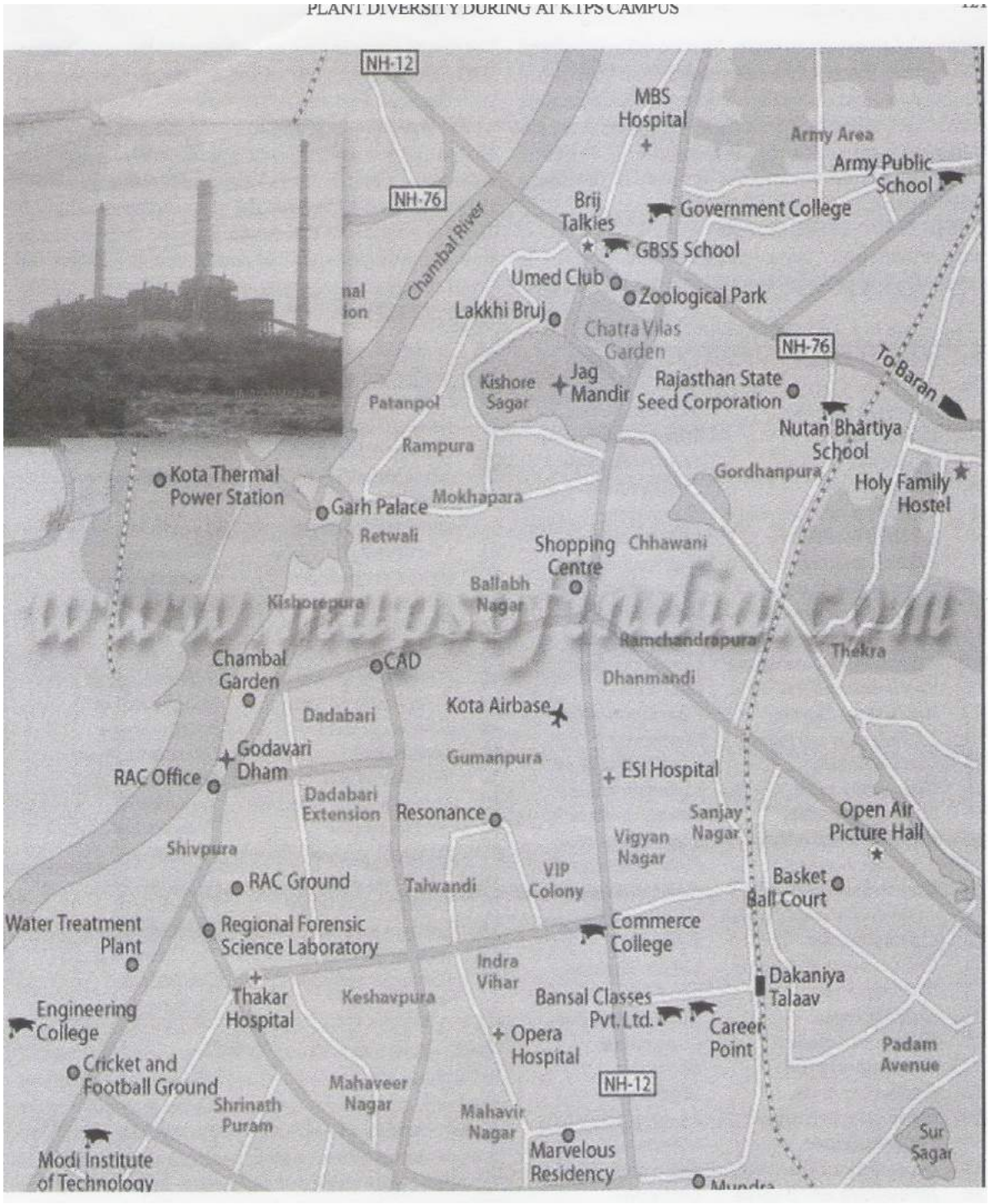

PLANT DIVERSITY DURING AT KTPS CAMPUS
NH-12
MBS Hospital
Army Area
Army Public School
NH-76
Brij Talkies
Government College
GBSS School
Chambal River
Umed Club
Zoological Park
Lakkhi Bruj
Chatra Vilas Garden
NH-76
To Baran
Kishore Sagar
Jag Mandir
Rajasthan State Seed Corporation
Patanpol
Nutan Bhartiya School
Rampura
Gordhanpura
Holy Family Hostel
Kota Thermal Power Station
Garh Palace
Mokhapara
Retwali
Shopping Centre
Chhawani
Ballabh Nagar
Kishorepura
Ramchandrapura
Thekra
Chambal Garden
CAD
Dhanmandi
Kota Airbase
Dadabari
Godavari Dham
Gumanpura
ESI Hospital
RAC Office
Dadabari Extension
Resonance
Sanjay Nagar
Open Air Picture Hall
Vigyan Nagar
Shivpura
RAC Ground
Talwandi
VIP Colony
Basket Ball Court
Water Treatment Plant
Regional Forensic Science Laboratory
Commerce College
Indra Vihar
Dakaniya Talaav
Thakar Hospital
Keshavpura
Bansal Classes Pvt. Ltd.
Engineering College
Opera Hospital
Career Point
Padam Avenue
Cricket and Football Ground
Mahaveer Nagar
NH-12
Shrinath Puram
Mahavir Nagar
Sur Sagar
Modi Institute of Technology
Marvelous Residency

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Table 2. Status of plant diversity at K.T.P.S. (1988-2011)

| Sr. No. | Familes | Phase -1 | Phase-2 |
|---|---|---|---|
| 1 | *Acanthaceae* | - | 1 |
| 2 | *Amaranthaceae* | 2 | 7 |
| 3 | *Annonaceae* | 1 | - |
| 4 | *Anacardiaceae* | 2 | 1 |
| 5 | *Arecaceae* | - | 1 |
| 6 | *Apocynaceae* | - | 2 |
| 7 | *Ascelpideaceae* | 1 | 1 |
| 8 | *Boraginaceae* | 1 | - |
| 9 | *Capparadaceae* | 1 | - |
| 10 | *Caryophyllaceae* | 2 | - |
| 11 | *Casurinaceae* | - | 1 |
| 12 | *Chenopodiaceae* | - | 1 |
| 13 | *Cleamaceae* | 1 | - |
| 14 | *Compositeae* | 7 | 6 |
| 15 | *Commelinaceae* | 1 | 1 |
| 16 | *Combretaceae* | - | 1 |
| 17 | *Convolvulaceae* | 4 | 2 |
| 18 | *Cucurbitaceae* | - | 1 |
| 19 | *Cyperaceae* | 1 | - |
| 20 | *Fabaceae* | 6 | 4 |
| 21 | *Euphorbiaceae* | 4 | 5 |
| 22 | *Liliaceae* | 1 | - |
| 23 | *Malvaceae* | 2 | 2 |
| 24 | *Mimosaceae* | 4 | 2 |
| 25 | *Meliaceae* | 1 | 1 |
| 26 | *Moraceae* | - | 2 |
| 27 | *Myrataceae* | 1 | 1 |
| 28 | *Nyctaginaceae* | 1 | - |
| 29 | *Nyctanthaceae* | 1 | - |
| 30 | *Oxalidaceae* | - | 1 |
| 31 | *Papaveraceae* | 1 | - |
| 32 | *Polygonaceae* | - | 2 |
| 33 | *Poaceae* | 5 | - |
| 34 | *Rhamnaceae* | 1 | 1 |
| 35 | *Rubiaceae* | - | 1 |
| 36 | *Scrophilariaceae* | 1 | - |
| 37 | *Solanaceae* | 1 | 1 |
| 38 | *Simaraubaceae* | - | 1 |
| 39 | *Tiliaceae* | - | 1 |
| 40 | *Verbenaceae* | - | 3 |
| 41 | *Ulmaceae* | - | 1 |
| 42 | *Zygophylaceae* | 1 | - |
| | *Total* | 27 | 29 |

*lebbeck* while *Mangifera indica* and *Azadirachta indica* show more resistance to emissions as compared to other tree species (Dadhich and Kasat 1988). In phase II, 21 tree species were observed which included approximately all tree species observed in phase I, with the exception of *Lannea coromandelich* and *Poinciana regia*. Amongst 21 tree species, *Cassia siamea, Cassia fistula, Azadirachta indica, Delonix regia, Bauhinia variegata* and *Albizzia lebbeck* were found near the campus while *Anoegissus spp., Acacia nilotica* and *Zizyphus spp.* were dominant far away from main plant and near to fly-ash dumping area (Table1). Thus compostion of tree species did not change much due to emission during last two decades. Shrub and herbs were also compared. In phase I, 13 shrub and 32 herb species were observed (Dadhich and Kasat 1988) while in phase II, 38 species of shrubs and herbs were observed . 7 shrubs present dominantly were *Ipomoea fistulosa, Lantana coromandelian, Thevetia peruviana, Ziziphus nummularia, Solanum xanthocarpum* and *Argemone maxicana*. Herbs were less in number and diversity in comparison to phase I. The dominant herbs in phase II were *Amaranthus spinosus, Acyranthes aspera, Chenopodium album, Euphorbia hirta, Phyllanthus fraternus, Croton banplandianum, Alternanthera pungens and Tridex procombens.*

Table (2) shows family wise distribution pattern of plants during two decades, changes were clearly observable during phase I, 27 families out of 42 were available, while during phase II, 29 families were observed, which is not the remarkable difference, but in phase I, dominant families were Compositeae, Fabaceae and Poaceae. In place of these in phase II, Amaranthaceae, Caesalpiniaceae, Compositeae and Euphorbiaceae were dominantly present in the study area. Tree and shrub species act as pollution sink due to their higher ecological amplitude of tolerance, although the sensitive plants show morphological, anatomical and biochemical changes and act as bioindicator of pollution levels. The herbaceous vegetation of an area depict the actual picture of pollution level caused by thermal power plant because only few tolerant species can survive there and the actually sensitive species disappear in that particular area by the damaging effect of various types of pollutions.

## CONCLUSION

Herbal species were the most susceptible while already established woody species could cope-up with the pollution,

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though under stress, they did not show any permanent visible injury. Plant species common to Phase I and II were *Cassia siamea, Cassia fistula, Azadirachta indica, Delonix regia, Bauhinia variegate, Albizzia lebbeck, Ipomoea fistulosa, Thevetia peruviana, Zizyphus nummularia, Solanum xanthocarpum Chenopodium album, Euphorbia hirta, Phyllanthus fraternus, Croton banplandianum Alternanthera pungens* and *Tridex procombens* and these may serve as a natural sink for pollutants of KTPS.

**REFERENCES**


Agarwal,M. and S.B Agarwal.1988. Phytomonitoring of air pollution around a thermal power plant. Proceedings Internation Conference on Tropical Micro-Meteorology and Air Pollution pp. 215-218.

Agrawal, S.B., A. Singh and M.M. Bhatt. 2004. Impact of fly ash amendment on seed germination, growth and yield of *Vigna mungo* L. Asian Journal Microbiological, Biotechnology and Environment Science 6(3):421-426.

Dadhich, L.K .1981. Recognition and monitoring of air pollutants with the aid of plants. WHO workshop on biological indicators and indices of environmental pollution. Osmania University, Hyderabad, pp. 27-32.

Dadhich,L.K and M.L.Kasat.1988. Studies on the effects of thermal power emissions on plant community in and around Kota. Trends in Pollution and Toxicology, pp.30.

Rao, D.N 1989.Plants as pollution monitoring device. Fertilizer News 24:25-28.

Shetty,B.V and V. Singh. 1991. Flora of Rajasthan. Botanical Survey of India, Kolkata.

Thakre, R.1983. Effect of thermal power plant emissions on nearby vegetation. J.I.P.H.E 9-20.

Thakre, R and M.V.Rao.1985. Physico- chemical analysis of soils amended with fly ash and their effect on germination of Wheat, Cotton, and Tomato . *Geobios.41-47*

Thakre, R and A.L.Aggarwal. 1987. Plant community responses to thermal power station emissions. ENVIS no; 06/005. Vol. 3; 1. 21-31.

Woodwell, G.M. 1970. Effect of pollution on the structure and physiology of ecosystem. Science 168: 429-433.

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# IMPACT OF FLY ASH ON MORPHOLOGICAL, CULTURALAND BIOCHEMICAL CHARACTERISTICS OF *RHIZOBIUM MELILOTI* OF *MEDICAGO SATIVA*

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## ABSTRACT

The present investigation is an attempt to study the impact of fly ash on *Rhizobium* bacteria of root nodules isolated from legume plants and to study morphological, cultural, and biochemical characteristics of bacterial strain obtained from selected legume i.e.*Medicago sativa. Rhizobia* inhabited in root nodules of *Medicago* plant, grown in fly ash amended soil (60% soil + 40% FA), *Rhizobia* was isolated and inoculated on Yeast Extract Mannitol Agar (YEMA) medium and its morphological, cultural and biochemical characteristics were studied. It was observed that colonies were circular or irregular; light creamish, glistering, gelatinous, convex with entire margins. The bacteria was gram negative, rod shaped, aerobic, non spore forming and slow moving bacteria arranged single, in pairs and in clusters. It showed negative chemical reaction for indole, methyl red, Voges-Proskauer and hydrogen sulphide, while showed positive reaction for citrate, catalase, urease and nitrate reduction. By the help of biochemical characteristics it was confirmed that isolated bacterial culture was of *Rhizobium meliloti* and fly ash in the concentration of 40% does not have any negative effect on the characters of *Rhizobium*, our findings was supported by many earlier investigations.

## INTRODUCTION

Legumes are unique plants which have the ability to work with certain bacteria i.e. *Rhizobia,* (inhabit root nodules) to gather available nitrogen from the soil atmosphere and convert it into usable ammonia and make it available to the plant. Biological nitrogen fixation is a component of sustainable agriculture and Rhizobial inoculants have been applied frequently as bio-fertilizers. Each major legume group is nodulated by different species of *Rhizobium. Medicago sativa* - a common legume plant of Hadoti region is selected for study purpose. *Medicago sativa* also known as Alfalfa, Lucerne, Sativa ( Family- Fabaceae) is an erect, ascending, smooth herbaceous legume that is much-branched glabrous perennial, 30-90 cm, with alternate trifoliate leaves. It is deep-rooted, (2-4m or more) in well-drained soils. (Ecocrop. FAO.Org). It is one of the highest yielding forage legumes and requires deep, well-drained fertile soils to maximize its potential. The inherent growth characteristics and good yield response to infrequent cutting make lucerne a highly suitable species for conservation as hay or silage. It is mainly grown as a fodder crop. It is grow as a cover crop to reduce erosion. A very unique character It is a nitrogen fixer and estimates of annual rates range from 85 to 360 kg N/ha with a wide variation among sites. Similarly its fibres can be used in the manufacture of paper. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. A Deep-rooting ability is an important factor in drought tolerance and any adverse soil physical or chemical conditions, which restrict root growth, will reduce drought tolerance. Compared with many forage species, Lucerne is an efficient user of water supply largely as a result of its deep taproot system. During severe drought, plants become dormant but resume growth when moisture becomes available. So it is used as forage directly and as residues, this is used as manure and fuel. These properties make it a useful tool for agro forestry.

*Medicago sativa* is nodulated *by Rhizobium meliloti* (Subba Rao1977). Fred et al. (1932) recognized eight cross inoculants group in legumes. The genus *Rhizobium* was erected by Frank (1890) based on its characters to form nodules on roots of legume plants. This property is the only valid test in the identification of the organism. Apart from it some diagnostic features of *Rhizobium* could be conveniently not only determine and identify the organism but also delineate

different species (Graham and Parker 1964, Vincent 1970, Gaur 1975, Mahana 1981). Review of literature indicates that during last decade there is decrease in soil quality of Kota region. It is also observed that due to lower content of various ions, production of legumes is also decrease. Due to decrease in legume production fertility of soil is badly affected. It was observed that fly ash which is generated from Thermal Power Station situated in Kota District, affect soil quality of study area.

The impact of coal residues on environment and health consequences has been reviewed extensively, conventional disposal methods for Fly ash lead to degradation of arable land and contamination of ground water (Jala and Goyal 2006). Now we move towards the utilization of fly ash in soil amendment and agronomy for wealth generation as well as pollution control because various previous researches and chemical analysis support that fly ash is a potential source of many macro and micro nutrients (Aggarwal and Gupta 1993, Singh et al. 1997). Whitish grey colour fly ash is mostly alkaline (pH 7.5-8.2) and hydrophilic in nature so that fly ash is a useful ameliorant that may improve the physico-chemical and biological properties of soil (Shridharan and Pandian 1998, Haynes 2009). Although it contain almost all the plant nutrients but deficient in Organic Carbon i.e. N and P (Rai et al. 2002). An integrated biotechnological approach to revegetation seems appropriate and should be investigated further. This problem may overcome by addition of organic manure and microbial inoculants in the fly ash and use of inoculated legumes to add N. Fly ash has impact on soil quality of study area. Soil quality may affect microbial population. In the light of above facts present research is undertaken to know the impact of fly ash on cultural, morphological and biochemical characteristics of *Rhizobium meliloti* of *Medicago sativa*.

## MATERIALS AND METHODS

Isolation and Purification: Isolation and Purification of *Rhizobium* strain was done as described by Graham and Parker (1964). Healthy and mature pink colored nodules of selected Plant grown over fly ash amended soil (40% Fly ash + 60% soil) were collected and were washed thoroughly under tap water and surface sterilized with 0.1 % mercuric chloride and then 95% ethanol and crushed aseptically in sterile water blank. This nodule suspension was then serial diluted (10-5 to 10-7) streaked on the sterilized yeast extract mannitol agar (YEMA) medium plates containing Congo red and incubated at 26 to 30ÚC temperature for 5-7 days. After incubation for 4-6 day transparent to white single colonies were transferred to YEMA slants described by Graham and Parker (1964).

**Characterization of isolates:**

The cultural and morphological as well as bio-chemical characteristics of the isolates were studied following the procedure given by Aneja (2008).

**Cultural characteristics:**

The shape, colour, opacity, margin and elevation of the colonies of the test isolates grown on standard YEMA plates were observed.

**Morphological characteristics:**

The shape, oxygen demand, motility, spore formation and Gram stain reaction of *Rhizobial* cells were observed under microscope using standard procedure.

**Biochemical characteristics**

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja (2008).

To analyse the impact of fly ash on Biochemical characteristics of *the Rhizobium meliloti,* of *Medicago sativa,* it was treated with 40% concentration of fly ash collected from Thermal Power Plant area.

## RESULTS AND DISCUSSION

The experimental results depict that *Medicago sativa* demonstrate that no marked difference in response to 40% concentration of fly ash under pot conditions. Table-1 elaborate that colonies were circular or irregular; white creamish, gelatinous, opaque, convex with entire margins. The bacterium was gram negative, rod shaped, aerobic, non spore forming and motile. From Table-1 it was clearly observed that Indole was not produced after incubation of isolated Rhizobial inoculants in tryptophan broth. Similarly Methyl red and Voges-proskauer reaction were examined in glucose phosphate broth by adding methyl red and α-napthol solution with KOH respectively. Citrate was utilized as a sole carbon source in Simon's citrate medium. Ammonia was produced by degradation of urea available in to the urea broth containing phenol red as an indicator by the bacterium inoculated. Catalase activity was observed by testing the

Table1. Cultural, Morphological and Biochemical Character of *Rhizobium meliloti*

| Sr. No. | Characters | Result |
|---|---|---|
| 1. | Shape | Circular |
| 2. | Colour | White creamish |
| 3. | Opacity | Opaque |
| 4. | Margin | Regular/entire |
| 5. | Elevation | Convex |
| 6. | Shape | Rod shaped |
| 7. | Oxygen demand | Aerobic |
| 8. | Motility | Motile |
| 9. | Spore formation | Non spore forming |
| 10. | Gram's nature | Gram Negative |
| 11. | Production of Indole from tryptophan | Negative |
| 12. | Methyl red test | Negative |
| 13. | Voges-Proskauer test | Negative |
| 14. | Citrate utilization as source of carbon | Positive |
| 15. | Production of ammonia from urea | Positive |
| 16. | Catalase test | Positive |
| 17. | Nitrate Reduction | Positive |
| 18. | Production of Hydrogen peroxide | Negative |

culture in a drop of hydrogen peroxide (10% by W/V). Nitrate was converted to nitrite by inoculants of Rhizobial strain. Production of hydrogen sulphide gas examined by SIM Agar method. It showed negative chemical reaction for indole, methyl red, voges-proskauer and hydrogen sulphide, while showed positive reaction for citrate, catalase, urease and nitrate reduction.

Morphological, cultural and biochemical characteristics of different *Rhizobial* strains have been studied by investigators like Allen and Allen (1981), Bisset (1959), Muthuswamy et al. (1973), Mahana (1981), Garg et al. (1991) and Oblisami 1974). Staining reactions of *Rhizobial* strains showed that *Rhizobium* is gram negative (Allen and Allen 1950, Graham and Parker 1964, Mahana 1981, Garg 1991). Our findings also supported these results but report of Bisset (1959) showed that isolates of *Rhizobium* from tropical legume were gram positive. Similar to the work of various workers Graham and Parker 1964, Basak and Goyal 1980 and Garg 991) we observed that the colonies of *Rhizobium* were circular, white glistening and attained normal growth within even days growth when grown on YEMA medium. The morphological, cultural and biochemical characteristics of the purified *Rhizobial* strain which were purified after treatment with 40% of fly ash, indicate that there was no significant difference in *Rhizobial* strain collected from control. But it may possible that higher concentration of fly ash may alter the cultural, morphological and biochemical characters of *Rhizobium meliloti*. So that further study is required in this direction.

## REFERENCES


Aggarwal, S. K. and R.Gupta.1993. Impact of fly ash amended soils on pigment characteristics of seedlings of two varieties of Glycine max. Acta Ecology 15(2):73-78.

Allen, E. K. and O.N Allen.1950. Biochemical and symbiotic properties of the *Rhizobia*. Bacterial Review 14:273-330.

Allen, O. N. and E. K Allen. 1981. The Leguminosae. A Source Book of Characteristics, Uses and Nodulation. The University of Wisconsin Press, Madison, Wisc. U.S.A. pp. 341-351

Aneja, K.R. 2008. Experiments in Microbiology, Plant Pathology and Biotechnology Fourth Edition. New Age International Publishers Limited.

Basak, M. K. and S.K.Goyal.1980. Studies on tree legumes-*Rhizobium* symbiosis: Nodulation pattern and cross inoculation trials with tree legumes and cultivated legumes. Annals of arid Zone19: 427-431.

Bisset, K.A.1959. Some characters of *Rhizobium* strains from tropical legumes. Journal of Microbiology 20:89-90.

Frank, B. 1890. Uber die Pilzsymbiose der leguminosen. Landw Jb 19:523-640. Chilton, Frank B., Papers (1906-1911, updated Dolph Briscoe Center for American History, The University of Texas at Austin.

Fred, E.B., I.L.Baldwin and E. McCoy. 1932. Root nodule bacteria and leguminous plants. pp. 32-48. Studies Science University of Wisconsin 5 Madison. USA.

Garg, R. 1991. Induced Mutations in Legumes with Special Reference to Nitrogen Metabolism. Ph.D. Thesis. University of Ajmer, Rajasthan, India.

Gaur, Y.D. 1975. Studies on root nodule bacteria of *Cicer arietinum* L (Chickpea). Ph. D. Thesis, University of Rajasthan, Jaipur, India.

Graham, P.H. and C. A. Parker. 1964. Diagnostic features in the characterization of the root nodule bacteria of legumes. Plant and Soil 20:383-395.

Haynes, R. J. 2009. Reclamation and revegetation of fly ash disposal sites, challenges and research needs. Journal of Environmental Management 90(1): 43-53.

Jala, S. and D. Goyal. 2006. Fly ash as a soil ameliorant for improving crop production–a review. Bio resource Technology 97(9):1136-1147.

Mahana, V. K. 1981. Studies on causal organism, origin, development and biology of root nodules of tree legumes.Ph.D.Thesis,University of Rajasthan, Jaipur, India.

Muthusamy,S., P.Vidhyasekaran and C.V. Govindsamy. 1973. Studies on *Rhizobium* strains of groundnut. Indian Journal of Microbiology 13: 143-148.

Oblisami, G. 1974. Studies on Rhizobium and nodulation pattern in a forage legume, *Clitoria ternate* isolated from paddy field. Indian National Science Academy Proceeding B 40: 618-623.

Rai, U.N., R.D Tripathi, and N. Singh. 2002. Integrated biotechnological approach for phytoremediation of fly ash dykes. pp.35. Abstract: Second International Conference on Plants and Environmental Pollution (ICPEP – 2). NBRI, Lucknow.

Shridharan, A. and N.S.Pandian.1998.Fly ash Characterization. Regional Workshop cum Symposium on Fly ash Disposal and Utilization at K.T.P.S., Kota (15-16 Sept.).

Singh, S.N., K. Kulshreshtha and K.J.Ahmad. 1997. Impact of fly ash soil amendment on seed germination, seedling growth and metal composition of *Vicia faba* L. Ecological Engineering 9: 203-208.

Subba Rao, N.S.1977. Soil Microbiology. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 166-228.

Vincent, J. M. 1970. A Manual for the Practical Study of Root Nodule Bacteria. IBP Handbook and Blackwell Scientific Publications, Oxford.

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# Impact of Pollutant Emission of KTPS on Growth Performance of Selected Herbaceous Flora of Kota District

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The present study was aimed to identified the herbaceous vegetation at Kota Thermal Power Station *i.e.*, KTPS campus and to compare some eco-morphological characters of selected terrestrial perennial herbs growing in the vicinity of KTPS, Kota with non-polluted site. The selected site for the study purpose referred as site-1 polluted area which is 1-2 km in the vicinity of KTPS, Kota ; site-2 non-polluted area (JDB College) which is 3 - 4 cm away from KTPS Kota respectively. Six most dominate herbs species were selected for comparative studies *as Croton banpladianum, Chenopodium alba, Euphoriba hirta, Acyranthes aspera, Amaranthus hybridus* and *Tridex procombens*. Different parameters were selected for the eco-morphological studies for herbs as percentage foliar damage, aggregation and association of species, fresh weight, length of plant, number of leaves, size of inflorescence, size of leaves etc. The result indicates that site-1 represents as disturbed area, because of deposition of fly ash, and pollutants directly emitted through chimneys of KTPS. Very less *i.e.* only 35 herbaceous plant types found near the pollution emission source, and biodiversity confined up to some common plants present in some specific patches, some plants show slight degradation of size, fresh weight, and increased foliar damage, as *Croton banplandianum* and *Euphorbia hirta*, while others showed resistance to comulative effect of fly ash deposition and other air pollutants, as *Chenopodium alba, Tridex procombens, Acyranthus aspera*. These results indicate that reduced quantity and biodiversity is observed at polluted site as compared to non-polluted. Because only tolerant species can survice there and the actually sensitive species disappear in that particular area due to KTPS.

**Keywords :** Fly ash, *Chenopodium, Euphorbia, Croton, Tridex, Acyranthes.*

## INTRODUCTION

Kota is famous for its coal based thermal power plant. The fly ash is a by product from thermal power station causing pollution to the local area of power plant as well as fly ash dumping station and transporting tracts, on the other hand air pollutants directly emitted through chimneys of thermal power plant due to the combustion of coal also spread in the local environment. The emission from thermal power plant adversely affects the plant species growing near the source (Gordon and Gorham, 1963). Thirdly the road transport vehicles like trucks, tankers, trailers involve in transportation of fly ash to the industries as cement industry, also contribute in enhancement of pollution level through automobile emission by increasing level of particulate matter and gaseous pollution in the environment. These pollutants affect the local vegetation directly or indirectly. As the result of cumulative effect of all these factors the biodiversity of that particular area is comparatively less and confined to limited species. In polluted air, changes in plant community can be expected to occur both in species composition and in community productivity (Shrivastava and Joshi, 2003). Plant communities act as pollution sink due to their higher ecological amplitude of tolerance, although, the sensitive plants show morphological, anatomical and biochemical changes and act as bioindicator of pollution levels (Chapekar, 1981). Plant communities to the introduction of pollutant stress by selectively eliminating the susceptible species (Dadhich and Kasat, 1988). The herbaceous vegetation of an area depict the actual picture of pollution level caused by thermal power plant, because herbs differ considerably in their responses towards pollutants, some are highly sensitive and show immediate symptoms while others are hardy and tolerant, they can withstand the stress of pollution aptly well. Only few tolerent species can survive there and the actually sensitive species are disappearing in that particular area by the damaging effect of various types of pollutions. While at lower dose plant community may not show any symptoms, still there can be substantial production losses. A few observations have been made to study the responses of a predominantly deciduous plant community as a whole to various air pollutants in tropical conditions. (Dadhich, 1981; 1982).

The present study is aimed to find out the resistant species in polluted area, tolerant species may be used as bio-monitor for monitoring the quantitative and qualitative level of pollutants. For this purpose few abundantly found herbaceous plants are selected for eco-morphological studies. Selection of plants was done by keeping in mind that decided criteria can easily be applied to all plants, like length of plant, inflorescence, size of leaves, petiole etc. Criteria which were decided for the study purpose are logically feasible to perform on the spot or carry out in the laboratory very easily, The results give the approximate picture of the effect of various pollutants on selected herb plants and suggested that some plants are highly resistant to the pollution load when they are compared with the same plant species growing at other locality that is considered as non polluted site, on the other hand some plants show damaging effect in general observation like scatted growth, less aggregation and association, confined to limited patches, under shrub position, higher leaf damage area, necrosis, chlorosis, stunted growth, reduced leaf area etc. Most of the plants listed below are of great medicinal importance.

## MATERIAL AND METHODS

**Area of study :** For the present investigation material was collected from two sites, polluted site which is 1 - 2 km in the vicinity of Kota thermal power plant and other is non-polluted site 3 - 4 km away from pollution emission source *i.e.* J.D.B. College, Kota.

**Survey and Collection of sample :** Survey of selected site has been done periodically; five different plots were established for the study of ecosystem at each site. The land selected was not previously in use for agriculture purposes and hence the impact fo human activity was eliminated, the dominant plant species in the site were marked and recorded (Shetty and Singh 1991). Foliar damage was the morphological criterion for knowing about the impact of emissions on plants. The area experienced the conditions of drought except for a wet spell of two or three months. Herbaceous annuals had luxuriant growth during the rainy period. Therefore, ground layer vegetation was included for determine species richness.

The dominant plant species was selected for study purpose. The collected samples will be of approximately same hight and size, herbaceous samples were collected separately from 4 separate microhabitats in polythene bags for the study of different parameters and immediately after collection they were placed in iceboxes and brought to the laboratory. Samples were collected in Triplets and mean value of every parameter has been taken.

**Parameter studied**

(1) Fresh weight of plant - Above ground part only are selected for the collection and bring to the laboratory, fresh weight is evaluated by physical balance.
(2) Length of plant - The maximum length of each shoot was recorded in cm (from apex to ground) and mean shoot length was calculated.
(3) Length of inflorescence - Total length of inflorescence was taken (from apex to origin of peduncle) and mean value of inforescence length was calculated.
(4) Length of third internode - The length from base to second internode up to the base of third internodes was measured and means length was calculated.
(5) Total number of leaves - Total number of leaves from apex to third internodes was recorded and means value was calculated.

**Table 1 : Herbaceous Vegetation near KTPS, Kota.**

| Sr. No. | NAME OF PLANTS | FAMILY |
|---|---|---|
| 1. | *Abutilion indicum* | Malvaceae |
| 2. | *Acalypha* spp | Euphorbiaceae |
| 3. | *Acyranthes aspera* | Amaranthaceae |
| 4. | *Ageratum conyzoides* | Compositae |
| 5. | *Alternanthera pungens* | Amaranthaceae |
| 6. | *Amaranthus hybridus* | Amaranthaceae |
| 7. | *Amaranthus spinosus* | Amaranthaceae |
| 8. | *Cassia tora* | Caesalpiniaceae |
| 9. | *Celosia argentea* | Amaranthaceae |
| 10. | *Chenopodium album* | Chenopodiaceae |
| 11. | *Coccinia grandis* | Cucurbitaceae |
| 12. | *Commelina attenuata* | Commelinaceae |
| 13. | *Concolvulus microphyllus* | Convolvulaceae |
| 14. | *Corchorus* spp | Tiliaceae |
| 15. | *Croton bonpladianum* | Euphorbiaceae |
| 16. | *Desmodium trifolium* | Papilionceae |
| 17. | *Eclipta prostrata* | Compositae |
| 18. | *Euphorbia hirta* | Euphorbiaceae |
| 19. | *Evolvulus* spp | Convolvulaceae |
| 20. | *Gomphrena celosoides* | Amaranthaceae |
| 21. | *Indigofera* spp | Papilionaceae |
| 22. | *Ipomoea* spp | Convolvulaceae |
| 23. | *Launea asplenifolia* | Compositae |
| 24. | *Malavastrum coromandelianum* | Malvaceae |
| 25. | *Oxalis corymbosa* | Oxalidaceae |
| 26. | *Peristophe paniculata* | Acanthaceae |
| 27. | *Phyllanthus fraternus* | Euphorbiaceae |
| 28. | *Polygonum barbatum* | Polygonaceae |
| 29. | *Rumax dentalis* | Polygonaceae |
| 30. | *Sida cordifolia* | Malaceae |
| 31. | *Solanum xanthocarpum* | Solanaceae |
| 32. | *Sonchus asper* | Compositae |
| 33. | *Taphrosia hamiltonil* | Papilionaceae |
| 34. | *Tridex procombens* | Compositae |
| 35. | *Vernonia albicans* | Compositae |

Table 2 : Selected plants for comparative study.

| Sr. No. | Species | Family | Leaf Shape | Phyllotaxy |
|---|---|---|---|---|
| 1. | *Chenopodium alba* | Chenopodiaceae | Ovate | Opposite |
| 2. | *Croton bonplandianum* | Euphorbiaceae | Lanceolate | Opposite |
| 3. | *Euphorbia hirta* | Euphorbiaceae | Lanceolate | Opposite |
| 4. | *Acyranthes aspera.* | Amaranthaceae | Lanceolate | Opposite |
| 5. | *Tridex procombens* | Compositae | Lanceolate | Opposite |
| 6. | *Amaranthus hybridus* | Amaranthaceae | Lanceolate | Opposite |

Table 3 : Parameters selected for the study.

| Sr. No. | Name of plant | | *Acyranthes* spp. | *Tridex* spp. | *Amaranthus* spp. | *Chenopodium* spp. | *Euphorbia* spp. | *Croton* spp. |
|---|---|---|---|---|---|---|---|---|
| 1. | Fresh weight | Site-1 | 2.34 | 1.82 | 3.02 | 3.9 | 1.65 | 10.8 |
| | (gram) | Site-2 | 2.56 | 2.5 | 3.56 | 4.4 | 2.42 | 11.6 |
| 2. | Length of plant | Site-1 | 30.7 | 11.9 | 11.2 | 18.4 | 18 | 35.2 |
| | (above ground) (cm) | Site-2 | 30.8 | 15.6 | 11 | 23 | 18.7 | 37.5 |
| 3. | Length of | Site-1 | 10 | 7.3 | 6.2 | 5.2 | 0.9 | 5.5 |
| | inflorosecnce | Site-2 | 11 | 9.8 | 6.3 | 7 | 0.9 | 5.6 |
| 4. | Length of 3rd | Site-1 | 2.1 | 2.4 | 2.3 | 2.3 | 1.8 | 5.2 |
| | internode | Site-2 | 2.1 | 3.2 | 2.4 | 2.3 | 1.9 | 5.5 |
| 5. | Length of petiole | Site-1 | 1.9 | 0.5 | 1.5 | 1.2 | 0.3 | 1.8 |
| | | Site-2 | 2.1 | 0.8 | 1.9 | 1.2 | 0.5 | 1.9 |
| 6. | Total no. of leaf | Site-1 | 18 | 20 | 42 | 56 | 34 | 76 |
| | | Site-2 | 18 | 32 | 48 | 52 | 38 | 80 |
| 7. | Length of 3rd | Site-1 | 3.24 | 3.03 | 3.32 | 2.82 | 2.72 | 2.79 |
| | leaf lamina | Site-2 | 3.1 | 3.3 | 3.5 | 3.2 | 2.7 | 3.8 |
| 8. | Breadth of | Site-1 | 1.92 | 1.42 | 1.9 | 1.11 | 0.9 | 1.44 |
| | 3rd leaf | Site-2 | 2 | 1.5 | 2 | 1.3 | 1.2 | 1.4 |
| 9. | L/B | Site-1 | 1.69 | 2.13 | 1.75 | 2.54 | 3.02 | 1.94 |
| | | Site-2 | 1.6 | 2.19 | 1.75 | 2.46 | 2.25 | 2.71 |
| 10. | L*B | Site-1 | 6.22 | 4.3 | 6.31 | 3.13 | 2.45 | 4.02 |
| | | Site-2 | 6.08 | 4.98 | 7 | 4.22 | 3.24 | 5.32 |

(6) Length of lamina of third leaf - The mean length of third leaf was taken and expressed in cm.
(7) Breadth of lamina of third leaf - The breadth of third leaf was measured and expressed in cm.
(8) L/B ratio of third leaf - The length of third leaf was divided by the breadth of third leaf.
(9) Calculated area of third leaf - Calculated area was found by multiplying the length by the breadth of third leaf.
(10) Length of petiole - The length of third leaf petiole was measured, the mean value of length of petiole of all the plants was calculated.

## RESULTS AND DISCUSSION

The data clearly show that, plants from site-1 are more affected from pollutants as compared to the plants from site-2. Same species were studied from both the sites, and their eco-morphological performance can be attributed to the polluted atmosphere. The abiotic factors in the given area remained more or less constant. The better performance of plants from site-2 is may be due to more distance from the source of pollution. Lal and Roa (1980), Shrivastava and Joshi (2002), Dadhich and Kasat (1988), Thakre and Aggarwal, (1987), have recorded the list of plants which are highly damaged at polluted areas. Dadhich and Kasat (1988) found that *Mangifera indica* and *Azadirechta indica* showed more resistance to emissions of thermal power plant as compared to ten listed trees, more then fifty herbs and shrub species. Shrivastava and Joshi (2003), have compared same morphological parameters of plant growing in polluted and non polluted areas in Kota, the data indicated that from ten different parameters studied, *Riccinus communis* is the most sensitive

plants showing maximum inhibition. *Quisqualis indica* and *Ipomea fistulosa* seems are to be least affected.

Herbaceous vegetation found in abundance at rainy season, exhibit normal growth with moderate association and aggregation, but in summer areal part show deposition of flyash and leaves show little sign of damage, Result show that 35 herbaceous species were found at KTPS campus, maximum plants belong to the family Amaranthaceae followed by Euphorbiaceae amd Compositae. *Acyranthes aspera* show slight reduction in general plant growth parameters, but find in abundance in each plot, *Tridex procumbans* show marked difference in all ecomorphological criteria specially length of plant, fresh weight, and total number of leaves etc. Amaranthus not show visible symptoms of reductions except the number of leaves per plants. Result indicate that *Euphorbia hirta* show slight degradation of size and fresh weight found prostate in position with dark brownish green coloration of areal parts of plant, due to it's under shrub position. Less aggregation and association was found in comparison of non-polluted site. Though the length of plant, length of inflorescence, internodes and petiole were not showing significant differences, but slight reduction in number of leaves and area of leaf was observed at site-1. *Croton banplandianum* also show slight reduction in length of plant, fresh weight along with all other parameters. On the other side *Chenopodium alba* and *Acyranthes aspera* appear dominance over other herbs with moer aggregations and sociability In *Chenopodium alba* number of leaves per plant was more then site-2, though size of plant, fresh weight with other criterion show slight reduction in compare with site-2. Thus, Plantation of some plants as pollution indicators may also be use for monitoring the biological changes caused by thermal power pollution in the polluted areas.

## REFERENCES


**Anderson, K. and Scott, R. 1981.** Fundamentals of Industrial toxicology. Ann. ARBOR, Michigan.

**Chpekar, S.B. 1981.** Significance of growth changes in plants in pollution indication. WHO Workshop on biological indicators and indices of environmental pollution. Cont. Bd. prev. cont. Water poll. Osm Uni, Hyderabad. 27 - 32.

**Dadhich, L.K. and Kasat, M.L. 1988.** Studies on the effects of thermal power emissions on plant community in and around Kota. Trends in pollution and Toxicology pp.30 (229 - 230)

**Dadhich, L.K. 1981.** Recognition and monitoring of air pollutants with the aid of plants. WHO workshop on biological indicators and indices of environmental pollution. Cont.Bd.prev. cont. Water poll. Osm Univ, Hyderabad. 27 - 32.

**Dadhich, L.K. 1982.** Plants for air pollution monitoring. Acta Ecol. 4 (1) : 5 - 8.

**Kaushik, P. and Dhiman, A.K. 2000.** Medicinal plants and Raw Drugs of India. Bishan Singh Mahendra Pal Singh, 23A Connaught Place, Dehradun, Pp XII+I-623.

**Lal, B. and Ambasht, R.S. 1980.** Effect of cement dust pollution on plants of *Psidium guava*. Indian J. Environ Hith **22,** 231 - 237.

**Lal, K.N. and Rao, M.S. 1980.** Nature; 1162 - 1172.

**Gordon, A.G. and Gorham, E. 1963.** Ecological aspects of air pollution from an iron sintering plant at Wawa. Ontario. Can. J. Bot. **41** : 1063 - 1070.

**Pandey, S.N. and Rao, D.N. 1979.** Effects of coal smoke sulphur dioxide pollution on the accumulation of certain minerals and chlorophyll contents of wheat plants. Trop. Ecoll. **19** : 155 - 162.

**Shrivastava, N. and Joshi, S. 2002.** Effect of particulate pollutants from Automobile air pollution at Kota on the growth performances of some plants. Geobios. **29 (4)** : 281 - 282.

**Shrivastava, N. and Joshi, S. 2003.** Comparative studies on growth performances of some plants affected by automobile pollution. Poll. Res. **22 (4)** : 591 - 593.

**Shetty, B.V. and Singh, V. 1991.** 'Flora of Rajasthan', Botanical survey of India. Kolkata.

**Singh, R.N. 2003.** Need for fly ash management concern and achievement of NLC (Neyveli lignite corporation, LTD) in fly ash management.. Director, power, N.L.C Source; planning commission.

**Thakre, R. and Aggarwal , A.L. 1987.** Plant community responses to thermal power station emission ENVIS no; 06/005. **Vol. 3 (1).**


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# COMPARATIVE STUDY ON GROWTH PERFORMANCE OF SOME PERENNIAL TERRESTRIAL ANGIOSPERMS, GROWING IN NON POLLUTED AREA AND POLLUTED AREA AROUND THE KOTA THERMAL POWER STATION, KOTA, RAJASTHAN, INDIA

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**ABSTRACT**

Present comparative study is based on eco-morphological characteristics of some perennial terrestrial angiosperms facing harmful effects of pollutant emitted by Kota Thermal Power Station (KTPS), Kota, Rajasthan to plants which are growing in non-polluted area away from KTPS. KTPS is situated at 24° 52′ 20″ N and 75° 36′ 50″ E in South West of Kota district. Area around KTPS of 1-1.5 kilometer radius with pollution was taken as sub-study area first. And another area 4-5 kilometer away from KTPS was taken as sub-study area second denoted as non-polluted area. Most dominant plants selected for comparative studies are *Acacia nilotica, A. senegal Albizia labbek, Dalbergia· sisso, Eucalyptus rudis, Mangifera indica, Azadirachta indica Prosopis cineraria, Ziziphus mauritiana, Bauhinia variegata, Callistemon lanceolatus,* etc. Parameters for eco-morphological studies were characteristics of tree and these were basal area, structure of canopy, branching pattern, percentage foliar damage and aggregation & association of species. Observations and results indicate that first study area with pollution shows disturbed vegetation because of deposition of various pollutants emitted by KTPS. Here vegetation shows some arid characters simultaneously. There was a reduction in quantity of biodiversity, also observed in comparison to non-polluted site i.e. study area second.There was no marketable difference observed in basal area, canopy structure, and branching pattern of the selected tree species in both the study areas.

**KEY WORDS:** Eco-morphological characters, KTPS, Pollution, Terrestrial Angiosperms.

## INTRODUCTION

Pollutants affect local vegetation directly or indirectly. Emission from thermal power plant adversely affects the plant species growing near the source, (Gordon and Gorham, 1963). Due to polluted air, changes in plant community can be expected both in species composition and in community productivity (Rao, 1977). Trees and shrubs act as pollution sink due to their higher ecological amplitude of tolerance, although the sensitive plants show morphological, anatomical and biochemical changes and act as "Bio-indicator" of pollution levels (Chapekar, 1981). Plant communities can be introduced for the study of stress due pollutant (Dadhich and Kasat,1988).Observations have been made to study the responses of a predominantly deciduous plant community as a whole to various air pollutants in tropical conditions (Dadhich, 1981, 1982). Present study is also in the series of studies on Effect of pollutants on terrestrial angiosperms. Here pollutants emitted by thermal power plants have been studied and tried to find out their effect on plants. There are 82 thermal power stations are operating in the country, Kota Thermal Power Station(KTPS) is first in Rajasthan as 'coal based power station' located on the left bank of river Chambal and presently it is running with installed capacity of 1045 MW. Beside power generation KTP generates large number of particulates and gaseous pollutants like oxides of carbon and oxides of nitrogen in form of 'Fly-ash' those effect on local vegetation harmfully. Transportation of fly-ash by trucks, tankers, trailers without precautionary measures and local cement industries also contribute in enhancement of pollution level through automobile emission and gaseous pollution into the environment. These pollutants affect the local vegetation directly or indirectly. During the study these harmful effect were studied an observations were carried out.

## METHODOLOGY

### Study Area

For the purpose of this comparative study two study areas were selected. First study area is around KTPS with 1-1.5 kilometer radius that is showing dangerous pollution and another area 4-5 kilometer away from KTPS that is 'Chhatravilas Garden' was taken as study area second denoted as non-polluted area. Study was done during the month of January to march.

### Data Collection

Four different plots were established for the study of ecosystem at each site corresponding to each direction. Efforts have been made to select site point was not previously in use for agriculture purpose and hence the impact of human activity was eliminated. Dominant tree species was selected for study purpose. The selected trees were taken approximately with same height and size. Parameter selected for the eco-morphological studies were basal area, canopy, branching pattern, number of main

branches and aggregation & association of plants. These parameters were observed and recorded on the spot, tree plant selected randomly and in multiplication of five at both the sites. After the collection of data mean value was calculated. Followings parameters for deciduous trees at both the study area were selected-

1. **Basal Area**- Basal area refers to ground actually penetrated by the stem. Five plants of same species selected at random from five different locations near the KTPS. By using a centimeter tape circumference of each tree at breast height was measured then radius of each plant was calculated with the formula.

$r = c / 2\pi$ $\pi = 3.14$

Basal Area = $\pi r^2$ or $4\pi c^2$

r = Radius

c = Circumference

2. **Canopy cover**- Area occupied by the above ground part of the plant, it is called herbage cover or canopy cover, it includes branches, leaves inflorescence etc. To calculate canopy cover distance from stem to the extreme boundary of shed is taken, this distance is the radius (r) for canopy cover, reading should be taken at mid-noon when sun is over the head, it is helpful in taking perfect shadow of the plant, reading should be taken from 4 sides of stem then mean of radius will be calculated.

$r^* = r1+r2+r3+r4.....$ Canopy= $\pi r^2$.

3. **Abundance**- Abundance may not be expressed in quantitative terms, but expressed as qualitative character, because tree plants are not found uniformly distributed in an area, they found in smaller patches or groups. Differing in number at each place, abundance is divided into five arbitrary groups depending upon the number of plants. These groups are- Very rare, Rare, Common, Frequent and Very frequent.

4. **Sociability**- It denotes the proximity of plant to another. Plants generally grow as isolated individuals, in patches, colonies or groups. Each species or some species may differ in sociability value. Thus sociability expresses the degree of association between species. Braun Blanquet (1932) divided plant into five sociability groups. These are-

S1-Plant (stem) found quite separately from each other, growing singly

S2-A group of 4-6 plants at one place

S3-Many smaller and scattered groups at one place

S4-Several bigger groups of many plants at one place

S5-A large group occupying larger area.

5. **Total number of main branches**- All selected trees show dichotomous branching pattern, to calculate the number of main branches in a tree binocular is used and start the counting from very first branching to the upper most branch After the counting of number the branches trees are categories in to following categories,

i. Highly branched – More than 12 branches
ii. Moderate branches – 8 to 12 branches
iii. Less branches – 4 to 8 branches
iv. Very less branches – 0 to 4 branches

6. **Branching pattern of tree**- Branches play role in formation of canopy architecture of the tree species. According to branching pattern various types of canopy structure are formed as sparse, irregular, globular, spreading crown, open, semi-dense etc.

## RESULTS & DISCUSSION

In present study **21** tree species were observed which include approximately all tree species observed by previous workers during last two decades, except *Lannea coromandelich, Poinciana regia* (Dadhich and Kasat 1988). In these 21 tree species (Table-1) *Cassia siamea, Cassia fistula, Azadirachta indica, Delonix regia, Bauhinia variegate, Albizia lebbeck* were found near the campus while *Anoegissus spp, Acacia nilotica* and, *Zizyphus spp* were dominant far away from main plant and near to fly-ash dumping area. Results indicates that trees found in most abundance at first sub area are *Acacia nilotica Albizia lebbek and Ziziphus spp* in comparison to *Azadirechta indica* and *Dalbergia sissoo* of sub area second. *Ficus banghalensis* was rare plant for first study area while present commonly in sub area second.*Eucalyptus rudis, Cassia siamea* distributed equally at both the sites. According to sociability only *Acacia nilotica* and *Ziziphus mauritiana* show slight grouping patterns at sub-area I. Maximum branches, dense branching pattern and spreading dense canopy architecture was observed in *Ficus banghalensis*, minimum branches with sparse canopy observed in *Eucalyptus rudis* at both the sites. Maximum basal area occupied by *Ficus banghalensis* at subarea I which is more than the same plant present at subarea II. Minimum basal area occupied by *Eucalyptus rudis* followed by *Cassia siamea* at subarea I, while minimum basal area at subarea II occupied by *Cassia siamea* and *Ziziphus mauritiana* .At both the sites largest canopy cover was of *Ficus banghalensis* followed by *Azadirachta indica.* While a remarkable difference was observed in canopy cover of *Eucalyptus rudis* and *Cassia siamea* at both sub-study areas. The overall picture shows that the small canopy covers ware present at subarea I in comparison to subarea II. Lynch.1951 documented the reductions in annual diameter growth in trees of polluted area. But in this study the relationship between total basal area of tree species and distance from the power plant was poor, however the extent and type of damage to leaves was observed in the tree species, which is affected mostly by direction of wind, distance from chimneys, fly ash hopper and dumping area. Maximum symptoms were observed at south east direction of power plant. *Prosopis cinereria, Bougainvillea, Ziziphus mauritaina, Bauhinia variegata, Callistemon lanceolatus* show some leaves injury symptoms, may be due to combined effect of all the factors discussed earlier. It was observed that the trees like *Mangifera indica, Azadirachta indica, Acacia nilotica, Eucalyptus rudis* show more resistance to emissions as compare to other trees. It can further be discussed that the vegetation around thermal power station, though not exhibiting the apparent symptoms of pollution injury, but was constantly under stress due to pollution. Herbal species were the most susceptible while already established woody perennials could cope up with the stress, so that they did not show any permanent visible injury, the work of Thakre and Aggarwal (1987), & Dadhich and Kasat (1988) confirm this view. Such studies are very significant as the information furnished can be

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utilized in making a pollution free industrial complex, which is suitable for human habilitations. The main objective of present study is to identify pollution tolerant species and to suggest an ecological model in the form of green belt around industrial complex to mitigate pollution. *Mangifera indica, Azadirachta indica, Acacia nilotica, Eucalyptus rudis Albizia lebbek and Ziziphus spp* may provide a natural sink for them. Though under stress, they did not show any permanent visible injury. In the comparative study between two decades on some common plants were identified that were remain unchanged e.g. *Cassia siamea, Cassia fistula, Azadirachta indica, Delonix regia, Bauhinia variegate, Albizia lebbeck, Ipomoea fistulosa, Thevetia peruviana, Zizyphus nummularia, Solanum xanthocarpum Chenopodium album, Euphorbia hirta, Phyllanthus fraternus, Croton banplandianum Alternanthera pungens* and *Tridex procombens.* These plants may provide a natural sink for pollutants of KTPS. In general plants growing in polluted area were stunted in comparative to unpolluted area, some visual injury symptoms like burning of leaf margins and tip, necrosis and chlorosis were observed, the dust covered the leaves show brown necrotic lesions starting at the tip and progressing down the lamina, maximum necrosis and chlorosis observed in *Ziziphus* and *Cassia* (Varshney and Garg, 1980).

**TABLE:-1**

| S. No. | Name of Tree with Family | Study Subareas | Circumference in meter (m) | Basal area In Sq. meters ($m^2$) | Radius (meter) | Canopy Area | Abundance | Sociability | Total number of main branching |
|---|---|---|---|---|---|---|---|---|---|
| 1. | *Eucalyptus rudis* (Myrtaceae) | Subarea I | 20.096 | 32.154 | 3.2 | 32.2 | Common | S2 | 5 to 7 |
| | | Subarea II | 27.632 | 60.790 | 4.4 | 60.8 | | | & 7 to 8 |
| 2. | *Azadirachta indica* (Miliaceae ) | Subarea I | 36.424 | 105.629 | 5.8 | 105.6 | Common | S1 | 7 to 8 |
| | | Subarea II | 37.052 | 109.303 | 5.9 | 109.3 | | | |
| 3. | *Albizia lebbek* (Mimosaceae) | Subarea I | 31.4 | 78.5 | 5 | 78.5 | Frequent | S3 | 8 to 10 |
| | | Subarea II | 35.168 | 98.470 | 5.6 | 98.5 | | | |
| 4. | *Ficus banghalensis* (Moraceae) | Subarea I | 37.68 | 113.04 | 6 | 113.0 | Rare | S1 | 12 to 18 |
| | | Subarea II | 50.24 | 200.96 | 8 | 201.0 | | | |
| 5 | *Ficus glomerata* (Moraceae) | Subarea I | 27.004 | 58.059 | 4.3 | 110.0 | Rare | S1 | 10 to15 |
| | | Subarea II | 35.168 | 98.470 | 5.6 | 191.0 | | | |
| 6. | *Acacia nilotica* (Mimosaceae) | Subarea I | 28.888 | 66.442 | 4.6 | 66.4 | Very much frequent | S4 | 8 to 12 |
| | | Subarea II | 30.144 | 72.346 | 4.8 | 72.3 | | | |
| 7 | *Acacia senegal* (Mimosaceae) | Subarea I | 28.888 | 66.442 | 4.6 | 59.4 | Frequent | S4 | 8 to 12 |
| | | Subarea II | 28.888 | 66.442 | 4.6 | 69.2 | | | |
| 8. | *Cassia siamea* (Caesalpineaceae) | Subarea I | 25.748 | 52.783 | 4.1 | 55.4 | Common | S3 | 8 to 12 |
| | | Subarea II | 32.656 | 84.906 | 5.2 | 84.9 | | | |
| 9. | *Ziziphus mauritaina* (Rhamneeae) | Subarea I | 22.608 | 40.694 | 3.6 | 40.7 | Very much frequent | S4 | 8 to 12 |
| | | Subarea II | 20.096 | 32.154 | 3.2 | 32.2 | | | |
| 1 0. | *Dalbergia sissoo* (Fabaceae) | Subarea I | 27.632 | 60.790 | 4.4 | 60.8 | Frequent | S3 | 8 to 12 |
| | | Subarea II | 30.144 | 72.346 | 4.8 | 72.3 | | | |
| 1 1. | *Albizia lebbek* (Mimosaceae) | Subarea I | 31.4 | 78.5 | 5 | 78.5 | Frequent | S3 | 8 to 10 |
| | | Subarea II | 35.168 | 98.470 | 5.6 | 98.5 | | | |
| 1 2. | *Holoptelea integrifolia* (Ulmaceae) | Subarea I | 28.26 | 63.585 | 4.5 | 101.0 | Frequent | S1 | 10 to 15 |
| | | Subarea II | 36.424 | 105.630 | 5.8 | 184.0 | | | |

**REFERENCES**


Agarwal, M. and Agarwal, S. B. (1988) Phytomonitoring of air pollution around a thermal power plant. *Proceedings of International conf. on Trop. Micro-Meteorology and Air Pollution*, 215-218.

Agarwal, S. K. (1985) Foliar biochemical changes in response to air pollution. *Acta Ecologia*. 7:2:39-42

Agarwal, S. K. and Sharma, A. P. (1984) Air pollution induced the ranged physiology in plants. I. Changes pertaining to chlorophyll, carotenoid and phenol contents in *Cassia fistula*, L. *Dalbergia sissoo*. Acta. Ecol. 6:2:37:-43

Arivazhgan, K. (2011) Project report submitted to NTPC, Simhadri Dadri Talcher Thermal and Vindhyachal Entitled-Show Case Project on Ash Utilization in agriculture in and around the thermal power station area.

Banerrji, A. K. & Chaphekar, S. B. (1978) Observations of foliar injury to plant by Air pollution Geobios. 5:8-11

Chapekar, S. B. (1981) Significance of growth changes in plants in pollution indication. WHO workshop on biological indicators and indices of environmental pollution. Cont.Bd.prev. cont. Water poll. Osm Univ, Hyderabad.pp27-32.

Dadhich, L. K. (1981) Recognition and monitoring of air pollutants with the aid of plants. WHO workshop on biological indicators and indices of environmental pollution. Cont. Bd. prev. cont. Water pollution. Osmania University Hyderabad 19-26 27-32

Dadhich, L. K. (1982) Plants for air pollution monitoring *Acta Ecol.4(1): 5-8.*

Dadhich, L. K. and Kasat M. L. (1988) Studies on the effects of thermal power emissions on plant community in and around Kota. *Trends in pollution and toxicology* pp.30 (229-230).

Dubey, P.S., Trivedi, L. & Shringi, S.K. (1982) Pollution studies of betul forest area to satpura Thermal Power Station aerial discharge. Final Report D.O. En. Project No. 19/27/78-Env. Pp. 53.

Furukawa, A., Isoda, C., Iwaki, H. and Totsuka, T. (1980) Interspecific differences in resistant to sulphur dioxide. Studies on the effects of air pollution on the plant, and mechanism of phytotoxicity. Res. Rep. Natal. Environ. Stud. Japa. 11:113-126

Gordon, A. G. and Gorham, E. (1963) Ecological aspects of air pollution from an iron sintering plant at Wawa. Ontario. *Can.j .Bot. 41. 1063-1078.*

Gupta, D. K., Rai, U. N., Tripathi, R. D. and Msahiro Inouhe (2002) Impact of fly ash on soil and plant responses. *Journal of plant research.* Springer, Japan. Volume-115 issue6, pp 401-409. N.B.R.I. Lucknow.

Gupta, D.K., Rai, U. N., Tripathi, R. D., MsahiroInouhe (2002) Impact of fly ash on soil and plant responses. *Journal of plant research.* Springer, Japan. Volume-115 issue6, pp401-409. N.B.R.I. Lucknow.

Gupta, M. C., Solanki, M. and Singh, M. P. (1988) Anatomical response of *Mangifera indica* (L.) to coal smoke pollution. Acta. Ecol. 10:1:29-32.

Gupta, M.C., Solanki, M. and Singh, M. P. (1988) Anatomical response of *Mangifera indica* (L). to coal smoke pollution. *Acta. Ecol.* 10:1:29-32.

Journey from food bowl to ash dykes, Lesson from Punjab; India beyond www.coal.org. Accessed June 2013, 22-5-2013.

Lynch, O. F. (1951) Diameter growth of *Pondrosa* pine in relation to spoken pipe blight problem. *Northwest sci* 25:157-163.

Rao, D.N. (1977) Phyto-monitoring of Air pollution; Possibilities and Methods. J.I.P.H.E; 9-20.

Rao, D.N (1989) Plants as pollution monitoring device fertilizer news 24; 25-28.

Sharma, S.K., Kalra, N. and Singh, G. R. (2001) Impact of coal-burnt ash on environment and crop productivity sci. Ind. Res. 60;580-585.

Shetty, B. V and Singh, V. (1991) 'Flora of Rajasthan', Botanical survey of India. Kolkata.

Shrivastava, N. and Joshi, S. (2002-2003) Effect of particulate pollutants from Automobile air pollution at Kota on the growth performances of some plants. *Geobios.* 30.

Sikka, R. and Kansai, B. D. (1994) Characterization of thermal power plant flyash for agronomic purposes and to identify pollution hazards, *Bio-resource Technol.* 50 pp. 269-273.

Singh H. and Kolay, P. K. (2009) Analysis of coal for trace elements and their geo environmental implications. *Water Aira nd Soil Pollution,* 1988(1-4); 87-94.

Singh, N. and Yunus, M. (2000) Environmental impacts of fly ash. Pp.60-79. In: M. Iqbql, P. S. Srivastava and T.O. Siddiqui (esd). Environmental Hazards-Plants and people. C. B. S. Publishers and distributors, New Delhi.

Singh, S.K. and D.N. Rao (1979 & 1985) Evaluation of plants for their tolerance to air pollution. Vol. 1:218-224. Indian Association for Air Pollution Control, New Delhi.

Sirohi, A. and Singh, D. (1989) Anatomical response on *Salvadora persica* L. to coal smoke pollution. Acta ecol. 11:2:83-85.

Thakre, R. and Aggarwal, A.L. (1987) Plant community responses to thermal power station emissions ENVIS no; 06/005. Vol. 3; 1.21-31.

Thakre, R. and Rao, M.V. (1985) Physico- chemical analysis of soils amended with fly ash and their effect on germination of Wheat, Cotton, and Tomato .*Geobios.41-47.*

Thakre, R. (1983) Effect of thermal power plant emissions on nearby vegetation, J.I.P.H.E; 9-20.

Thakre, R. and Aggarwal, A. L. (1987) plant community responses to thermal power station emissions ENVIS no; 06/005. Vol. 3; 1.21-31.

Thomas, M. D. & Hendricks, R.H. (1956) Effect of air pollutants on plants. In P.L. Magil (Ed.), Air pollution Handbook: 1-44: New York McGraw-Hill.

Varshney, C. K. and, Garg, J. K. (1979) Plant response to sulphurdioxide pollution, CRC critical review environmental control 9:27-49

Varshney, C.K. & Garg, J. K. (1980) Significance of leaf surface characteristic in plant response to air pollution. *Water Air & Soil pollution.*14:429-433.

Verma, S. & Sharma, K.C. (2009) Air pollution tolerance index of some plant species of Ajmer, Rajasthan.

Wentzel, K.F. (1956) The part played by industrial and domestic smoke in damage to forest by flumes and in atmospheric pollution. *Forstarchiv* 27:84-89