CHAPTER - 4

POT EXPERIMENTS AND STUDIES OF TRACE ELEMENTS UPTAKE
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Cicer arietinum is an erect or spreading, much branched annual herb of family Leguminosae. Gram is the most important pulse crop in India. In Madhya Pradesh, gram cultivation is concentrated in the districts of Hoshangabad, Jabalpur, Chhindwara, Sagar, Betul and Mandla. In Chhattisgarh region, it is grown mainly in Takhatpur and Mungeli for want of appropriate soil characteristics and its amelioration.

Gram is nutritive pulse, extensively used as a protein adjunct to starchy diets. Whole gram has 9.8% protein, 17.1% fat (ether extract), 5.3% mineral matter, 2.7% fibre, 3.9% carbohydrates, 61.2% Ca, 0.19% P and 0.24% Carotene.

The protein content of gram is greatly influenced by soil and locality. Three crystalline products have been isolated from the fresh whole germ of sprouting gram. They are:

(1) Biochanin A \( (C_{16}H_{12}O_5) \) = M.P. 212°C.

(2) Biochanin B \( (C_{15}H_{12}O_4) \) = M.P. 250°C

(3) Biochanin C \( (C_{6}H_{13}O_3N_3) \) = M.P. 310°C
Biochanin A is a non-glycosidic isoflavone isolated from a plant body. Crystalline Biochanin C accelerates growth of yeast cells and a 100% increase has been isolated when its concentration in 1% is the nutrient medium [2].

The micronutrients Zn, Cu, Fe, Mn, B, Mo etc. are essential to plant growth but are utilised in only minute quantities. But Zn and Mo are the nutrients more deficient in soils of acid regions. B is deficient in humid areas of Chhattisgarh due to leaching phenomenon [71]. Helianthus annuus is a drought resistant and frost resistant crop and it can withstand several degrees of frost but it does not thrive on acid soils, water logged lands or steep slopes [71].

So the pot experiments on the acid soil of Rehar basin major irrigation project were conducted in the successive two years of 1998 and 1999. Soil and fly ash were analysed for trace elements, pH, electrical conductivity etc. In the pot, different combination of soil and fly ash were taken with a view to study the effect of amelioration, neutralisation and optimisation of the acid soil for maximum uptake of Zn and Mo. The details of pot experiments have been displayed ahead.
MICRONUTRIENTS IN PLANTS:

Chemical analysis of plants has revealed the presence of some sixty elements in the plant tissue. However, all are not essential for plant growth and development. Extensive research carried out in plant nutrition has established the essentiality of sixteen elements. Nine of these elements are required in relatively larger amounts and are designated as macronutrients. The remaining seven are needed by plants in smaller amounts and in certain cases only traces of the nutrients are required. These are called micronutrients or trace elements. They are B, Fe, Mn, Mo, Zn, Cl and Cu. Each of these nutrients plays a specific role in the growth and development of a plant. Many of these micro-nutrients play the role as Metalloenzymes. Some elements like Se, Mo, As, Fe and Li, because of their toxicity to animals, have come to acquire a special importance.

In India, the deficiency of micronutrients has been observed in light textured and calcareous soils. The situation has been aggravated with the introduction of high-yielding crop varieties and intensive cropping system. The accompanied changes in our soil management practices frequently alter micronutrient availability. In order to maintain the level of productivity of our soils, we have to keep watch on such
changes. As the demands of nutrients for higher yields increase and plant requirements for macronutrients are met with more efficiently, micronutrient deficiencies are likely to become more acute with increased awareness of crop quality and maximum production, a renewed interest in the role of micronutrients in plants, animal and human nutrition is anticipated. The pollution effect of micronutrients also cannot be ignored. Keeping in view these facts, a part of work on delineation of areas of micronutrient deficiency has been completed and for other areas the work is in progress. This will help not only in forecasting the micronutrient deficiency areas, but also help in the production and distribution strategy of fertilizers containing micronutrients.

In some areas, it may be quite uneconomical to make up the deficiency or to overcome the toxicity of a particular micronutrient. This can be overcome by evolving suitable varieties which can withstand low and high levels of that micronutrient. Use of soil analysis is most common for predicting the availability of micronutrients. The threshold values provides better indication about the nutrient stress as its vary not only with the crops but also with different varieties of the same crop.
It may eventually be proved that some of the fatal diseases of animals and human beings are the outcome of toxicity or deficiency of trace elements. The five elements which have been proved to be essential in human health are Mn, Fe, Co, Cu and Zn. Many metalloenzymes have been found to be essential in animal and human nutrition.

**ROLE OF MICRONUTRIENTS IN CROPS:**

The role of micronutrients in the plant has been reviewed by several authors, in the last ten years [56, 57, 39].

**Boron:**

Boron is an essential plant nutrient. Generally in the soils of arid zones and particularly in the saline-alkali, soils, its toxicity and in the acid and highly leached soils of humid regions its deficiency is well known. The most pronounced effect of B deficiency on metabolism is a disrupted RNA synthesis. The disruption of RNA metabolism has been studied largerly using isotopically labelled RNA precursors in normal and B-deficient plants. An interesting observation is that the results of B deficiency are very similar to those from the
application of some plant harmones. This suggests a link between B metabolism and plant harmones. However, our theories of how B functions in plants still have little supporting evidence, and B remains the least understood of the micronutrients.

**Copper**:

Copper like most cations, is primarily involved with enzyme activity. Several enzymes that are affected directly by copper deficiency have been identified. The physiological result of copper deficiency is an apparent wilting of leaves. Graham, [40] suggests that this wilted appearance is the result of structural weakness of the cell walls in copper deficient plants and is not related to water stress. However, copper deficiency also reduces root growth more than shoot growth, creating an unfavorable shoot : root ratio [28, 38, 1]. This increased shoot : root ratio could lead to plant/water stress. The two phenomena may work together to create the wilted appearance.

Copper deficiency usually delays maturity and reduces yield by reducing grain size. [50, 41]. This reduced grain size is attributed to a lower photosynthetic efficiency caused by increased closure of stomata in Cu - deficient plants. When stomata are closed, the diffusion
of carbon-di-oxide into the leaves is slowed or stopped. Also studies with carbon-14 labelled carbon-di-oxide show that less carbon is fixed in C₆ sugars, the types that are translocated to other parts of the plant in the phloem [9]

The essentiality of copper as a plant nutrient was established in 1931.

**Iron :**

The role of iron in the formation of chlorophyll in plants was discovered over 100 years ago. Since that time, deficiency of iron in plants has been recognized. Beneficial effects of its application have been reported from, Punjab, Delhi, Rajasthan and U.P. Iron was considered indispensable for the normal growth of plants and designated as macronutrient, its requirement by plants is so small that it is grouped in micronutrients. A peculiarity of the iron nutrition in plants grown on soils is the fact that the limitation of growth under conditions of iron deficiency is usually, a consequence of the quality rather than the quantity of iron within the plants. Iron has a tendency to become unavailable within the plants.
Iron is readily oxidised and reduced between its two oxidation states $\text{Fe}^{3+}$ and $\text{Fe}^{2+}$. Its role in plant metabolism is closely linked to this reversible $\text{Fe}^{2+}/\text{Fe}^{3+}$ oxidation. Many of the reactions associated with Fe are the redox reaction of chloroplasts, mitochondria and peroxisomes [26,42]. These reactions include coupled electron-transfer reactions (Cytochromes a & b) oxidases (Cytochrome oxidase) and peroxidases (catalase and peroxidase) [53]. Also Fe, plays a role in the formation of amino levulinic acid, which is a precursor of chlorophyll synthesis [63] In addition, a strong correlation exists, between the leaf chlorophyll and Fe content [64]. When Fe becomes limiting, thyalkaloid development slows or stops. Then, as the leaf continues to expand, the thyalkaloid constituents such as Fe and several type of chlorophyll are diluted, resulting in the pale yellow colour typical Fe deficiency.

**Molybdenum:**

Molybdenum is needed in the least amount of all the essential micronutrients it is involved in several enzyme systems including nitrogeneous nitrate, reductase, xanthine oxidase, aldehyde oxidase, and sulfate oxidase [39]. The result of Mo deficiency is a disrupted nitrogen metabolism. Nitrate reductase activity is reduced by Mo
deficiency. Nitrate reductase is involved in the first step of incorporating inorganic \( \text{NO}_3^- \) into organic N compounds. Das Gupta and Basuchaudhari [44] demonstrated that Mo applications to rice increased the amount of reduced nitrogen in the plant tissue.

Mo deficiency results in reduced chlorophyll concentration in the leaves [46, 44] which leads to decreased photosynthetic efficiency. The lack of chlorophyll production is most likely a secondary effect of the disrupted N metabolism since Mo has not been shown to play a direct role in chlorophyll synthesis.

Another role of Mo in N metabolism is in \( \text{N}_2 \) fixation by legume-Rhizobium symbiosis. Nitrogenase is a Mo containing enzyme [29, 30]. It catalyzes the fixation of denitrogen gas to ammonia, which can be utilized by the host plant. However examples of Mo deficiencies on legumes in the tropics are rare.

Increased use of N-Fertilizer increases the need for Mo. The production of nitrate reductase by plant is induced by the nitrate availability. Evans [7] showed that \( \text{NO}_3^- \) grown plants required more molybdenum and that all the additional uptake was accounted or by the nitrate reductase enzyme.
**Zinc:**

Some researchers furnished first convincing evidence that zinc is essential for higher plants. Zinc was indispensable for normal growth of plant. Zinc serves both some structural and some regulatory roles in enzyme activity in plant tissue. Only a few enzymes have clearly been identified as requiring Zn. However, for several others there is considerable indirect evidence of Zn involvement [53, 45]. Zn deficiency is characterized by a reduction in the number of ribosomes and RNA in the plant cells. Plant growth is stunted by Zn deficiency. Like most micronutrient deficiencies, Zn deficiency results in an overall reduction in photosynthetic efficiency and disrupted metabolism.

**Manganese:**

The role of Mn in plant metabolism is very unclear. Manganese has properties similar to Mg and can substitute for Mg in some enzyme systems. This is probably not a common occurrence in nature since Mg is taken up by crops in much greater quantities than in Mn. Only one system, albeit a very important one, has been shown to require Mn. This is the splitting of water by photolysis in photosystem II [31]. This reaction probably involves a change in the oxidation state of Mn, but the exact structure or functional role of Mn in photosynthesis has not yet been defined.
**PATH OF MICRONUTRIENT UPTAKE:**

The various micronutrients have different mobility characteristics in the soil, and they are needed in widely differing quantities, in addition the plant vary in their ability to redistribute the micronutrients from older tissue to new growing points or for grain filling. The mobility of nutrients in the soil and plant, the quantities of micronutrients in the seed, and environmental conditions all affect the quantity and timing of micronutrient needs and uptake.

The amount of uptake of micronutrients from the soil solution is dependent on several factors including:

1. The rate and distance of movement of nutrients in the soil.
2. The volume of soil the plant is exploiting and
3. The plant's capacity to absorb the nutrient.
4. Nutrients move to the plant by diffusion and mass flow[17].

Diffusion occurs along a concentration gradient. As plants roots absorb nutrients, the soil Environment near the root is depleted of the nutrient and the gradient is thus created. As long as the root uptake exceed the rates of movement of nutrients toward the root diffusion will continue.
The other mode of micronutrient movement to the plant is by mass flow. The plant and this induces a flow of water to the root where it is absorbed. The water moving to the root has nutrients dissolved in it. Thus the rate of supply of nutrients to the nutrients to the rate is related to the plants transpiration rate. Very high transpiration rates would increase the flow of nutrients to the root, & lower transpiration rates would decrease the nutrient flow. This is an over simplification, though since soil moisture also limits transpiration.

The factor influencing micronutrient availability is the volume of soil being exploited by the crop. The rooting pattern of crops grown in is different. One more factor is the plant's capacity to absorb and utilize the micronutrients that are available. The uptake process is best described by the membrane carrier site mode. This model depicts sites located on the membrane which actively transport ions from outside the membrane to the cytoplasm. This process utilizes metabolic energy, thus uptake is affected by temperature, water stress, and respiration. It seem that Zn, Cu, Mn, and B are absorbed by active uptake [58].

All of the micronutrients can move through the xylem tissue from the roots to the arial portions of the plants. Boron is virtually
immobile. Once it reaches a plant organ [36]. Thus a continuous supply of B is needed by a crop to avoid deficiency. Mo is readily redistributed via the phloem tissue to the other parts of the plants where it is needed [3] The other micronutrients Zn, Cu, Fe and Mn are intermediate in mobility. The deficiency symptoms of all four of these appear first in the youngest leaves, Although same redistribution is evident, it is probable that a continuous supply of each micronutrient except Mo is needed to avoid deficiency and a reduction in yield.

CRITICAL VALUE OF MICRONUTRIENTS IN PLANTS:

The concept of critical micronutrient concentration in plants is the basis for using plant analysis as a diagnostic tool.

The critical concentration of a nutrient element in plant or soil refers to a level at or below which the plant either develops deficiency symptoms or causes significant and or 5% to 10% reduction in its yield as compared to optimum.

The most commonly used technique to determine critical limit is the establishment of a relationship between plant responses or Bray's percent yield (i.e. yield without nutrient divided by the yield with optimum nutrients x 100) and the nutrient concentration in plant.
In chick pea, symptoms of Zn deficiency first appear on the upper one third part of the leaflets of middle leaves as light yellow colour. This gradually turns pinkish/reddish brown, followed by bronzing and necrosis. The leaflet size also decreases. The plants are stunted and hence have fewer branches in red yellow soil, the critical limit of Zn in Chick pea is 26.8 ppm [52].

SOIL ANALYSIS:

Soil analysis is an extremely useful and a convenient diagnostic tool for quick and timely assessing micronutrient availability. The critical limit in soil for proper growth of chick pea is 0.70 ppm and 0.52 ppm for black gram. Mean, Mn deficiency is 42.8 ppm in Surguja districts. The soils belong to Entisols, Alfisols and inceptisols with texture ranging from loamy sand to clay. The physico-chemical properties were found to be as:

1. pH - 4.3 to 7.8
2. E.C. - 0.10 to 1.00 ds/m
3. Org.C - 0.12 to 2.70 %

During soil analysis, we found, the DTPA-Zn content in soils ranged from 0.10 to 5.46 (mean 0.61) ppm in Ambikapur, 0.15 to 3.51 (mean 0.65) ppm in Baikunthpur, 0.10 to 3.62 (mean 0.63) ppm.
in Bharatpur, 0.10 to 2.67 (mean 0.73) ppm in Manendragarh and 0.12 to 2.68 (mean 0.74) ppm in Ramanujganj.

Zn deficiency in soils were:
1. 71% in Semeri and Surajpur
2. 64% in Manendragarh
3. 61% in Bharatpur
4. 65% in Ambikapur
5. 49% in Baikunthpur and Ramanujganj.

Available Mo, B and Status of Soils:

The available Mo in soils varied from 0.01 to 2.03 ppm with a mean value of 0.20 ppm. Available B in there soils varied from 0.03 to 3.82 ppm with a mean value of 0.48 ppm. Boron deficiency in soils of Surguja is 1.3% [47,48].

These deficiencies are described in the maps ahead, in the next pages. Available nitrogen status is below 250 kg N/ha. Available K status is medium (400 kg/ha) P deficiency is low as shown in the map. Available "S" is very low.

In general, red and yellow soils are formed in situ from indurated and metamorphosed rocks, such as granite, granitic gneiss
LEGEND
(\% SOIL SAMPLES DEFICIENT)

- BELOW 25
- 25 - 50
- 50 - 75
- ABOVE 75
- AREA NOT SURVEYED

Fig. 4. Status of available zinc in soils of Madhya Pradesh
LEGEND
(\% SOIL SAMPLES DEFICIENT)

<table>
<thead>
<tr>
<th></th>
<th>NIL</th>
<th>BELOW 25</th>
<th>25 - 50</th>
<th>50 - 75</th>
<th>AREA NOT SURVEYED</th>
</tr>
</thead>
</table>

**Fig. 2** Status of available iron in soils of Madhya Pradesh
LEGEND
(% SOIL SAMPLES DEFICIENT)

<table>
<thead>
<tr>
<th>Nil.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below 25</td>
</tr>
<tr>
<td>25 - 50</td>
</tr>
<tr>
<td>50 - 75</td>
</tr>
<tr>
<td>Area not surveyed</td>
</tr>
</tbody>
</table>

Fig. 4. Status of available manganese in soils of Madhya Pradesh
LEGEND
(% SOIL SAMPLES DEFICIENT)

- BELOW 25
- 25 - 50
- 50 - 75
- ABOVE 75
- AREA NOT SURVEYED

Fig 4.4 Status of available sulphur in soils of Madhya Pradesh
LEGEND
FERTILITY STATUS (N)

LOW
MEDIUM

Fig 4.5 Status of available nitrogen in soils of Madhya Pradesh
Fig 4. Status of available phosphorus in soils of Madhya Pradesh.

LEGEND

HIGH

MEDIUM

LOW

FERTILITY STATUS (P)
Fig 4. Status of available potassium in soils of Madhya Pradesh
and schists. Major characteristics are:

1) Red colour is due to coatings of \( \text{Fe}_2\text{O}_3 \) on the soils particles. The colour is yellow when coating is due to hydrated \( \text{Fe}_2\text{O}_3 \).

2) Soils are generally loam to clay-loam in texture.

3) Soils are acidic in most of the cases. \( pH = (6.4 - 6.8) \).

4) Soils are severely deficient in organic matter, N, P, lime and S. K is medium.

5) C/N Ratio is around 10.

**WORK PLAN:**

In the light of these informations obtained after series of soil analysis, pot experiments were conducted. These are as mentioned below:

i) Soil

(ii) Soil + NPK

(iii) Soil 90% + fly ash 10% + NPK (200 : 500 : 300) mg

(iv) Soil 80% + fly ash 20% + NPK (200 : 500 : 300) mg

(v) Soil 70% + fly ash 30% + NPK (200 : 500 : 300) mg

(vi) Soil 60% + fly ash 40% + NPK (200 : 500 : 300) mg

(vii) Soil 50% + fly ash 50% + NPK (200 : 500 : 300) mg
The object of using these combinations is to establish the use of fly ash on soil to ameliorate soil for:

1. Proper Growth of plant as there are some plant species which do not grow on acidic soil [54].

2. From acidic soil, Zn and Mo, essential micronutrients are not available to plants.

3. N:P:K, (200mg : 500mg : 300mg) per pot, were mixed with different ratio of soil and fly ash. This helps to increase uptake of trace elements from soil [62].

**Determination of pH**:

Determination of Chemical properties of fly ash, soil and combinations:

**Requirements** : pH meter, electrodes, beakers, samples etc.

**Procedure**:

The pH-meter was stabilised for half an hour, the electrodes are cleaned and dipped firstly in distilled water and setted it with 'CAL' control and checked the instrument with buffer solutions of
pH = 4 and pH = 9. Repeated the same procedure for unknown samples and observed the reading till a constant reach.

For determination of pH in soil, fly ash and its different combinations. Firstly solution of the sample in 1:5 ratio is prepared and observed.

**Determination of Electrical Conductivity** :

**Requirements** : Conductometer, electrode, samples, beaker, etc.

**Procedure** :

The conductometer was warmed for 10-15 min. The temperature was setted and also the cell constant range. Observed the reading till the meter needle reached the red line. Repeated the procedure for different unknown samples.

For determination of conductivity in soil, fly ash or its different combination, 1:5 ratio solution of soil or sample is to be prepared.

**Determination of Water Holding Capacity (W.H.C.)**

**Requirements** : Sample, electric oven and Tared tin plate.
Procedure:

Approximately 5 g of sample are accurately weighed into a tared tin and then dried in an electric oven at a temperature of 105-110°C for 8 hours after, cooling in a dessicator, the loss in weight is determined.

If the sample is dried then wet the sample with water and let it for overnight and than analyse when analyzing a fresh, wet soil, it is imperative to weigh the sample for moisture determination at the same time as for the analysis, for easy determination the empty tared tin plate must be pre-weighed, then with sample and after also empty tared tin must be weighed.

pH and conductivity (m. mhos/cm) of fly ash, soil and their different combinations:
Table No. 4.1

pH and E.C. of Fly ash, soil and their different combinations

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment</th>
<th>pH</th>
<th>Electrical conductivity (in m mhos/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fly ash</td>
<td>8.81</td>
<td>0.217</td>
</tr>
<tr>
<td>2.</td>
<td>Soil</td>
<td>6.54</td>
<td>0.082</td>
</tr>
<tr>
<td>3.</td>
<td>Soil + NPK</td>
<td>6.55</td>
<td>0.091</td>
</tr>
<tr>
<td>4.</td>
<td>90% soil + 10% fly ash + NPK</td>
<td>6.58</td>
<td>0.106</td>
</tr>
<tr>
<td>5.</td>
<td>80% soil + 20% fly ash + NPK</td>
<td>6.62</td>
<td>0.118</td>
</tr>
<tr>
<td>6.</td>
<td>70% soil + 30% fly ash + NPK</td>
<td>6.92</td>
<td>0.131</td>
</tr>
<tr>
<td>7.</td>
<td>60% soil + 40% fly ash + NPK</td>
<td>7.35</td>
<td>0.154</td>
</tr>
<tr>
<td>8.</td>
<td>50% soil + 50% fly ash + NPK</td>
<td>7.39</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Concentration of compounds (in percentage) and trace elements (in ppm) in fly ash, soil and in the different cobinations of fly ash and soil, is as mentioned below:
Table No. 4.2
Concentration of Compounds and trace elements in fly ash, soil and in their different combinations

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Details of Combination</th>
<th>Compounds Concentration (in %)</th>
<th>Concentration (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SiO₂</td>
<td>Al₂O₃</td>
</tr>
<tr>
<td>1.</td>
<td>Fly ash</td>
<td>62.10</td>
<td>18.87</td>
</tr>
<tr>
<td>2.</td>
<td>Soil</td>
<td>78.99</td>
<td>8.54</td>
</tr>
<tr>
<td>3.</td>
<td>Soil + Fly ash + NPK</td>
<td>76.55</td>
<td>8.82</td>
</tr>
<tr>
<td>4.</td>
<td>Soil + Fly ash + NPK</td>
<td>76.55</td>
<td>8.82</td>
</tr>
<tr>
<td>5.</td>
<td>Soil + Fly ash + NPK</td>
<td>72.80</td>
<td>10.83</td>
</tr>
<tr>
<td>6.</td>
<td>Soil + Fly ash + NPK</td>
<td>71.06</td>
<td>11.25</td>
</tr>
<tr>
<td>7.</td>
<td>Soil + Fly ash + NPK</td>
<td>69.9</td>
<td>12.41</td>
</tr>
</tbody>
</table>
Soil and fly ash analysis was done as per details mentioned by Hesse, P.R. [70] and Vogel, A.E. [68]. The details have been tabulated in the previous Table No. 4.2. Trace and toxic elements were determined spectrophotometrically [70, 68]. For estimation of Mo, method of A.I. Busev et al was employed [59]. Details are as mentioned below.

**Determination of Trace Elements in the Soil and Plant samples**

**Preparation of Solution of the sample**:

Accurately weighed 1 gm over dry substance (soil, fly ash or plant sample) was taken into a 250 cm³ tall form beaker and 20 cm³ of conc. HNO₃ was added. Cautiously heated. 10 c.c. of 60% HClO₄ was added. Extra HClO₄ was added for complex combustion. The solution was acidified with 10 c.c. conc. HCl. The solution was heated for 15 minutes and diluted with 25 c.c. warm water and filtered through Whatman 41 into a volumetric flask. Silica residue in the filter paper was washed with 0.5 M HCl. The residue was used to determine SiO₂. x H₂O.

The filtrate was used for the determination of trace and toxic elements.
**Determination of B :**

**Reagents :**

1. Preparation of curcumin reagent - Dissolve 0.4 gm curcumin and 5.0 gm oxalic acid in 100 ml ethanol.
2. Preparation of standard boron solution - 0.5716 gm Boric acid per litre.
3. Ca(OH)\(_3\) suspension 0.4g/100 cc. of water.

**Procedure :**

To prepare the colibration curve, aliquots of the dilute standard boron solution into evaporating dish and 5 c.c. of Ca(OH)\(_3\) suspension were evaporated to dryness. After cooling, 1 drop of phenolphthalein solution and then 2 M HCl were added until the pink colour is discharged. 0.5 c.c. of acid was added in excess 4 c.c. of curcumin solution was added. Saffron red colour obtained. It was stable for two hours. Optical density was measured at 540 nm.

Concentration of B obtained has been displayed for various combinations of fly ash and soil, in the back page.
Estimation of Zn:

Reagents:

(1) Standard Zn solution  Dissolve 0.1 g of pure metallic Zn in 50 c.c. of water containing 1 c.c. of conc. H$_2$SO$_4$. Dilute to giving a solution containing 100 µg cm$^{-3}$ Zn.

(2) Ammonium citrate buffer solution 25% - Dissolve 225 gm ammonium citrate in water and dilute to (1 litre) add strong NH$_4$OH solution (45 c.c.) to give a pH of 8.5 Extract the solution with 20 c.c. of dithiozone in CCl$_4$ untill the organic phase remains green and the aqueous phase is orange. This orange phase is extracted with pure CCl$_4$.

(3) Dithizone solution - Dissolve 0.25 gm of diphenylthiocarbazone in 1 litre of pure CCl$_4$. Add 2 litre of 0.02 M NH$_4$OH solution and shake. Discard the CCl$_4$ phase and extract the solution with 50 c.c. portions of pure CCl$_4$ untill the extract is clear green. Add 500 c.c. of CCl$_4$ and 50 c.c. of 1.0 M HCl. Shake and discard the aqueous layer. Dilute with CCl$_4$ to 4 litres and store in a brown glass bottle. Add 25 c.c. of water, partly saturated with SO$_2$.
An aliquot of test solution was pipetted containing up to 2 
µg./cc. of Zn into 125 c.c. separating funnel. Added 5 c.c. of 
ammonium citrate buffer and bring to pH 2.5 with NH₄OH. Using 
three drops of the acid form of bromothynol blue as indicator. Added 
5 c.c. of dithizone solution and shake the mixture for 5 mnts. The 
CCl₄ phase was runned into a clean funnel and repeat the extraction 
of the sample. Combined the two extracts. The aqueous phase was 
taken for analysis.

5 c.c. of ammonium citrate buffer solution was added to 
the aqueous phase and adjusted the pH to 8.3 with NH₄OH solution, 
using phenolphthalein as indicator. Extracted the Zn with two 10 cm³ 
portions of dithizone solution. The aqueous phase becomes orange 
in colour when all the Zn has been extracted. The aqueous phase 
was extracted once more, this time with CCl₄. Discarded the aqueous 
phase. Placed the combined organic phases in to a clean separating 
funnel. Shaked for 2 minutes with 50 c.c. of 0.02 M HCl, discarded 
the organic phase and washed the aqueous layer once with CCl₄.
5 c.c. of citrate buffer solution was added and raise the pH to 8.3 with NH₄OH. 10 c.c. of dithizone solution and 10 c.c. of carbamate solution was added and shaked the mixture for two minutes. Transferred the zinc dithiozonate phase to a clean funnel and shaked with 25 c.c. of 0.01 M NH₄OH solution. Removed 5 c.c. of zinc dithiozonate solution by inserting a pipette through the aqueous phase and the optical density at 535 nm was read. Accuracy of this method is 98-99%. Results are tabulated in the Table No. 4.2.

**Determination of Mo :**

**Reagents :**

(1) KI solution, 50%, w/v aqueous.

(2) Ascorbic acid solution, 5% w/v aqueous, freshly prepared.

(3) Tartaric acid solution, 10% w/v, aqueous.

(4) Thiourea solution 10% w/v, aqueous, freshly prepared filtered.

(5) Dithiozone solution: To 0.2 gm of melted dithiol (m.p. 51°C) add 100 c.c. of 1% w/v NaOH solution and keep the mixture at 51°C with stirring for 15 minutes. 1.8 c.c. of thioglycollic acid was added.

(6) Iso-amyl acetate.
Procedure:

(1) An aliquot of test solution was added containing up to 1 μg/c.c. Mo and treated with an excess of KI solution clear the liberated iodine by adding ascorbic acid solution dropwise. 1 c.c. of tartaric acid solution, 2 c.c. of thiourea solution and 4 c.c. of dithiol solution were added and allowed the mixture to stand for 30 minutes. Extracted the complex into iso-amyl acetate by shaking vigorous for 30 seconds with 5 c.c. of acetate. Dried the organic phase and measured the optical density at 680 nm. Results are tabulated in the Table No. 4.2.

Determination of toxic metals in soil and plant samples:

Determination of Pb:

Reagents:

(i) Standard lead solution - Dissolve 0.1599 gm of oven-dried (110°C), recrystallized Pb(NO₃)₂ in 1% HNO₃ and dilute to 1 litre. This solution contains 100 μg./c.c. of Pb.
**Procedure:**

1 gm of 0.15 mm. soil was digested with acids as described for trace elements determination. After separating $\text{SiO}_2 \times \text{H}_2\text{O}$, the solution was taken in dilute HCl.

An aliquot of sample was taken in separating funnel. 5 c.c. of ammonium citrate buffer was added and the pH was adjusted to 2.5 with NH$_4$OH using 3 drops of the acid form of bromthymol blue as indicator. When the colour turns yellow, 5 c.c. of dithizone solution was added and shaked for 3 minutes. The CCl$_4$ phase is discarded and the extraction repeated with 5 c.c. more dithizone solution. To the aqueous phase 5 c.c. of ammonium citrate buffer solution and 10 c.c. of 10% potassium cyanide solution was added. Raised the pH to 9.3 with NH$_4$OH, using thymol blue as indicator. 10 c.c. of dithizone solution was added and shaked for 3 minutes. The CCl$_4$ phase was transferred to a clean funnel, washed with 50 c.c. of 0.01 M NH$_4$OH solution and filtered into a spectrophotometer cell.

Measured the optical density of the solution at 520 nm. Results for soil, fly ash combination and plant extract have been clear in the Table No. 4.2.
**Determination of Cd:**

It is estimated by dithiozone method \([13]\). 25 ml. of 1:17 H\(_2\)SO\(_4\) was added and heated to dissolve the ash. It was neutralised by NH\(_4\)OH (1:14 v/v). Then 4 ml of 1:11 (v/v) HCl was added. The volume was made to 100 ml. The pH of the sample was adjusted to 6 by adding ammonium hydroxide. 1 ml of sample solution was pipetted out. 0.1 ml of dithiozone solution added and concentration of Cd estimated spectrophotometrically. Stock solution of Cd was prepared by dissolving, 9.8 µg Cd/ml contain. Standard solutions were prepared by dilution method to contain 0.2 to 3.5 ppm of Cd.

Results are tabulated in the Table No. 4.2 in the back page.

**Determination of Mn:**

**Reagents:**

1. HNO\(_3\) concentrated.
2. H\(_2\)SO\(_4\) concentrated.
3. HClO\(_4\) 60%.
4. 8-Phosphoric acid
5. Sodium periodate

[138]
**Procedure:**

Approximately 3 gm of KMnO$_4$ was added to 1 litre distilled water and gently boiled for 20 minutes in a covered beaker. Cooled and filtered in a brown glass bottle. It was standardised with As$_2$O$_3$ and diluted a suitable aliquot to give a solution containing 1000 µg/c.c. of Mn standard solution were prepared to cover the range of 0.5 µg./c.c. to 10 µg./c.c. of Mn.

1 gm of soil fly ash combination or plant sample was digested in 250 c.c. beaker with 20 c.c. HNO$_3$, 10 c.c. H$_2$SO$_4$ and 2 c.c. HClO$_4$. Cooled the digest, diluted with water and filtered into a volumetric flask. Transferred suitable aliquots of solution to small beakers and added 2 c.c. of H$_3$PO$_4$ and 0.5 gm of sodium periodate. heated the mixture until just below boiling point. After the appearance of the permanganate colour, continued heating for a further 30 minutes, adding more periodate. Cooled the solution and transferred to a 100 c.c. measuring flask. Transmittance was read at 545 nm.
RESULTS AND DISCUSSION:

Boron:

Boron is remarkable in soils by its very narrow range of deficiency and toxicity. Less than 1 μg/gm of Boron may mean a deficiency and 3 μg/gm will be toxic. Boron deficiency decreases the permeability of the plasma membrane and boron deficient plants may accumulate N as NO₃⁻. Toxicity of excess boron is connected with transpiration. The content of water soluble boron in soils is influenced by pH, organic matter and amount of colloids.

Boron remain to be least understood of the micronutrients. Total B in soils of tropical India varies between 5 to 80 ppm. Some researchers [10] have reported that the average B content of soils changed on the basis of the surface geology as follows:

- Acid lavas: 22 ppm
- Basalt: 36 ppm
- Limestone: 13 ppm
- Aluminium: 62 ppm
- Slate: 42 ppm
- Gneiss: 7 ppm
- Laterite: 25 ppm

[140]
In humid region Boron content in parts per million is 34.5. In Madhya Pradesh, Chhattisgarh region has not been surveyed for B contents [49]. A Research Scholar [20] obtained a significant positive correlation between available B and pH. available of B was comparatively more between pH 6.0 and 8 [20]. Soil of Rehar basin in Surguja district is acid prone and fly ash should be used to ameliorate soil for increase in boron content.

Catalase activity of boron in the leaves of rice has been found to increase with increase in boron concentration from 0.5 to 1.0 ppm [5].

All the carbohydrate fraction in tomato plant had been found to decrease with the increasing concentration of the element. Positive correlation between boron concentration and nitrogen content have been reported by [51]. Boron concentration in the leaves of legumes have been found to be between 32-95 ppm [14]. Zn+B treatment increased the yields of gram by 89.6% [11].

In the present experiment, when acidic soil was treated with fly ash. Boron content have been found to increase as suggested by the table No. 4.2 and accordingly yield of gram crop have been found to increase in the two years successive experiments.
**Molybdenum:**

Micro organisms use ATP and a powerful reductant to convert N$_2$ into NH$_4^+$ as:

Electron from reduced Ferrodoxin

\[
\text{Reductase (Fe Protein)} \quad \rightarrow \quad \text{Nitrogenase Mo-Fe Protein}
\]

\[
\text{N}_2 + 6e^- + 12\text{ATP} + 12\text{H}_2\text{O} \rightarrow 2\text{NH}_4^- + 12\text{ADP} + 12\text{Pi} + 4\text{H}^+
\]

Reductase Provides electrons with high reducing power and nitrogenase uses these electrons to reduce N$_2$ to NH$_4^+$. Nitrogenase is Mo-Fe Protein structure shown in the next page. The conversion of N$_2$ into NH$_4^+$ by the nitrogenase complex requires ATP and a powerful reductant.

The stoichiometry of the reaction catalyzed by the nitrogenase complex is:

N$_2$ + 6e$^-$ + 12ATP + 12H$_2$O $\rightarrow$ 2NH$_4^-$ + 12ADP + 12 Pi + 4H$^+$
\( \text{NH}_4^+ \) is assimilated into amino acids by way of glutamine and glutamate [60].

Below mentioned table describes the ppm. dry weight of trace elements in herbage plants.

**Table No. 4.3**

**Range of Contents of trace elements in herbage plants**

<table>
<thead>
<tr>
<th>Element</th>
<th>ppm dry wt.</th>
<th>Herbage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>1</td>
<td>grass</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>alfaalfa</td>
</tr>
<tr>
<td>Mo</td>
<td>0.01</td>
<td>red clover</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>clover</td>
</tr>
<tr>
<td>Mn</td>
<td>9</td>
<td>meadow grass</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>alfaalfa</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>grasses</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>alfaalfa</td>
</tr>
</tbody>
</table>
Mo in plant tissues may vary from less than 0.1 ppm to more than 300 ppm does not affect the plant growth. However, if plants containing more than 15-20 ppm Mo are eaten by livestock, animal develops molybdenosis (Cu deficiency) [18].

The average amount of Mo found in agricultural soils is generally reported to vary from 0.5-3.5 ppm.

Soil pH was found to be of greatest importance in the availability of soil Mo to plants [8]. A good response of berseem to Mo application at the rate gap of 0.58 kg/ha [21]. 0.5 ppm of Mo application gave better yield. There is an increase in the yield of berseem by applying 0.5 ppm of Mo. Molybdenum deficiency have definite influence on the growth of plants. Its deficiency depressed nodulation, nitrogen fixation and lowered the yields of legumes. It has role in transformation and translocation of the intermediate nitrogen compounds [22].

In the present study, application of (Mo high) fly ash increased the crop yield. Yield parameters, % germination, increase in protein contents were observed. This is due to two reasons (i) Amelioration of acidic soil increased the nitrogen uptake by plant. as
Mo concentration increased by 50% by the increase of fly ash (ii) pH increased from 6.8 to 7.2. Yield parameters have been displayed in the Table No. 4.4.

**Zinc:**

Zinc is not available in acidic soil, to the plants. Plant, lucerne is a strong Zn - extracting plant and hence is a good cover crop for Zn deficient soils. Shortage of Zn in soil leads to decrease in plant protein synthesis and a decrease in uptake of P and N. Mn uptake increase. Adequate Zn is absolutely necessary for the synthesis of alanine, glycine, proline, threonine, serine, Valine, Lencine, aspartic and glutamic acid. Zn has specific role in the synthesis of fyrozine, tryptophan and phenylamine [15].

Zinc shortage leads to decrease in protein synthesis. Zn serves both some structural and some regulatory roles in enzyme activity in plant tissue. Few enzymes have been indentified as requiring Zn [45] [53].

Zn in Indian soil ranges between 80-89 ppm [65] In Madhya Pradesh, red yellow soil contains 41-74 ppm of Zn [49]. But
extractable Zn is only 4.09 ppm [49]. In acid soil of Rehar basin, major irrigation project, soil is acidic, which hinders the Zn uptake from soil [71].

In the light of above mentioned situation the acidic soil was ameliorated by fly ash from NTPC, Korba Zn was taken by plants and this helped in the increase in the yield of Cicer crop. Zn uptake by plant was estimated by plant analysis. Results are tabulated in the table No. 4.4.

Manganese:

The nature and distribution of Mn in soils is largely dependent upon pH and redox potential. An increasing pH increases oxidation of Mn, thus at high pH, Mn is relatively unavailable to plants [69].

In plants, Mn is a constituent of enzymes, is involved in protein synthesis. Mn is involved in sugar translocation [16].

Some investigators [27] have found the manganese deficiencies characteristically occurring in soils of pH 6.5 to 8.0 and
# Table No. 4.4

## PLANT GROWTH PARAMETER AFTER POT EXPERIMENTS

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments</th>
<th>Percentage Germination</th>
<th>Root Length in cm.</th>
<th>Plant Height in cm</th>
<th>Leaf area in cm$^2$ [Compound leaf]</th>
<th>Chlorophyll content (a)</th>
<th>Chlorophyll content (b)</th>
<th>Weight of 100 Seeds (in gm.)</th>
<th>Amount of N$_2$ (in gm.)</th>
<th>Amount of Protein (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control [plain soil]</td>
<td>60%</td>
<td>9.20</td>
<td>28</td>
<td>3.78</td>
<td>0.201</td>
<td>1.099</td>
<td>31.26</td>
<td>3 mg.</td>
<td>18.752</td>
</tr>
<tr>
<td>2</td>
<td>Control + NPK</td>
<td>60%</td>
<td>10.50</td>
<td>27</td>
<td>3.64</td>
<td>0.440</td>
<td>1.894</td>
<td>33.35</td>
<td>3.21</td>
<td>20.075</td>
</tr>
<tr>
<td>3</td>
<td>Control + NPK + 10% F.A.(90%)</td>
<td>46%</td>
<td>10.87</td>
<td>28</td>
<td>5.10</td>
<td>0.500</td>
<td>1.916</td>
<td>40.23</td>
<td>4.02</td>
<td>25.150</td>
</tr>
<tr>
<td>4</td>
<td>Control + NPK + 20% F.A.(80%)</td>
<td>66%</td>
<td>10.96</td>
<td>29</td>
<td>4.06</td>
<td>0.686</td>
<td>2.071</td>
<td>41.25</td>
<td>4.13</td>
<td>25.956</td>
</tr>
<tr>
<td>5</td>
<td>Control + NPK + 30% F.A.(70%)</td>
<td>73%</td>
<td>11.34</td>
<td>30</td>
<td>3.50</td>
<td>0.705</td>
<td>2.304</td>
<td>42.37</td>
<td>4.23</td>
<td>26.501</td>
</tr>
<tr>
<td>6</td>
<td>Control + NPK + 40% F.A.(60%)</td>
<td>70%</td>
<td>12.42</td>
<td>32</td>
<td>3.38</td>
<td>0.889</td>
<td>3.208</td>
<td>43.47</td>
<td>4.25</td>
<td>26.85</td>
</tr>
<tr>
<td>7</td>
<td>Control + NPK + 50% F.A.(50%)</td>
<td>86%</td>
<td>14.03</td>
<td>34</td>
<td>5.55</td>
<td>0.955</td>
<td>4.570</td>
<td>44.57</td>
<td>4.87</td>
<td>30.53</td>
</tr>
</tbody>
</table>
sometimes occurring in peaty and sandy soils [27]. Photolysis of water, requires Mn [32].

Raychaudhary and Dutta Biswas [23] recorded an average of 1270, 500, 805 and 368 ppm Mn in black, red, lateritic and alluvial soils, respectively. Mn in different Indian soils varied from 6 to 820 ppm [6].

Despite soil is not deficient in Mn, acidic nature of soil prevents uptake. After the use of fly ash, its uptake was found to be optimum for plant growth. Crop yield is increased. This is the importance of fly ash in the agriculture.

**PLANT ANALYSIS**

Plant analysis is a procedure by which the nutritional requirement or status of an element, an inorganic fraction of the element, or some related organic compound or enzyme activity that is associated with the metabolism of the plant. Plant analysis involving the micronutrients B, Cu, Fe, Mn, Mo, and Zn is generally believed to be more useful for some kind of crops.
Generally, plant analysis is used: (1) To make fertilizer recommendations for the current crop; (2) To identify causes of poor growth, due to either deficiencies or toxicities, under field conditions; (3) To identify possible problems associated with introduction of new crops into an area; (4) To evaluate the effectiveness of fertilizer programs; (5) To survey the nutrient status of a crop within a region; (6) To compliment soil test programs; and (7) To gain an understanding of interactions among elements. Plant analysis also has an important role to play in comparing nutrient utilisation by different cultivars and species.

**Theory:**

The underlying assumption behind the use of plant analysis as a diagnostic tool is that there is some relationship between levels of chemical constituents in the plant and health of plants.

**Relevant Chemical Constituents:**

Total concentration of B, Cu, Fe, Mn, Mo and Zn in plant tissue are normally used for diagnostic purposes. However, "active" Fe fractions, enzyme activities associated with an element, and ratios
of elements are also used. A new technique based on the quantity of Fe$^{2+}$ which reacts with o-phenanthroline was found, unlike total Fe, to differentiate between the chlorotic and green leaves of rice and possibly other crops [55]. An attractive feature of this test is that washing of leaves prior to analysis is not needed.

**NUTRIENT LEVEL AND PLANT GROWTH**:

Knowledge of the relationship between nutrient concentration and yield is essential for interpretative purposes and for selection of the most suitable tissue for analysis. On the basis of reports [19, 43] the curve between yield and nutrient concentration is helpful in determining critical values.

**TISSUE SELECTION AND TIME OF SAMPLING**:

When plant analysis is used for making diagnosis or fertilizer recommendations, a part of the plant, usually recently matured leaves, is preferred for determining B, Cu, Fe, Mn, Mo and Zn [24, 4] However, whole-plant analysis at the boot stage for some crops is also favoured by few researchers.
PREPARATION OF SAMPLE FOR ANALYSIS:

During sample preparation following points must be kept in mind: (1) Respiration losses must be avoided, (2) Adequate sub-sampling techniques must be used both before and after grinding, (3) Samples must be effectively ground to the required particle size, and (4) Effective drying and storage techniques must be employed contamination must be guarded against at all stages.

(1) Rotten samples should be discarded. Samples that cannot be processed within 4 hours should be loosely placed in polyethylene bags and kept refrigerated until clearing and oven drying can be done [24].

(2) Sub-sampling errors due to separation of veinal and interveinal tissue should be avoided. If necessary, fresh samples can be cut with stainless steel knives, uniformly mixed and then sub-sampled by quartering.

(3) Both fine grinding and careful mixing are essential. Material for analysis should be ground to pass sieves with either 0.8 mm or 0.4 mm openings.

(4) Enzyme activity is usually stopped in fresh plant tissue by

[151]
heating at maintained rate 60-70°C for 24-48 hours plant tissue is hygroscopic, the finely ground powder must be re-dried after grinding.

CHEMICAL ANALYSIS OF PLANT TISSUE:

Contamination Problems:

An understanding of likely causes of contamination is essential for increasing the efficacy of any plant analysis program, particularly one involving micronutrients. Great care must be taken to (1) remove any surface contamination; (2) avoid contamination during the collection, drying, grinding and storage of plant tissue; and (3) avoid contamination during the analysis.

(1) Any surface contamination likely to interfere with the analysis must be removed soon after the relevant tissue has been collected and before it is dried or wilted.

(2) Care should be taken for harvesting and preparing samples for analysis. Instead of polythylene bags, glass containers should be used to decrease contamination.
Extreme care is needed to avoid accidental or systematic introduction of foreign elements in various analytical operations [34]. The reagents should be stored in polyethylene containers. The glassware must be acid washed followed by rinsing in distilled water essentially.

Rubber used in tubing, stoppers, clamps of shakers etc. can cause Zn contamination. Rubber bulbs attached to transfer pipettes can cause contamination with Zn, Cu and Fe [25].

**Method of Analysis** :

Chemical methods for determination of total elements based on emission spectroscopy, atomic absorption spectrophotometry (AAS), induced coupled argon plasma emission spectroscopy (ICAP), and colorimetry require that the organic matter be removed and the elements be solubilized before analysis. Both wet and dry digestion techniques are commonly used. Choice of methods depends on such factors as convenience, safety, available equipment and element to be analysed.

Where large number of plant samples are analysed for diagnostic purposes, there is tendency to favour dry-ashing techniques. One advantage of the dry-ashing techniques is that a separate digestion
is not required for B, which is difficult to analyse by wet-digestion methods because of possible contamination associated with use of pyrex glassware [61] and volatalization during digestion [12].

Wet- digestion methods involving use of $\text{HNO}_3-\text{HClO}_4$ to destroy organic matter appear reasonably efficacious for recovery of Cu, Zn, Mn and possible Mo, but not for recovery of Fe [67] or B [35] described a wet-digestion method for B determination involving heating of plant tissue with $\text{H}_2\text{SO}_4.\text{H}_2\text{O}_2$ in Vycor tubes. Gestring and soltanpour [61] also concluded that B was not lost from $\text{HNO}_3$ digests heated at 90°C in bottles.

RESULTS:

The deficiency of Zn, B were compensated by the use of fly ash when 50% fly ash was used. Plant analysis for trace and toxic elements have been depicted in the table No. 4.5.

OBJECTIVES OF THE EXPERIMENT:

The experiment work was started with the following objects:

1. Physico-chemical and Mineralogical characterization of fly ash, soil sample and their different combinations, including analysis of trace and toxic metals.
Table No. 4.5

Concentration of trace and toxic elements in Cicer *auretinum* (Concentration expressed in Mg/gm)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Details of Combination</th>
<th>Trace Elements</th>
<th></th>
<th></th>
<th></th>
<th>Toxic Elements</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mn</td>
<td>Zn</td>
<td>Mo</td>
<td>B</td>
<td>Pb</td>
<td>Cd</td>
<td></td>
</tr>
<tr>
<td>A.</td>
<td>Soil</td>
<td>40</td>
<td>35</td>
<td>0.30</td>
<td>28</td>
<td>0.05</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td>Soil + NPK</td>
<td>44</td>
<td>38</td>
<td>0.31</td>
<td>29</td>
<td>0.08</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td>Soil + Flyash 10% + NPK</td>
<td>46</td>
<td>38</td>
<td>0.35</td>
<td>30</td>
<td>0.10</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td>Soil 80% + Flyash 20% + NPK</td>
<td>47</td>
<td>40</td>
<td>0.34</td>
<td>35</td>
<td>0.11</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>E.</td>
<td>Soil 70% + Flyash 30% + NPK</td>
<td>50</td>
<td>41</td>
<td>0.36</td>
<td>37</td>
<td>0.12</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td>Soil 60% + Flyash 40% + NPK</td>
<td>51</td>
<td>43</td>
<td>0.36</td>
<td>40</td>
<td>0.13</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>G.</td>
<td>Soil 50% + Flyash 50% + NPK</td>
<td>53</td>
<td>45</td>
<td>0.36</td>
<td>45</td>
<td>0.13</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>
2. Conduction of pot experiments on Cicer *areitum* with varying proportions of fly ash from NTPC Korba, and soil of Rehar basin irrigation project.


4. Detailed physico-chemical characterisation of soil and plant after harvesting of crop.

5. Studies on trace elements carry over, due to fly ash application, to soil and plants.

6. HPLC (High Precision Liquid Chromatography) of the protein hydrolysate was analysed for different amino acid contents. Results have been incorporated ahead.

**Experimental details:**

The experiment, consisting of 8 fertility levels, was laid out with 2 replications. The details of the experiment are as follows:
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (No Fertilizer applied)</td>
<td>A</td>
</tr>
<tr>
<td>NPK</td>
<td>B</td>
</tr>
<tr>
<td>NPK + 10% F.A.</td>
<td>C</td>
</tr>
<tr>
<td>NPK + 20% F.A.</td>
<td>D</td>
</tr>
<tr>
<td>NPK + 30% F.A.</td>
<td>E</td>
</tr>
<tr>
<td>NPK + 40% F.A.</td>
<td>F</td>
</tr>
<tr>
<td>NPK + 50% F.A.</td>
<td>G</td>
</tr>
</tbody>
</table>

Name of crop            Cicer *areitinum*

Total No. of treatments  8
No. of replications      2
Fertilizer dose (basal)  N:P:K=200:500:300mg/pot.
No. of seed/pot          10
Area of pot              1 sq. ft.

**Schedule of Operations:**

Schedule of operations during the course of experiment is as follows:
<table>
<thead>
<tr>
<th>Operations</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil preparation</td>
<td>25.10.99</td>
</tr>
<tr>
<td>Fertilizer application</td>
<td>10.11.99</td>
</tr>
<tr>
<td>Sowing</td>
<td>15.11.99</td>
</tr>
<tr>
<td>Irrigation/watering</td>
<td>As per requirements</td>
</tr>
<tr>
<td>Insecticide application</td>
<td>As per requirements</td>
</tr>
<tr>
<td>Harvesting</td>
<td>20.02.2000</td>
</tr>
</tbody>
</table>

**DETAILS OF FIELD OPERATIONS:**

(1) **Soil Preparation**:

Soil sample collected from Rehar basin was dried, ground and sieved, then mixed with fly ash in different proportions, homogenised and filled in the pots.

(2) **Fertilizer application**:

Basal dose of fertilizer i.e. 200 mg N, 500 mg P and 300 mg K was given as urea, single super phosphate and Muriate of potash, respectively. It was incorporated well in the soil before sowing.

(3) **Seed Sowing**:

Seeds of Cicer *areitinum* was sown at 1 cm depth.
of seed sown in each pot was 10, at equidistance from each other on the soil surface.

(4) **Irrigation/Watering :**

The crop required watering until germination of seeds. Intensity of watering was decreased during the growth of plants is undesirable for its health.

(5) **Insecticide application :**

Melathian 5% solution was sprayed on the plants, when plants were affected by insects.

(6) **Harvesting and Threshing :**

When the crop was fully matured and becomes golden yellow colours, it was harvested by uprooting gently, and then threshed by hand. The yield of both grain and straw were recorded as on received (air-dried) basis.

**OBSERVATIONS :**

(A) **Growth and Development studies :**

Observations were made for different plant growth stages. viz- Germination, budding, flowering and at maturity stage.
1. **Germination (%)**:

Germination of seeds were observed after sowing. The average Germination was reported as shown below:

**Table No. 4.6**

**Percentage germination after pot experiments**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment (symbol)</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>82</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>82</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>83</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>82</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>85</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>81</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>90</td>
</tr>
</tbody>
</table>

2. **Plant Height**:

Height was recorded from the base in the apex, of the plants, at an interval of 15, 30, 45 days after sowing. The average plant height was reported as shown below:

[160]
Table No. 4.7

Plant height after pot experiments

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments (symbol)</th>
<th>Plant Height (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day after sowing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>1.</td>
<td>A</td>
<td>06</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>06</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>06</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>06</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>06</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>06</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>06</td>
</tr>
</tbody>
</table>

3. Number of branches:

Number of branches were counted at an interval of 30, 60, 90 days after sowing. The observation are as follows:
Table No. 4.8

Number of branches after pot experiments

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatments (symbol)</th>
<th>No. of Branches Days after sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>1.</td>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>7</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>8</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>9</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>12</td>
</tr>
</tbody>
</table>

4. Area of leaves:

Area of leaves were measured at the time of harvesting.

Average leaf area was observed as below:
### Table No. 4.9

#### Leaf area after pot experiments

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatments (symbol)</th>
<th>Leaf area (sq.cm) In full grown plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>45</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>46</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>48</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>48</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>50</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>55</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>60</td>
</tr>
</tbody>
</table>

5. **Chlorophyll Content**:  

Chlorophyll content of the leaves was observed and calculated in full grown plants and are as follows:
### Table No. 4.10

**Chlorophyll contents after pot experiments**

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatments (symbol)</th>
<th>Chlorophyll in full grown plant</th>
<th>Chlorophyll (a)</th>
<th>Chlorophyll (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td></td>
<td>0.201</td>
<td>1.099</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td></td>
<td>0.440</td>
<td>1.894</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td></td>
<td>0.550</td>
<td>1.916</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td></td>
<td>0.686</td>
<td>2.071</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td></td>
<td>0.705</td>
<td>2.304</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td></td>
<td>0.889</td>
<td>3.208</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td></td>
<td>0.955</td>
<td>4.570</td>
</tr>
</tbody>
</table>

(B) **Post-Harvest studies:**

Observations were made after Harvest of plants, viz. Grain yield & straw yield.
1 Grain yield:

Crop was uprooted manually and the grains were detached from these harvested plants and a small of the grain was taken out and then kept in oven at 65% for 24 hours for drying. Thereafter grains were weighed and the yield was worked out in gms for different treatments.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment (symbol)</th>
<th>Grain yield/pot (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>32</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>33</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>35</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>34</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>40</td>
</tr>
</tbody>
</table>
2. Straw yield:

The harvested straw was dried in oven at 65% for 24 hours and weighed.

Table No. 4.12

Straw yield after pot experiments

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatments (symbol)</th>
<th>Straw yield (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>24</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>E</td>
<td>23</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>25</td>
</tr>
<tr>
<td>7.</td>
<td>G</td>
<td>28</td>
</tr>
</tbody>
</table>

Experimental Results:

The results on the cultivation of the Cicer *areitinum* with fly ash are described as follows:

(A) Growth and development studies:

1. Germination:

The sign of germination was seen in some pots only after 5 days of sowing. Maximum germination, in all the treatments was attained on completion of 30 days after sowing. At 30 days after
sowing, clearly observable maximum Germination was attained in "G" (50% soil and 50% fly ash) treatment in comparison with all the remaining treatments.

2. **Plant Height:**

Plant Height increase with advancement in crop age. Maximum height, in all the treatments, was attained on completion of 45 days after sowing. The rate of increase in height was observed to be maximum during 30-45 days sowing. At 45 days after sowing, clearly observable maximum height was attained in treatment "G" in comparison with all the remaining treatments.

3. **Number of Branches:**

The number of branches increased with maturation of plants. Maximum number of branched, was observed on completion of 90 days after sowing. The maximum no. of branches was observed in treatment "G" in comparison with all the remaining treatments at 90 days after sowing.

4. **Area of Leaves:**

The maximum leaf area was observed in a full grown plant in "G" treatment in comparison with all the remaining treatments.
5. **Chlorophyll content**:

   The maximum chlorophyll content in the leaves are in a fully grown plant in "G" treatment in comparison with all the remaining treatments.

(B) **Post harvest Studies**:

1. **Grain Yield**:

   50% soil + 50% fly ash had highest grain yield followed by 40% fly ash treatment. However, all the treatment were significant over control, but non-significant with each other including NPK.

2. **Straw Yield**:

   The straw yield at harvest was observed to be significantly affected by different fertility treatments. The treatments at 50% soil + 50% fly ash significantly increased yield of straw as compared to rest of the fertility treatments including control.

   A critical examination of the results presented above in tables lead to the following observations:

   1. Physico-chemical properties of soil with the application of increasing proportions of fly ash (10-50%) resulted in dramatic improvement in respect of water holding capacity. [168]
organic carbon and available nutrients, all of which have obviously helped in greatly improving the soil texture and fertility cumulatively resulting eventually in significant increase effect on crop growth.

2. No significant change in the concentration of heavy metals i.e. Pb, Cd in Cicer areitinum (Bengal gram) crop was observed even after maximum dose of fly ash being (50%).

3. Trace elements concentration in soil, then in plants increased due to pH = 7 and due to enhancement of trace elements from fly ash to the soil.

★ ★ ★ ★ ★
REFERENCE


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