ACKNOWLEDGEMENT

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I heartily thank my parents for their continued blessings.

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BACKGROUND

Micronutrients play a vital role in soil – plant – animal – human environment in reaching the targeted quantity and quality of food to maintain a good health. After green revolution, soils are showing deficiencies of micronutrients due to the low supplementation of exhausted micronutrients either through fertilizers or manures. Out of 2, 48, 786 soil samples analysed from different states for micronutrient status. 48, 12, 5, 2.5, 33, 13 percent of soils were found deficit in Zn, Fe, Mn, Cu, B and Mo respectively (Singh 1999). Whereas in Karnataka , out of 52,275 soil samples analysed all over the state showed 80, 31, 9, 3 & 25 percent deficiency in Zn, Fe, Mn, Cu & B respectively (State Dept. of Agricultural report).

To correct the micronutrient deficiency and soils, micronutrient fertilizers and organic manures are widely used. Commercial crops in general, fruit and vegetable crops in particular need regular application of micronutrients to get quality and valued produce. As per the survey report of Ministry of Agriculture. Govt. of India (1991 – 92). The consumption of total micronutrient fertilizers in India were 1, 66,225 and 8, 93,437 thousand metric tonnes respectively. With an increase in deficiencies of micronutrients in soils, the use of micronutrient fertilizers is increasing day by day and so also the cost of these fertilizers. Hence, to minimize the cost of input and to meet the demand of micronutrient fertilizers. There is a need to go for application of the micronutrient rich agricultural/industrial wastes and mine waste as source of micronutrients to the crops without affecting the soil wealth and environment.

Karnataka has abundant minerals / ore like Iron, Manganese, Gold, Chromite, Copper, Limestone, Bauxite etc and it stands fourth as per the mineral resource in the country. After the extraction of element from the mine ore, the leftout solid wastes are generally dumped without being used. These solid wastes do contain unextracted and other elements, depending upon the composition of ore. Some of the elements present in the ore wastes are considered as essential plant nutrients. There is a need to study the nutrient composition and their solubility in soil and to use these as source of micronutrient to the crops[-5].

Research on the use of Hutti Gold Mine ore waste in agricultural was initiated during 2006 at the K.L.E's S.Nijalingappa College, Bangalore.

A successful study Use of mining waste (GOT) as a source of micronutrient in agriculture, a pot level work carried out by our senior students (CBBt – 2007) Nitya .J, Nayana S. Raddi, Sushma J. Bhat, Manjunath .S, Vijay Kumar, Srinivasa .R, Shweta K. Divakar, Ashwini G. Salgar, Nethravati .U, Rajeshwari K. Kadam, Nadaf Abrar Ahamad .P encouraged us to take up field level study and the outcome of our almost one year three months study is presented in this

report. Beans, Bengal gram were grown they found 80:20 ratio (Soil to GOT ratio) successful in increased yield, reduced time for complete growth encouraged us to carry the research at field level. The Hutti Gold Ore mine is situated at Raichur district, extracts gold from the ore mineral Auxite embedded in schistose rocks. After the extraction of gold the processed mine ore is called **Gold Ore Tailings (GOT)**. It is defined as the waste minerals generated by the grinding and processing of gold ore and containing materials like schistose rock and economically retrievable minerals. This waste is heaped at the mining site without any use which looks like large hillock. The estimated quantity of the waste available is around crores of tonnes. Therefore, the Indian Gold Development centre, Kolkata has financed the project for utilization in agriculture with the following objectives.

1. To know the nutrient composition, Physico – chemical properties of GOT and its solubility in soil.
2. To know the response of different crops to various level of GOT application.
3. To fix up the optimum level of COT application to different crops and to know the residual effect of nutrients in soil for the succeeding crop.
4. To know the micronutrient uptake by the crop and its effect on the quality of produce.
5. To know the heavy metal content in the waste and its accumulation in plants.

The results of laboratory and field investigations carried to determine the usefulness of Gold Ore Tailings as a source of micronutrient in agriculture is reported here.

When Cabbages and Cauliflowers yield Gold!

Scientists from NewZealand has invented a way of recovering gold from the soil by planting cabbages, however gold has to be in the soil first. (Annexure - 1)

Dr. Chris Anderson, who has been working on the project for years, is now confident that his procedure for extracting gold by growing plants like cabbages on old mine tailing (the residue after they have been stripped of their best ore) will prove commercially viable. By following his method metallurgists can now extract more than a kilogram of gold from a cabbage grown on a hectare of tailings. Massive amounts of tailing are lying near Kolar and Hutti Gold mines in Karnataka. As of today they are a waste product.

The method appears to be successful in the laboratory. Field tests are being carried out to prove in the field. Dr. Anderson discovered the system by accident in September 1997. The ability of the plants to extract some metals from the earth by a process called phytomining was

well known but it did not seem to work for gold. He stumbled on a chemical that could do the trick for gold during his research.

Observations made by Dr. Chris Anderson such as cabbages, cauliflower and turnips were the best. They were planted on tailings and when they have reached their maximum growth the chemical is added to the soil. The plants then suck up the gold particles and die. The withered plants are burned up to extract the gold.

Using some plants and flowers to soak heavy metals from the soil make sense. It is a lit cheaper than the solutions used currently. Most plants find nickel to be a bad material. But Streptanthus polygaloids, a member of mustard family, flourishes in nickel – rich soil. Mining engineers in the US are planning to extract the nickel by this method. They planted a million Streptanthus polygaloids plants on a hectare of wasteland rich in nickel. When the crop was ready for harvesting, it was cut and dried like grass. The dried plants were burnt in an incinerator and the nickel extracted by leaching the ash, which yielded 15 to 20 percent nickel by weight. Such plants and flowers, which for some unknown reason take up significant amounts of metals like gold, copper, nickel, lead, selenium, uranium and zinc along with other nutrients essential for their normal growth are called hyper – accumulators. They store the metals in their roots and leaves. The same technique can be used to clean up soils contaminated with heavy metals.

In India, there are vast tracts of land such as abandoned mines, chemical, units, metal processing units where the soil has been contaminated with heavy metals like chromium, copper, lead, zinc, nickel and manganese. Traces of these metals have gone deep into the soil and no useful crops grown on them.

GENERALISED SYMPTOMS OF PLANT NUTRIENT DEFICIENCY OR EXCESS

(Table – 1)

NUTRIENTS	DEFICIENCY	EXCESS
N	Light green to yellow appearance of leaves, especially older leaves, stunted growth, poor fruit development	Dark green foliage, which may be susceptible to lodging drought, disease & insect invasion, fruit & seed crop, may fail to yield.
P	Leaves may develop purple coloration, stunted plant growth & delay in plant development. Poor root & seed formation.	Excess potassium may cause deficiency in iron & possibly zinc.
K	Older leaves turn yellow initially around margins & die; irregular fruit development. Can not sustain crop growth.	Excess potassium may cause deficiency in magnesium & possibly calcium.
Ca	Reduced growth death of growing tips, blossom rot of tomato, poor fruit development & appearance.	Excess calcium may leads to calcareous soil prevents uptake of other nutrients especially potassium in addition it effects phosphorous and micronutrient availability due to fixation in the soil & thus, creating deficiency of many nutrients. Excess calcium may cause deficiency in either magnesium or potassium
Mg	Initial yellow of older leaves between veins spreading to younger leaves; poor fruit development & production.	High concentration tolerated in plant however imbalance with calcium and Potassium may reduce growth.

Fe	Initial distinct yellow or white areas between veins of young leave leading to spots of dead leaf tissue. Chlorosis of the younger leaves characterizes an iron deficiency. The tissue between the veins gradually turns yellow. While the veins tend to stay green. The tips and margins of some leaves may turn brown & become dry & brittle. Deficiency seen on high pH soil because lime reduces the availability of iron.	Possible bronzing of leaves with tiny brown spots.
Mn	Inter veinal yellowing of young leaves; while veins remain green-white to grey flecks or specks first appear & become more sever on mature leaves half way up the shoot. If a deficiency persist symptoms spread to old leaves then to the youngest leaves .The specked condition is referred to as grey speck & will appear in the inter veinal area of the lower half of older leaves & extend toward the tip as symptoms develop. A mild inter veinal chlorosis develops in the mid section of the leaf & spreads rapidly becoming pale yellow green.	Older leaves have brown spots surrounded by chlorotic circle or zone
Zn	Results in stunted beans, older leaves are smaller and narrower. The older leaves may have light blotches between the veins; younger leaves will have a more normal healthy green color but may be smaller. In flax, a zinc deficiency can cause grayish brown spots in the younger leaves with shortened internodes spaces and stunted appearance. Zinc deficiency symptoms are similar to those of Mn and Fe in some crops. In corn, symptoms occur within a few weeks of emergence as light yellow bands on young leaves. The most severe symptoms occur on youngest leaves from the unfolding bud, referred to as 'white bud'; old leaves remain dark green and appear healthy. In prolonged case of deficiency, the middle leaves develop pale yellowing interveinal chlorosis near the tips. A zinc deficiency prevents the elongation of internodes and leaves.	Excess zinc may cause iron deficiency in some plants.

B	Death of growing points and deformation of leaves with areas of discoloration stunted growth of young plants. The youngest leaves are affected first. They will be misshapen, thick, brittle and small. Because boron is not easily transferred from old to young leaves, older leaves usually remain green and appear healthy. Often dark brown, irregular lesions appear followed by pale yellow chlorosis of young leaves. Stems are short and growing points may die. It is similar to Sulphur deficiency. Boron deficiency leads to reduce seed yield without any evidence of severe deficiency symptoms during vegetative growth.	Leaf tips become yellow followed by necrosis; leaves get a scorched appearance and later fall off. Pea and bean yields have declined by 10-20% due to boron toxicity after a 2lb/ac application of boron.
Cu	High levels of soil phosphorous or heavy application of manure are often associated with a copper deficiency on these soils.	Copper is not readily transferred from old to young leaves, so older leaves remain darker and relatively healthy and deficiency symptoms occur on younger leaves. The visual symptoms includes wilting, pigtailing (whip tailing or curling) of the upper leaves and kenking of the leaf tips, excessive tillering, aborted heads, delayed maturity, poor grain development, irregular patches. Copper deficiency associated with diseases of stem and increased incidence of ergot Chelated forms of copper are very effective.

OBJECTIVES

a. *To use the Processed Gold Ore Tailings (P - GOT) as a source of micronutrients for agriculture.*

b. *To study the influence of the micronutrients over the growth of Green gram, Cauliflower, Cabbage.*

c. *To study the safe disposal of GOT dumped for decades without being used.*

d. *To know the nutrient composition, Physico-chemical properties of GOT and its solubility in soil.*

e. *To fix the optimum level of ore waste application to different crops and to know the residual effect of nutrients in soils for the succeeding crops like Onion, Tulip etc.*

f. *To the heavy metal content and its accumulation in plants.*

MATERIALS

I. **(GOT):** Hutti Gold Mines Ltd in Raichur District of Karnataka is only the leading gold producer in India. The rich grade ore contains about 4 – 6 g of gold per ton. The ore is available at the depth of 3,000-3,500 feet below the ground level. The ore from deep mines are shifted to the surface is crushed and finely ground to about -200 mesh. This when processed yields the precious metal but leaves behind, a huge amount of solid residue called Gold Ore Tailings (GOT).

The extensive joint research work done by the faculty member of Department of Chemistry of K.L.E's S.Nijalingappa College, Bangalore and Surface Chem Finishers, Peenya, Bangalore on GOT showed that GOT still contained economically recoverable quantity of gold present in it. The continuous "Unmanned Heaped Leaching" of GOT carried out yielded 0.68g per ton of GOT. The residue now left behind after the recovery of the precious metal from GOT is termed as "Processed GOT". The Cyanide content still associated with the processed GOT is neutralized and then used as the material for the supply of micronutrients for the present research project. This with suspected Cyanide scientifically and completely killed is used as a basic raw material.

The above mentioned researchers provided us the processed GOT to study it as a source of micronutrients for agriculture. This is the basic raw material for the present research project.

II. **(SOIL):** The typical red soil used to cultivate Green gram, Cauliflower and Cabbage by the farmers of Dabaspet area near Tumkur is used for the present study.

III. **(SEEDS):** **a)** Chemically treated seeds were selected for Green gram. **(Fig - 2)**

b) Namdhari certified seeds (Export quality) for Cauliflower. **(Fig - 16)**

c) Maharani ISI certified seeds for Cabbage. **(Fig - 24)**

IV. **(WATER):** Borewell water was used to irrigate the crops till the cultivation was complete.

METHODOLOGY

The chosen land for study was divided into six strips each measuring 37.4 x 10 foot (420 square foot). Two adjoining strips were identified for each crop. Alternate strip was mixed with GOT by sprinkling in such a way that every square foot of Soil had about 0.5kg of GOT. Soil and GOT were thoroughly mixed. **(Fig – 1)**

Homogeneity of mixture was ensured by ploughing. All the strips were irrigated and later exposed to air for drying. The strips were identified by erecting sign boards. First two adjacent strips were identified to grow Green gram, next two for Cauliflower and the last two for Cabbage. Green gram was cultivated by directly sowing the seeds. Seeds of Cauliflower and Cabbage were sent to Sri Rama Nursery, where the ISI Certified seeds were sown in a Coco – peat. One tray of Coco – peat contains 1 kg of the coco – peat. Before loading the coco – peat, mixture is sterilized at 100º C and then loaded to trays. The loaded seed trays are kept in Green House for the germination of saplings for about 25 days. Water sprat is done only once in a day.

(Fig – 17 and 18)

Saplings of Cauliflower and Cabbage were grown in nursery which later on was replanted in the identified strips **(Fig – 19)**

Periodically plants were irrigated, removal of weeds and other required care like spraying of insecticide was carried out for the crops grown both in Soil and Soil + GOT.

Particular Insecticides were used for the crops grown for the present study for the better yield and to prevent invasion of pests.

- Green gram – ***Imidacloprid 17.8 %*** **(Fig – 37)**
- Cauliflower **– *Success*** **(Fig - 36)**
- Cabbage **– *Parrysulphan 35 EC*** **(Fig – 38)**

Equipments were used to spray the above insecticides. **(Fig – 39)**

Green gram was harvested after about three months, Cauliflower was harvested after two months and Cabbage was harvested after two months.

BLOCK DIAGRAM OF CHOSEN LAND

Table - 2

1 Plot GREEN GRAM SOIL + GOT 37.4'x10' = 374 square feet area		**2 Plot GREEN GRAM SOIL 37.4'x10' = 374 square feet area**		**3 Plot CAULI FLOWER SOIL + GOT 37.4'x10' = 374 square feet area**		**4 Plot CAULI FLOWER SOIL 37.4'x10' = 374 square feet area**		**5 Plot CABBAGE SOIL+ GOT 37.4'x10' = 374 square feet area**		**6 Plot CABBAGE SOIL 37.4'x10' = 374 square feet area**

About 200kgs of GOT was sprinkled over the alternate strips measuring 37.4×10 square feet.
(Fig – 1)

PREPARATION OF LAND AND ADDITION OF GOT

There are different methods adopted for mixing of ore tailings

a. Broadcast mixing
b. Band placement
c. Ring method

BROADCAST MIXING

This method is by sprinkling GOT by hand by covering maximum area of the plot. We have used this method in our present study. Recommended level of GOT is to be applied directly or mixing with farm yard manure at the time of land preparation or with fertilize at the time of sowing.

1. EFFECT OF GOT ON THE GROWTH OF GEEEN GRAM (Phaseolus aureus)

1.1 A Brief account on Green gram plant (Phaseolus aureus)

Kingdom:	Plantae
Class:	Magnoliopsida
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Tribe:	Phaseoleae
Genus:	***Phaseolus***

Green gram is an erect bushy annual widely cultivated in warm regions of India and Indonesia and United States for forage and especially its edible seeds; chief source of bean sprouts used in Chinese cookery; sometimes placed in genus Phaseolus.

Phaseolus species are used as food plants by the larvae of some Lepidoptera species including Common Swift, Garden Dart, Ghost Moth Hypercompe albicornis, Hypercompe icasia, the Nutmeg and various caterpillar species. Snail Flower was discovered in 1753 and in 1970 moved from the genus family of Phaseolus to the Vigna family.

Mung bean, also known as **green bean**, **mung**, **moong**, **mash bean**, **munggo** or **monggo**, **green gram**, **golden gram**, and **green soy**, is the seed of *Vigna radiata* which is native to India. The split bean is known as **moong dal**, which is green with the husk, and yellow when dehusked. The beans are small, ovoid in shape, and green in color. The mung bean is one of many species recently moved from the genus *Phaseolus* to *Vigna* and is still often seen cited as *Phaseolus aureus* or *Phaseolus radiatus*. These are all the same plant.

Mung beans are mainly cultivated in India, Indonesia, China, Burma, and Bangladesh. They can also be cultivated in other hot and dry regions of South Europe and Southern USA. In India and Bangladesh, they are grown during two seasons. One is the Rabi season (starting November), and the other is the Kharif season (starting March). Mung beans are tropical (or sub-tropical) crops, and require warm temperatures (optimal at 30-35°C). Loamy soil is best for mung bean cultivation[-4].

1.2 Brief note on Nitrogen Fixation by leguminous plants

The process by which N_2 is reduced to NH_4^+ is called Nitrogen fixation. It is, so far as we know, carried out only by prokaryotic micro – organisms. Principal N_2 – fixers include certain free – living soil bacteria, free – living cyanobacteria on soil surfaces or in water, cyanobacteria in symbiotic associations with fungi in lichens or with ferns, mosses and liverworts and bacteria or other microbes associated symbiotically with roots, especially those of legumes. Their role in nitrogen fixation is of great importance to the food chain in forests, freshwater and marine environments and even artic regions. Furthermore, the activation of the roots of nitrogen fixing plants benefits the roots of surrounding plants, either

through excretion of nitrogen from nodules or through microbial decomposition of nodules or even whole plants. This contribution is important in agriculture, in which mixed legumes often are used as pastures[-2]

About 15% of the nearly 20,000 species in the family Fabaceae (Leguminosae) have been examined for N_2 fixation and approximately 90% of these have root nodules in which fixation occurs.

The micro – organisms responsible for nitrogen Fixation in roots of many species have been identified. In sole tropical trees it is various cyanobacteria. In legumes, bacterial species of the closely related genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* are responsible. A particular Rhizobium or Rhizobium – like species is generally effective with only one legume species. All rhizobia are aerobic bacteria that persist saprophytically in the soil until they infect the root hair or sometimes a damaged epidermal cell. Root hairs usually respond to invasion first by curling and surrounding the bacterium; curling is caused by unidentified molecules released from the bacteria. Yet another interesting finding is that genes of the rhizobia that control the production of the molecules that cause curling are activated first by compounds released by the roots, probably root hairs. In alfaalfa, green gram, faba bean, the most abundant compounds are certain flavonoids. These results emphasize that various specific chemical signals are probably sent from the root hair and are somehow recognized by the invading bacteria[-3].

Next, enzymes from the bacteria degrade part of the cell wall allow bacterial entry into the root hair cell itself. Then the root hair produces a thread like structure called the *Infection thread*, which consists of an infolded and extended plasma membrane of the cell being invaded, along with new cellulose formed on the inside of this membrane. The bacteria multiply extensively inside the thread, which extends inwardly and penetrates through and between the cortex cells.

In the inner cortex cells the bacteria are released into the cytoplasm and stimulate some cells (especially tetraploid cells) to divide. These divisions lead to a proliferation of tissues, eventually forming a mature **root nodule made largely** of tetraploid cells containing bacteria, as some diploid cells without bacteria. Each enlarged, non – motile bacterium is referred to as a **bacteroid**. A typical root nodule cell contains several thousand Bacteriods. The Bacteriods usually occur in the cytoplasm in groups, each group surrounded by a membrane called **peribacteroid membrane**. Between the peribacteroid membrane and the bacteroid group is a region called the **peribacteroid space**. Outside the peribacteroid space in the plant cytosol is a protein called **Leghaemoglobin**. This molecule is red because of a heme group attached as a prosthetic group to the colorless globin protein. Leghaemoglobin give legume nodules a pink color, although it is much more dilute in nodules of non – legumes. Leghaemoglobin is thought to help transport O_2 into the bacteroids at carefully controlled rates. Too much O_2

inactivates the enzyme that catalyzes nitrogen fixation, yet some O_2 is essential for bacteroid respiration.

Nitrogen fixation in root nodules occurs directly with in the bacteroids. The host plant provides bacteroids with carbohydrates, which they oxidize and from which they obtain energy. These carbohydrates are first formed in leaves during photosynthesis and then are translocated through phloem to the root nodules. Sucrose is the most abundant carbohydrate translocated, at least in legumes. Some of the electrons and ATP obtained during oxidation by the bacteroids are used to reduce N_2 to NH_4^+.

1.3 Study of Nitrogen fixation in Green gram by isolation of Rhizobium bacteria from Root Nodules.

AIM:

To isolate Rhizobium from root nodules of leguminous plants i.e. Green gram grown in our research land with Soil and Soil + GOT were taken. (**Fig 5 and 7)**

INTRODUCTION:

Rhizobium is a gram negative, rod, shaped aerobic non – sporing bacteria that symbiotically forms nodules with root of leguminous plants. Where it fixes atmospheric nitrogen. The process of nitrogen fixation is catalyzed by enzyme "Nitrogenase" in the cells of Rhizobium. The Nitrogenase enzyme is sensitive to oxygen and Nitrogenase enzyme is protected by Leghaemoglobin which is synthesized or formed by only the symbiotic relationship i.e. neither of the symbiontal are able to synthesize it individually.

In the laboratory, Rhizobium is isolated from the nodule using the yeast extract mannitol agar.

PRINCIPLE:

Entry of rhizobia into the plant root system is rather peculiar. Studies on Rhizobium provided the first reaction of the root system to the presence of rhizobia is a curling and deformation of root hair.

On entering the cortical cells they lodge themselves in the polyploidy cells of nodules called Bacteriods. Bacteriods surrounded by Leghaemoglobin which are ready to fix nitrogen and make available ammonium to plants in the form of amminoacid. Once this bacteria enters through the root the formation of a thread like structure visible inside the root hair called injection thread.The curling effect has been attributed to production of Indole Acetic Acid (IAA), a hormone produced in the root region by rhizobia. Once the bacterium gains entry into the root system, they divide randomly and become plueromorphic in shape(X, Y, I, V, W). At this point, they are referred to as Bacteriods. Their division of cortical cells, it takes place rapidly and gives rise to a structure called Nodule. Thus rhizobia can be observed in nodules as Bacteriods of various shapes.

REQUIREMENTS:

YEMA media, Petriplate, inoculation loop, Slides, Green gram root nodules, 0.1% Mercuric Chloride, 70% Alcohol, Sterile Distilled Water, Grams stain.

PROCEDURE:

1. Isolation of bacteroids from root nodules

I. Legume root nodule sample are selected and separated from the root end and were washed thoroughly using water.
II. They are surface sterilized with 0.1% of Mercuric Chloride for 5 minutes and washed thoroughly with distilled water.
III. They are transferred into 70% Alcohol for 1 minute.
IV. The roots are washed with sterile water for 3 - 4 times.
V. They are then transferred into clean glass slide with a drop of saline and crushed with the help of another slide.
VI. Some of the nodules that are crushed are streaked by taking loopful of inoculum on YEMA. Then plates are incubated at 37ºC for about 24 hours.

2. Microscopic observation of root nodules

I. A surface sterilized nodule is crushed between two clean glass slides.
II. The nodule suspension adhering on both the slides is smeared and air dried and heat fixed.
III. The heat fixed slid is subjected to gram's staining technique.
IV. It is air dried and observed under microscope.

RESULTS:

Bacteriods of various shapes such as X, Y, V, W, I were obtained which are stained pink i.e. Gram negative. Green gram grown with GOT shows maximum activity of Nitrogen fixation through Rhizobium bacteria. **(Fig 6 and 8)**

DISCUSSION:

The "Nif genes" (Nitrifying genes) are responsible for producing Nitrogenase enzymes in the cells of Rhizobium. The Nitrogenase enzyme is sensitive to oxygen and hence Leghaemoglobin prevents or controls the oxygen limit that enters Bacteriods. This Nitrogenase enzyme needs 16 ATP molecules to reduce one N_2 molecule to $2NH_3$ molecule.

$$\mathbf{N_2 + 3H_2 + 16\ ATP \rightarrow 2NH_3 + 16ADP + Pi}$$

1.4 Study of Extraction and Estimation of protein from Green gram

AIM:

To extract and estimate the amount of protein present in the Green gram grown in presence and absence of GOT in research field by Salt precipitation method.

INTRODUCTION:

Most pure proteins are insoluble in distilled water but they dissolve in dilute salt solutions. Additions of small amount of neutral salts increase the solubility of proteins. The added ion can cause small change in the ionization of side chains and also interfere with interaction between protein molecules. So the overall effect increases the interaction between the solute and the solvent. This is salting in phenomenon. This depends on the saturation of the salt solution. The addition of high concentration of the neutral salt to an aqueous solution of proteins causes it to precipitate. This is because at very high concentration it increases the interaction between added ion and water decreases the possibility for the protein water interaction. Hence proteins get precipitated from the solution. This is salting out phenomenon. Most of the proteins are soluble in 0.1M Ammonium Sulphate. But the concentration of the salt is increased to 3M to the protein precipitation. **(Fig – 11)**

PRINCIPLE:

Different types of proteins are present in all the cells and tissues. The proteins may vary in their solubility and extractability in different solvents. Depending on the procedure and the solvent used proteins get extracted from the tissues. Typical proteins are insoluble in low salt content. Most useful and general precipitation method is salt precipitation method using Ammonium sulphate. Salting out of proteins using high concentration of the salt is widely used procedure for protein purification. The amount of Ammonium sulphate used for precipitation is expressed in terms of saturation.

Lowry's method is one of the most sensitive method and widely used methods for the protein estimation. The reagent used here is Folin – cio – Chateaus (FC) reagent contains phosphomolybdic acid and phosphotungstic acid. The amminoacids contain Tyrosine and Tryptophan present in the protein react with these and produce blue colored complex. The Cu^{++} ions present in copper sulphate react with – CO – NH group of the protein in alkaline condition to give blue colored complex. The absorbance is read at 660nm.

REAGENTS REQUIRED:

I. **Alkaline Copper reagent**:
   a. Reagent A: Sodium Carbonate in 0.1N NaOH.
   b. Reagent B: 0.5 % Copper Sulphate solution and 1% Sodium potassium tartarate.
   c. Reagent C: Mix 50ml of Reagent A and 1ml of reagent B.

II. **Folin – Cio – Calteau (FC) reagent**: Use 1: 1 dilution.

III. **Standard protein**: Weigh 50mg of Bovine Serum Albumin (BSA) and dissolve in 50ml of distilled water. From this take 10ml and make it up to 50ml using volumetric flask. The working standard concentration should be 200µg per ml.

IV. **Unknown sample**: Sample of plant tissue extract.

PROCEDURE:

1. Preparation of plant tissue extract

Weigh 3 grams of Green gram and grind with 5ml of cold distilled water using mortar and pestle. The volume is made up to 15ml with cold distilled water. The tissues homogenate was taken in centrifuge tube and centrifuged at 1000rpm for 5 minutes and supernatant was taken. **(Fig – 10)**

To the collected supernatant 0.8g of solid Ammonium sulphate is added carefully to produce 45% saturation. The above solution is allowed to stand on ice for 45 minutes. The solution is centrifuged at 3000rpm for 10 minutes. The precipitate is collected and dissolved in 5ml of distilled water.

2. Estimation of proteins by Lowry's method[-1]

Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml aliquots of standard protein solution (200µg / ml) to different clean and dry boiling tubes. Make the volume in each case to 1.0ml with distilled water. Add 5.0ml of alkaline Copper reagent to all the tubes. Mix the solution well and allow the tubes to stand for 10 minutes. Then add 0.5ml of 1:1 diluted. Folin – cio – chateaus (FC) reagent to each tube and thoroughly mix the contents of each tube using cyclomixer. After keeping the test tubes for rest of about 30 minutes, read the absorbance at 660nm against the blank solution. Draw a graph by plotting amount of protein (µg) verses absorbance (nm).From the straight line obtained. Calculate the amount of protein present in the given unknown solution.

(Fig 12, 13 and 14)

RESULT:

- The 30% saturation of the given unknown sample grown in presence of GOT contains **83, 72000** µg of proteins.
- The 30% saturation of the given unknown sample grown in absence of GOT i.e. only in Pure soil contains **56, 76000** µg of proteins.

2. EFFECT OF GOT ON THE GROWTH OF CAULIFLOWER (Brassica oleracea botrytis)

2.1 Brief account of Cauliflower (Brassica oleracea botrytis)

Cauliflower (Hindi: Gobi) is one of several vegetables in the species ***Brassica oleracea***, in the family Brassicaceae. It is an annual plant that reproduces by seed. Typically, only the head (the *white curd*) is eaten while the stalk and surrounding thick, green leaves are used in vegetable broth or discarded. Cauliflower is very nutritious, and may be eaten cooked, raw or pickled.

Its name is a variation of cole flower or kale flower (cole and kale being variations of the same word), an acknowledgment of its unusual place among a family of food plants which normally produces only leafy greens for eating. *Brassica oleracea* also includes brussels sprouts, kale, broccoli and collard greens, though they are of different cultivar groups[-4].

2.2 Cultivation

The preferred garden soil for cauliflower is well drained, moist and has significant organic matter with a pH of 6 to 7. Cauliflower is typically started indoors six to eight weeks prior to setting out in the garden and is typically ready for harvest two months after transplanting. As with all brassicas, seeds germinate best with a soil temperature of 25°C (77 °F). The vegetable requires a cool, moist climate - if temperatures go too high, the plants will not produce flower heads, if too low a temperature is reached, and the plants might button, creating small heads. It is cold tolerant, but will not survive hard frosts. Of all the brassicas, cauliflowers have the largest number of growth requirements, and the ability to successfully grow cauliflower in a home garden is often thought to be the hallmark of well-managed soil and a good gardener.

The most common insect pests of cauliflower are cabbage worms, aphids and flea beetles. Diseases of cauliflower include black rot, club root and viral yellows. The viral diseases are primarily spread by insects.

2.3 Nutrition

Cauliflower, raw (edible parts) Nutritional value per 100g (3.5 oz)

Table - 3

Energy 20 kcal 100 kJ	
Carbohydrates	5 g
Sugars 2.4 g	
Dietary fiber 2.5 g	
Fat	0 g
Protein	2 g
Thiamin (Vit. B_1) 0.057 mg	4%
Riboflavin (Vit. B_2) 0.063 mg	4%
Niacin (Vit. B_3) 0.53 mg	4%
Pantothenic acid (B_5) 0.65 mg	13%
Vitamin B_6 0.22 mg	17%
Folate (Vit. B_9) 57 mg	14%
Vitamin C 46 mg	77%
Calcium 22 mg	2%
Iron 0.44 mg	4%
Magnesium 15 mg	4%
Phosphorus 44 mg	6%
Potassium 300 mg	6%
Zinc 0.28 mg	3%

Cauliflower is low in fat, high in dietary fiber, folate, water and vitamin C, possessing a very high nutritional density. As a member of the brassica family, cauliflower shares with broccoli and cabbage several phytochemicals which are beneficial to human health, including sulforaphane, an anti-cancer compound released when cauliflower is chopped or chewed. In addition, the compound indole-3-carbinol, which appears to work as an anti-estrogen, appears to slow or prevent the growth of tumors of the breast and prostate.

Cauliflower also contains other glucosinolates besides sulfurophane, substances which may improve the liver's ability to detoxify carcinogenic substances. A high intake of cauliflower has been found to reduce the risk of aggressive prostate cancer. Cauliflower possesses a single mutation in a gene called ***CAL***, controlling meristem differentiation into inflorescence. This causes the cells at the floral meristem to gain an undifferentiated identity, and instead of growing into a flower, they grow into a lump of undifferentiated cells. This mutation has been selected through domestication at least since the Greek empire. Cauliflower is one of several vegetables in the species Brassica oleracea, in the family Brassicaceae. It is an annual plant that reproduces by seed. Typically, only the head (the white curd) is eaten while the stalk and surrounding thick, green leaves are discarded. Cauliflower is very nutritious; Its name is a variation of cole flower or kale flower (cole and kale being variations of the same word), an acknowledgment of its unusual place among a family of food plants which normally produces only leafy greens for eating.

The Brassica oleracea species is the same species as cabbage, brussels sprouts, kale, broccoli and collard greens, though they are of different cultivar groups. The preferred garden soil for cauliflower is well drained, moist and has significant organic matter with a pH of 6 to 7. Cauliflower is typically started indoors six to eight weeks prior to setting out in the garden and is typically ready for harvest two months after transplanting. As with all brassicas, seeds germinate best with a soil temperature of 25 ± 1 °C (77 ± 2 °F). The vegetable requires a cool, moist climate - if temperatures go too high, the plants will not produce flower heads, if too low a temperature is reached, and the plants might button, creating small heads. It is cold tolerant, but will not survive hard frosts. Of all the brassicas, cauliflowers have the largest number of growth requirements, and the ability to successfully grow cauliflower in a home garden is often thought to be the hallmark of well-managed soil and a good gardener. The most common insect pests of cauliflower are cabbage worms. Aphids and flea beetles will also attack cauliflower. Control of pests can be achieved by using biological controls such as using geraniums and borage as companion plants to repel pests, the ***Bacillus thurengensis*** bacterium, predatory insects or chemical pesticides. Diseases of cauliflower include black rot, club root and viral yellows. Crop rotation helps to prevent fungal and bacterial diseases. The viral diseases are primarily spread by insects. Cauliflower is low in fat, high in dietary fiber, folate, water and vitamin C, possessing a very high nutritional density. As a member of the brassica family, cauliflower shares with broccoli and cabbage several phytochemicals which are beneficial to human health, including sulforaphane, an anti-cancer compound released when cauliflower is chopped or chewed. In addition, the compound indole-3-carbinol, which appears to work as an anti-estrogen, appears to slow or prevent the growth of tumors of the breast and prostate. Cauliflower also contains other glucosinolates besides sulfurophane,

substances which may improve the liver's ability to detoxify carcinogenic substances. A high intake of cauliflower has been found to reduce the risk of aggressive prostate cancer.

Cauliflower is a vegetable which can be produced abundantly from April until early December in the maritime Northwest. Rich, humusy soil is essential, as cauliflowers tend to have weak root systems. Heavy soils must be especially well amended with organic matter. With cauliflower only continuous *rapid* growth will produce high quality heads, so ideal soil conditions should be created and fertilizer used. One-quarter to 1/2 cup of complete organic fertilizer worked into the soil immediately below the plant will produce good results if the soil is light enough to permit good root development. A high soil pH of 6.5 - 7.5 is also important for best development.

2.4 Culture

Start transplants of the *early types* from March through June, or direct seed them if desired from mid-April through June. Sow seeds 1/2 inch deep in clumps of 4 or so seeds, 24 inches apart, and 30 inches between rows. Thin gradually to the best single plant. Start *late types* by direct seeding (or start transplants) during June. Start *overwintering* cauliflower during early July to early August, but do not fertilize Walcherin types heavily until spring regrowth begins, when they should be fertilized periodically so as to make a large plant by April. In spring, one teaspoonful of bloodmeal every 3 weeks sprinkled about the base of the plant will induce maximum growth.

2.5 Insects

Similar to cabbage, dealing with the root maggot during May/June can be discouraging. If plants will not establish by direct seeding despite following the above cultural directions, or if transplants become stunted and don't grow even if well-fertilized, the cause may be symphylans, which eat sprouting cole seeds before they emerge and chew off root hairs on cabbage family plants. The high ammonia content of fresh chicken manure may be the best "organic" symphylan control measure, especially for coles.

2.6 Disease

The common disease problems of cauliflower are similar to cabbage. Many disease and insect problems can be reduced if cole crops can be grown in a 4 - 5 year rotation with non-cruciferous crops.

2.7 Blanching

All varieties tend to self-wrap the flower; even so, to obtain completely white heads, especially from spring-sown varieties, tie the inner leaves around the curd when it starts to form, or break over some inside leaves so they lie flat on the flower and provide shade for it.

2.8 Harvest

When mature, the flowerettes are just beginning to separate and get slightly ricey. At this point the flavor is still at peak quality and the size is maximum.

3. EFFECT OF GOT ON THE GROWTH OF CABBAGE (Brassica oleracea capitata)

*3.1 A Brief account of Cabbage (*Brassica oleracea capitata)

Cabbage is from a group of plants known as the cole crops. The word "cole" derives from the Middle English word "col". The Romans called these crops "caulis", and the Greeks called them "kaulion". All these words mean "stem". This group of plants includes cabbage, cauliflower, broccoli, kale, collards, kohlrabi, and Brussels sprouts. Wild cole crops are found growing along the Mediterranean and Atlantic coasts of Europe. Cabbages and kale presumably originated in Western Europe; cauliflower and broccoli in the Mediterranean region. Cabbages and kale were the first of the cole crops to be domesticated, probably about 2,000 years ago. Before these crops were domesticated they were collected from the wild and used primarily as medicinal herbs. The other forms of the cole crops were domesticated at later dates, and Brussels sprouts are the most recent crop, having come into existence less than 500 years ago.

3.2 General Cabbage information

If you want a garden food supply you can depend on, cabbage is a must. Easy to grow and much sweeter than those bought in the grocery store, Territorial's selection of cabbages are harvestable almost 12 months of the year.

3.3 Culture

Cabbages can be grown as transplants or direct sown, although early sowings may fail in cold rainy Springs. Cabbage is a hardy, cool-season crop that does best under uniform cool, moist conditions. Sow early cabbage types March through June. Later maturing types should be sown late May to early June to allow for the heads to form during the relative cool of the Fall. Although it will withstand wide temperature variations, the optimum soil temperature range for growth is between 60 – 65 F.

Fertility requirements for cabbages are relatively high. 1-1/2 to 2 cups of complete organic fertilizer worked into the soil around each plant will provide the nutrition necessary for best production. The preferred pH is 6.5 and 1 to 1-1/2 inches of water per week is required for uninterrupted growth.

When direct sown, plant the seed 1/2 inch deep, 5 seeds per foot; thinned or transplanted plant spacings should be 18 inches for small-framed types, and 24 inches for large-framed varieties, in rows 2 - 4 feet apart.

3.4 Nutrition

Cabbage, raw (edible parts) Nutritional value per 100g (3.5 oz)

Table - 4

Energy 20 kcal 100 kJ	
Carbohydrates	5.8 g
Sugars 2.4 g	
Dietary fiber 2.5 g	
Fat	0.1 g
Protein	1.28 g
Thiamin (Vit. B_1) 0.061 mg	5%
Riboflavin (Vit. B_2) 0.040 mg	3%
Niacin (Vit. B_3) 0.234 mg	2%
Pantothenic acid (B_5) 0.212 mg	4%
Vitamin B_6 0.124 mg	10%
Folate (Vit. B_9) 53 µg	13%
Vitamin C 36.6 mg	61%
Calcium 40 mg	4%
Iron 0.47 mg	4%
Magnesium 12 mg	3%
Phosphorus 26 mg	4%
Potassium 170 mg	4%
Zinc 0.18 mg	2%

3.5 Insects

The first sign of cabbage worms will be white diamond-back moths fluttering close to the ground and over the plants. They will lay eggs in the soil around the plants, which hatch into worms that can cause severe root and head damage. To control light infestations simply spray plants with BT. For heavy infestations fill a water can with 1 tablespoon per gallon of BT solution, and drench the soil around the plants. Root maggots can be controlled by placing a physical barrier around each plant, such as a Reemay tent. This will prevent the fly from laying her eggs on the stem. Also see Chinese Cabbage. To bait cabbage worms, mix wheat bran into the above BT water solution until all water is absorbed by the bran. Hand sprinkle or broadcast bran mixture in and around the base of plants. Reapply as necessary. To control flea beetles, aphids, and symphylans see broccoli, Brussels sprouts, and cauliflower.

3.6 Disease

The home gardener growing cabbage can prevent many cole crop diseases by practicing crop rotations, using sterile starting mixes, and adopting strict garden sanitation methods.

3.7 Harvest

Early types mature fast and burst quickly so they must be harvested promptly. Later types, maturing in late Summer or Autumn when growth rates are slow, will hold in the field for much longer, often several months. When cutting the heads from the stems, leave two or three of the wrapper leaves to protect against bruising. Over-mature heads are subject to splitting, especially if they are exposed to moisture fluctuations. Successful storage starts with a good cultivar, free of diseases or injuries. Late storage types will keep for up to 6 months when kept at 32F and at 98-100% relative humidity, while early types will store for 1 - 2 months.

Common names:

Flowering Cabbage and Kale, Ornamental Cabbage and Kale, and Decorative Cabbage and Kale.

Scientific name: ***Brassica oleracea*** - **Explanation of scientific name:**

Brassica - the ancient Latin name for cabbage.

oleracea - vegetable –like[-4].

Flowering Cabbage and Kale are ornamental versions of the cabbage and kale that we use as edible vegetables. They have been hybridized and selected for ornamental features including bright foliage colors and compact growth. Growing best in cool weather, the seeds are sown in the early summer and several month old plants are transplanted into the garden in the late summer or early fall. As the weather cools, the purple, pink or white colors of the leaves become quite pronounced. The plants can tolerate cold weather, and in mild winters can look attractive up until spring arrives.

The name “flowering” cabbage and kale is a misnomer since the ornamental parts of the plant we value are really just fancy leaves that superficially resemble the petals of flowers. The plants can and often will eventually produce real flowers if left in the garden. As biennials they only bloom after experiencing winter conditions after their first season of

growth. Long stalks bearing small yellow flowers will develop when the weather warms in the spring. The flowers are not especially attractive and the foliage is usually quite ragged after surviving the winter. Most people remove the plants and deposit them in a compost pile long before they bloom.

The species to which flowering cabbage and kale belongs is truly fascinating. Wild Brassica oleracea is native to the coastal regions of Europe, from England to Italy, and still persists there today. The ancient Greeks and Romans grew it for food, but when it first was cultivated remains lost in history. The diversity of form within this species, that is the result of selection over the millennia by people, is almost unmatched in the plant world.

SAMPLE PREPARATION FOR ANALYSIS OF SOIL AND SOIL + GOT

Analysis of samples was carried out at Indian Institute of Horticultural Research (IIHR)

Coning and quartering is a method used by analytical chemists to reduce the sample size of a powder without creating a systematic bias. The technique involves pouring the sample so that it takes on a conical shape, and then flattening it out into a cake. The cake is then divided into quarters and two quarters which sit opposite one another are discarded, whilst the other two are combined and constitute the reduced sample. The same process is continued until a reasonable amount of material is left behind. Analyses are made with respect to the sample left behind.

In analytical chemistry, sub-sampling is a procedure by which a small, representative sample is taken from a larger sample. Good sub-sampling technique becomes important when the large sample is not homogeneous.

Coning and quartering involves pouring the sample so that it takes on a conical shape, and then flattening it out into a cake. The cake is then divided into quarters; two quarters which sit opposite one another are discarded, while the other two are combined and constitute the reduced sample. The same process is continued until an appropriate sample size remains. Analyses are made with respect to the sample left behind.

Riffle box splitting:

A riffle box is a box containing a number (between 3 and 12) of "chutes" - slotted paths through which particles of the sample may slide. The sample is dropped into the top, and the box produces two equally-divided subsamples. Riffle boxes are commonly used in mining.

The reduction in size of a granular or powdered sample by forming a conical heap which is spread out into a circular, flat cake. The cake is divided radially into quarters and two opposite quarters are combined. The other two quarters are discarded. The process is repeated as many times as necessary to obtain the quantity desired for some final use (e.g. as the laboratory sample or as the test sample).

If the process is performed only once, coning and quartering is no more efficient than taking alternate portions and discarding the others. Coning and quartering is a simple procedure useful with all powdered materials and with sample sizes ranging from a few grams to several hundred pounds.2 Oversized material, defined as > 0.6 millimeters (mm) (3/8 in.) in diameter,

should be removed before quartering and be weighed in a "tared" container (one for which its empty weight is known). Preferably, perform the coning and quartering operation on a floor covered with clean 10 mil plastic. Take care that the material is not contaminated by anything on the floor or that any portion is not lost through cracks or holes. Samples likely affected by moisture or drying must be handled rapidly, preferably in a controlled atmosphere, and sealed in a container to prevent further changes during transportation and storage.

The procedure for coning and quartering is illustrated. The following procedure should be used:

1. Mix the material and shovel it into a neat cone.
2. Flatten the cone by pressing the top without further mixing.
3. Divide the flat circular pile into equal quarters by cutting or scraping out 2 diameters at right angles
4. Discard 2 opposite quarters.
5. Thoroughly mix the 2 remaining quarters, shovel them into a cone, and repeat the quartering and discarding procedures until the sample is reduced to 0.4 to 1.8 kg (1 to 4 lb).

Coning and quartering is the method of reducing a gross sample to manageable size while still obtaining a representative sample. After thorough mixing, the material is formed into a cone that is then leveled into a flat circular heap. The heap is then divided diametrically into four equal quarters. Two opposite quarters are discarded and the remaining two opposite quarters are retained and formed into a second cone. The process is repeated until the four equal quarters contain the desired amount of sub sample.

Coning and quartering. The main problem with scoop and thief sampling is that the samples are removed from a static powder bed, and there is simply no effective way to sample a nonmoving powder and obtain a representative sample. One procedure that attempts to address this problem is that of coning and quartering. For this procedure, one begins by pouring a cone of material, which is then flattened as evenly as possible. The material is then divided into quarters, whereupon two opposite quarters are discarded. The remaining two quarters are recombined and poured into another conical pile, which is then flattened and divided. The quartering process is continued until one is left with an amount of material that is the desired sample Great care must be taken when obtaining a sample by coning and quartering. When the initial powder sample is poured, it will undergo the full range of

processes that result in segregation of particles according to their relative size. Because their flow ability is the poorest, the finer particles will collect at the center of the cone, and the coarser particles will flow toward the edges of the cone. Hence, each wedge of a poured cone will become severely segregated by the act of pouring, making the process of subdivision and recombination critical. It is not unusual to obtain large standard deviations in particle-size results when the coning and quartering method is used to obtain a sample. **(Fig - 31)**

BASIC PRINCIPLES AND TECHNIQUES AND ANALYTICAL METHODS INVOLVED IN CAULIFLOWER FLOWERS AND CABBAGE LEAF ANALYSIS

Nutrients are essential for productivity and quality of different crops. The perennial crops are different from seasonal crops in their nutrient requirement due to their size, population density rate of growth and rooting pattern. Determination of the nutritional needs of fruit trees must be made prior to the renewed growth or determination of potential yield. Orchards are normally fertilized on the basis of experience of successful growers, speculation or salesmanship. However, reliable information is needed to decide how much manures and fertilizers should be applied to fruit trees for an economic response. This can be obtained best by use of one or more of the diagnostic methods in consideration with a background of research results. The best diagnostic tool is one that recommends nutrient application, where a direct economic response is probable. Leaf analysis seems to be the best method for identifying the need for application of nutrients.

PRINCIPLE OF LEAF ANALYSIS:

Leaf analysis is based on the premise that plant behavior is related to the concentration of essential minerals in leaf tissue (Smith, 1966). Leaf analysis as a method for assessing the nutrient requirements of a given crop is based on the assumption that, within certain limits, there is a positive relation between doses of the nutrients supplied, leaf content and yield.

According to Bould (1966) leaf analysis is based on four assumptions:

1. Leaf is the principle site of plant metabolism.
2. Changes in nutrient supply are reflected on the composition of the leaf.
3. These changes are more pronounced at certain stages of development than at others.
4. The concentration of nutrients in the leaf at the specific growth stages are related to the performance of the crop.
5.

Leaf analysis is use full in many ways:

1. As an aid to understand the internal functions of the nutrients in the plant.
2. To confirm the deficiency detected by visual symptoms.
3. To distinguish between two nutrients which cause similar deficiency toxicity symptoms.
4. To identify mineral imbalance in the absence of a visible symptoms this is not correctable by addition of a simple nutrient.
5. To investigate toxicity of elements or nutrients.

6. To identify and indicate interactions or antagonism between nutrients.
7. For diagnostic use in cases of simultaneous multiple nutrient deficiencies or toxicities.
8. To ascertain whether applied nutrients have entered the plant system.
9. To identify whether hidden hunger is causing sub-optional plant performance, slow growth and lower yield/quality.
10. To prevent deficiencies rather than correct them after they develop.
11. In survey work to locate areas of incipient deficiencies.

The leaf analysis further can be useful on one of the following ways:

1. To develop a nutrient guide for recommendation of manures and fertilizers for economic optimum yield.
2. To correct defective manure and fertilizer application used by the growers, which often lead to soil, water and environmental problems.
3. To determine whether or not the supply of one or more nutrients is in adequate, satisfactory or unnecessarily high.
4. To provide a common denominator for making parallel comparison from solution, sand, soil and field cultures, location, year and climate.
5. To show that the lack of response to applied nutrients results from their failure to make into the leaves, there by preventing a wrong conclusion from being drawn and directing attention to the cause of the lack of absorption.

Thus, leaf analysis can be used for diagnostic testing, monitoring and predictive or prognostic testing. Diagnostic testing (some times called "Trouble shooting") is under taken to diagnose the cause of poor vigor (*e.g.*, patches of poor growth in other wise healthy crop, orchard) or to confirm a diagnosis made on the basis of plant symptoms or soil test.

Monitoring samples are collected to assess the adequacy of current fertilizer practice and related management factors (e.g. irrigation technique). Monitoring can be done to compare the nutritional status of crops grown in successive years and allow the fertilizer used to be adjusted according to trends in the chemical composition of the plant. It can also be used to monitor the nutritional status of an individual crop during its development (*i.e.*, crop logging) and thus ensure that its nutrient requirements are being met satisfactorily.

Predictive or prognostic testing may be used in three ways:

1. Analysis of samples collected during early crop development is used to predict the likely hood of nutrient deficiency occurring before crop maturity is reached.
2. Analysis of root is used to predict its likely behavior in storage.
3. Analysis of seeds is used to predict likely deficiencies in succeeding crops.

Each form of testing may identify latent deficiencies (also called "Hidden hunger"), which limits crop production while the plants exhibit no obvious symptom of deficiency. Distinction between three types of testing is necessary because the intensity and pattern of sampling varies considerably for each method of testing. However, in all testing methods there is a need to document details of site management and crop conditions before, and at the time of sampling.

Steps involved in leaf analysis:

1.Leaf sampling technique.

2.Sample handling and processing.

3.Leaf analysis methodology.

4.Developing leaf nutrients norms by interpretation of data and nutrient recommendation.

I. Leaf sampling technique:

The general principle in leaf sampling is to collect 'recently/youngest mature leaf or petiole'. Depending on rated of growth, the age of recently matured leaves varies. The nutrient concentration in the index tissue is influenced by factors associated with the plant or with environment and soil are those related to sample handling and processing.

Common guide lines for leaf and petiole sampling:

1. Select a vegetative terminal, unless otherwise specified.
2. Sample at chest height which is more than two meters height from ground level.
3. Collect a composite sample from north, east, west and south.
4. To monitor changes in nutrient status based on recommendation/modified practice, sample is collected from permanent sampling site.
5. Select leaves, which are fully exposed to sun light and avoid leaves in shade.
6. Collect samples prior to irrigation and fertilizer application.
7. Avoid sampling soiled, diseases and insect or mechanically damaged plants and exclude senescing and dead tissue from gathered material.
8. Avoid sampling plants growing within areas having unusual feature e.g. rocky areas etc.

9. Do not sample when plants are under water or temperature stress.
10. Avoid sample contamination.
11. Standardize sample collection during certain period of the day for elements whose concentration is known to vary diurnally.

II. Sample handling:

1. Field sampling should be carried out early in the morning.
2. Scientists carrying out sampling should was hands before sampling and use dispensable gloves. The collected material should be immediately shifted to cool container.
3. After transport from the field the samples can be stored in refrigerator at five degree Celsius till these are decontaminated. Samples covered with soil particles may be cleaned using deionised water. For micronutrients determination, avoid contact between plant materials and methods.
4. If it is difficult to send the samples to the laboratory within 12hours, these may be washed at sampling site and partially dried before mailing to the laboratory.

III. Decontamination:

1. The sample may be divided into four sub-samples for effective washing in minimum time.
2. One sub-sample may be pre-washed with deionised water, if it is soiled. If sprayed chemical deposits are visible, the deposits can be removed by cotton soaked in 0.2% detergent solution.
3. The sample should be passed through 4 solutions *viz.*, 0.2% detergent solution, N/1 HCl solution, distilled water and finally in deionised water.

IV. Sample drying:

Initial drying is designed to de-activate rapidly all plant enzymes there by minimizing weight loss and biochemical changes, and to remove all water from the tissues so that the sample reaches a oven dry state and its weight remains unchanged with revealed drying. The samples may be dried at $65^{o}C$ -$70^{o}C$ in stainless steel lined hot air oven, which allow adequate circulation of air between samples.

Final drying: initial dried samples are some times weighed, ground and stored for a period prior to the analysis when these may absorb atmospheric moisture. This necessitates second drying at $70^{o}c$ for 12hours immediately before analysis.

***V. Grinding and storage*:**

Dried samples are ground to reduce field samples to manageable size and this facilitates the preparation of homogeneous sub-samples for chemical analysis. During grinding, care must be taken to ensure that it does not segregate into cores and fine particles. Samples should be stored in clearly labeled, air-tight glass or poly-carbonate containers which can withstand a second drying cycle. This should prevent samples from being infected by insects during storage.

Laboratory analysis:

Standard and reproducible techniques should be used. With the advent of computer linked report generating instrument improvement in the efficiency and speed of quantitative plant analysis have become possible.

I. ***Determination of total nitrogen in plant samples:***

Principle:

A known quantity of powdered sample is digested with concentrated H_2SO_4 in presence of digestion mixture by heating in a fume chamber. During this process, organic nitrogen gets converted into ammonical. During the process of distillation, Ammonia gas released is trapped in basic acid which is then back titrated with standard acid and nitrogen is calculated.

Reagents and their preparation:

1. Concentrated Sulphuric acid.
2. Digestion mixture: 100gms of Potassium sulphate: 20gms of Copper sulphate: 1gm Selenium grind them to get fine powder.
3. 40% Sodium hydroxide solution: dissolve 40gms of Sodium hydroxide in about 80ml of water and make up the volume to 100ml.
4. Standard Sulphuric acid (0.05N): dilute 1.4ml of concentrated Sulphuric acid to 100ml and then standardize it with standard Sodium carbonate solution.
5. Standard Sodium carbonate (0.1N): dissolve 5.3gms of dried Sodium carbonate in some volume of distilled water and make upto 1litre.
6. Boric acid (4%): dissolve 40gms of Boric acid in hot distilled water, cool and then make upto 1litre.
7. Mixed indicator: mix 0.1gm of Bromocresol green with 0.07gm of Methyl red and dissolve this mixture in 100ml Ethanol.
8. Hydrogen peroxide solution.

Procedure for determination of Nitrogen in plant sample:

In the analysis of nitrogen two steps are involved

1. Digestion of the sample.
2. Distillation of the sample.

I. Digestion of sample using Block digester:

1. Weigh about 0.5 gm of plant sample into digestion tube (depending upon nitrogen level in the sample, the sample weight can be reduced or increased).
2. Add 10ml of concentrated Sulphuric acid to the sample that is into the digestion tubes along with 1gm of catalyst mixture and leave for half an hour.
3. Keep the tubes into the holes of block digester and put on the electrical switch for heating. When the tubes are heated, the contents in the tube become black in color. On heating, the contents of the tube may start foaming and may raise upto the top of the tubes. The tube can be lifted till the foaming subsides. Then add hydrogen peroxide in small increments (1ml-2ml at a time) to avoid frothing by excessive reaction (if you add more, the contents may come on to your hand and burn). When sufficient hydrogen peroxide (5ml) has been added, the contents in the tube become very light or even colorless which indicates that sample is digested.
4. After cooling the contents of the tube, make up the volume to 100ml.
5. Take 5ml from this 100ml and distill it to get the amount of nitrogen in the sample.

Kjeldahl method of digestion of the sample:

1. Weigh about 0.5gm of plant sample into 100ml of Kjeldahl flask.
2. Add 1-2gm of digestion mixture and 20ml of Sulphuric acid into the Kjeldahl flask swirl gently so as to mix the sample (to keep contact with sample) and keep for half an hour and then heat it slowly till a light bluish green residue is obtained. Then cool the contents and make up the volume to 100ml with distilled water.

II. Distillation:

1. Pipette out 10ml of digested sample into micro Kjeldahl distillation unit and add small unit of water (5ml).
2. In a conical flask, 10ml of 4% Boric acid solution is taken containing mixed indicator to which the condenser outlet of the flask is dipped.
3. Add about 10ml of 40% Sodium hydroxide to the sample in the distillation flask and distill it by heating. Check for completion of distillation with red litmus paper (if unchanged, it shows that the distillation is complete).

4. Take out the flask containing Boric acid and titrated against standard Sulphuric acid till it becomes pink in color which is the equivalence point.
5. Blank may be run and the titer value may be recorded.
   1ml of 0.1N H_2SO_4= 0.0014g N.

$$\%N = \text{Titre value} \times \frac{\text{Volume of digested}}{\text{Weight of sample}} \times \frac{1}{\text{Volume of aliquot taken}} \times 0.0014 \times 100.$$

$$R \times \frac{100}{0.05} \times \frac{1}{5} \times 0.0014 \times 100.$$

Calculation*:*

a. Weight of sample= 0.5gm.
b. Volume of digest= 100ml.
c. Normality of H_2SO_4= 0.1 N
d. Volume of aliquot taken= 5ml.
e. Titre value = (sample titre value – blank titre value) = R.

Digestion of sample for other nutrients (except Nitrogen):

The plant material can be digested in diacid mixture and make up volume to estimate different elements in the plant sample.

Preparation of diacid mixture:

It is prepared using Nitric acid and perchloric acid in the ratio of 9:4

Procedure for digestion of plant sample:

1. Weigh about 1-2gms ground leaf or plant material into 100ml volumetric flask and add 10ml diacid mixture and leave it in the digestion chamber for at least 1hour. Ad 5ml of Nitric acid for pre-digestion and leave for 1hour if required.
2. Now, keep them on hot plate and heat at low temperature till the frothing stops. Then rise the temperature of the hot plate. At higher temperature, the acid fumes disappear or ceases.
3. The contents are further evaporated until the volume is reduced to 3-5ml and the content become colorless.

4. After the contents are cooled, make up the volume to 100ml with distilled water and filter through What Mann's NO.1 filter paper for analysis of different elements in this solution. One can analyze P, K, Ca, Mg, S, Fe, Mn, Zn and Cu (and many more can be analyzed) from this digested sample.

I I. ***Phosphorus analysis:***

Vanadomolybdate and phosphates react to give yellow color complex in Nitric acid medium. This method is simple and stable.

Preparation of reagents:

Ammonium molybdate and Ammonium vanadate in HNO_3 medium: dissolve 25gm of Ammonium molybdate in 400ml of distilled water. Dissolve 1.25gms of Ammonium metavanadate in 300ml of boiling distilled water. Now add this boiled vanadate solution to the molybdate solution and cool to room temperature. Now add 250ml of conc.HNO_3 to the cooled solution and dilute to 1litre finally.

Preparation of standard curve:

Dissolve 0.2195gms of analytical grade (AR) Potassium dihydrogen phosphate (KH_2PO_4) in 200ml of distilled water and dilute to 1liter. This solution contains 50µg P/ml (50ppm).

Procedure:

1. Transfer 0,1,2,3,4,5ml of above solution through a pipette into 50ml volumetric flask to get 0,1,2,3,4 & 5ppm of Phosphorus respectively.
2. Add 10ml of Vanado-molybdate reagent to each flask and make up the volume with distilled deionised water; shake thoroughly and keep for 30min for development of yellow color.
3. This yellow color is measured at 470nm in spectrophotometer or colorimeter using blue filter.
4. Plot the absorbance recorded against the concentration and draw a curve called standard curve.
5. From the standard curve, we can determine the concentration if unknown solution.

Procedure for estimation of Phosphorus from plant digestion:

1. An aliquot of 5ml is pipette out into a 50ml volumetric flask.
2. Add 10ml of Vanado-molybdate reagent to each flask and make up the volume to 50ml using distilled water. Shake well and take reading after 30min. the yellow color is measured as described. The color is stable for 24hours or more.
3. From the standard curve, you can get the concentration of the sample for which you have to read the absorbance.

Calculation:

a. Weight of the sample= 1gm.

b. Final volume made= 100ml.

c. Volume of aliquot taken= 5ml.

d. Volume made to= 50ml.

e. Concentration from standard curve= R ppm.

$$\%P \text{ in the sample} = \frac{100}{1} \times \frac{50}{5} \times R \times \frac{100}{10^6} = \frac{R}{10}$$

Determination of Potassium in plant sample:

Potassium is estimated either though flame photometry or emission spectrophotometer or atomic absorption spectrophotometer in emission mode. When the plant digested solution is fed to the flame, the atoms of solution gets excited. While returning back to the ground state, the atoms emit radiation characteristic of that element which is measured. The intensity of radiation depends on the amount of that particular element in the solution and it is directly proportional.

Reagents:

100ppm Potassium: dissolve 1.9069gm of AR Potassium chloride (KCl) in 1litre volumetric flask and make up the volume to give 1000ppm of Potassium.

100ppm Potassium for working solution: Take 10ml of 1000ppm Potassium into a 100ml volumetric flask and make up the volume to get 100ppm of Potassium (0,191gm of KCl in 1liter gives 100ppm).

Preparation of standard curve:

Take 0, 1,2,3,4 &5ml of 100ppm Potassium solution into separate 50ml volumetric flask (which gives 0, 2,4,6,8 &10ppm Potassium) and make up the volume and mix well. Now take the readings from flame photometer by feeding these solutions into the flame. Plot flame photometer readings against concentration of standards and draw the standard curve.

Procedure for Potassium estimation of sample:

Take 1ml of digested sample and make upto 50ml with distilled water feed the sample to flame photometer and record the readings. From the standard curve, the concentration of sample can be known.

Calculation:

$$\%K = \text{ppm K from graph} \times \frac{\text{Volume made (Original)}}{\text{Weight of sample (1g)}} \times \frac{\text{volume made}}{\text{Wt, of sample (1g)}} \times \frac{100}{10^6}$$

Determination of Calcium and Magnesium:

Calcium and Magnesium can be determined by two methods:

1. Versenate method (EDTA method)
2. Estimation through atomic absorption spectrophotometer (emission mode).
3.

Versenate method:

Preparation of reagents:

1. Standard EDTA (Ethylene Diamine Tetra Acetic acid) solution (0.01N): dissolve 1.96gms EDTA in 900ml of distilled water and make up the volume to 1liter. This is to be standardizing with 0.01N calcium chloride.
2. Standard Calcium solution: dissolve 0.500gms of dried Calcium carbonate in minimum amount of 0.2N HCl solution and it is boiled to expel carbon dioxide and then dilute it to 1liter. This solution is used to standardize EDTA solution.
3. 10% sodium hydroxide solution: dissolve 10gms of Sodium hydroxide in some amount of water and make to 100ml.
4. Mureoxide indicator: mix 0.2gm of Mureoxide with 40gm of powdered Potassium sulphate.
5. Ammonium chloride- Ammonium hydroxide buffer (pH-10): dissolve 67.5gm of pure Ammonium chloride in 570ml of concentrated Ammonium hydroxide (Ammonia) and make to 1liter and adjust pH to 10. (Caution- Ammonia bottle may be cooled to freezing temperature in the refrigerator before opening. Otherwise it may blow off. This is very important precaution to be taken while opening Ammonia bottle).
6. Eriochrome black-T indicator: 0.5gm of Eriochrome black-T and 4.5gm of Hydroxylamine hydrochloric acid (AR) dissolve in 100ml of 95% ethanol.

Principle involved in Versenate estimation of Calcium and Magnesium:

Calcium and Magnesium gets complexed by EDTA. First Ca gets complexed at pH-12 and Mg at pH-10 afterwards. Ca is estimated by using Mureoxide indicator in presence of Sodium hydroxide. Then Ca and Mg is estimated by using Eriochrome black-T indicator in presence of Ammonium chloride-ammonium hydroxide buffer solution.

Estimation procedure:

Calcium alone:

1. Pipette out 5ml of diacid extract into a porcelain basin.
2. Add 10%Sodium hydroxide drop by drop to neutralize the acidity and then another 5ml excess to maintain pH at 12. Add a pinch of Mureoxide indicator and titrate against 0.01N EDTA till the color changes from pinkish red to violet color. From this titre value the amount of Ca present in the sample can be calculated.

Calcium and magnesium estimation:

1. Pipette out 5ml of diacid extract into a porcelain basin and about 10ml of Ammonium chloride-Ammonium hydroxide buffer solution.
2. Add 2-3 drops Eriochrome black-T indicator and titrate against 0.01N EDTA till the color changes from wine red to blue. This titre value represents both Ca and Mg. If you deduct the Ca titre value, you get the titre value of Mg.

Calculation:

a. Weight of sample= W gms (1gm).
b. Volume made= V ml (100ml)
c. Volume taken for titration= 5ml.
d. Volume of 0.01N EDTA used for Ca & Mg= A ml.
e. Volume of 0.01N EDTA used for Ca alone= B ml.
f. Volume of 0.01N EDTA used for Mg alone= (A-B) ml.

i. 1ml of 0.01N EDTA = 0.002g of Calcium.

$$Ca\% = \frac{\text{Volume made}}{\text{Wt. Of sample}} \times \frac{1}{5} \times B \times \frac{0.0002}{} \times 100$$

ii. 1ml of 0.01N EDTA = 0.00012g of Mg

$$Mg\% = \frac{\text{Volume made}}{\text{Wt. Of sample}} \times \frac{1}{5} \times (a-b) \times 0.00012 \times 100$$

Determination of Sulphur in plant sample:

Sulphur is estimated in plant sample by a method called turbidimetry method. The Sulphur content in the solution is precipitated by Barium ions as Barium sulphate (which develops turbidity). This turbidity is measured by turbidity meter or colorimeter which is proportional to the amount of Sulphur present in the sample.

Reagent preparation:

1. Barium chloride: A.R Barium chloride crystals should be used (avoid using powder). Half gram of Barium chloride is required per sample which can be kept ready by making into small packets to add just before taking readings to the solution.
2. Gum acacia solution: dissolve 0.5gm powdered gum acacia solution in a mixture containing 50ml glacial acetic acid and 50ml distilled water. Store the solution in refrigerator to avoid growth of microbes. This is adding to help in stabilizing turbidity.
3. Prepare 6N HCl acid.
4. Standard Sulphur solution: dissolve 0.543gm A.R Potassium sulphate in some amount of distilled and dilute to 1liter. This fives 100ppm Sulphur solution.

Preparation of standard curve:

1. Pipette out 0, 5,10,15,20 &25ml different aliquots of 100ppm Sulphur standard solution into 250ml volumetric flask to get 0, 2,4,6,8 &10ppm Sulphur solution respectively.
2. From this, pipette out 10ml of each solution separately into 50ml conical flask and add 1ml of 6N HCl and 1ml 0.5% gum acacia solution. Mix the contents by swirling and add 0.5g of Barium chloride crystals.
3. Allow the flask to stand for a minute and then swirl the contents gently by keeping on a table (without lifting) until the Barium chloride crystals are dissolved.
4. Now read the absorbance of the solution on spectrophotometer at 420nm.
5. The absorbance is plotted against concentration of Sulphur and the standard curve is drawn.

Procedure for estimation of Sulphur in the sample:

1. Pipette out 10ml of aliquot plant digest in to conical flask.
2. Add 1ml of 6N HCl and 1ml of gum acacia and mix well. Then add about 0.5gm of Barium chloride and keep for a minute and swirl gently and read the absorbance.

Calculation:

a. Weight of sample taken = 1gm.
b. Volume of digested sample = 100ml.
c. Volume taken = 10ml
d. Volume made to 1litre.

$$\%S = \text{Graph ppm} \times \frac{\text{Volume of digested sample}}{\text{Wt. Of sample}} \times \frac{\text{Volume made}}{10} \times \frac{100}{10^6}$$

Determination of Iron, Manganese, Zinc & Copper in plant sample:

These four elements are estimated by using atomic absorption spectrophotometer. The atomic absorption spectrophotometer is based on the principle that when the atoms of metallic elements are introduced into a flame, a large percentage of metal atoms will remain in the non-emitting ground state and are receptive of light radiation of their own specific resonance wavelength. The absorption of radiation is proportional to the concentration of the atoms of that element present in the solution.

Estimation of Iron:

The plant sample digested earlier in a diacid mixture can be used for estimating Fe, Mn, Zn and Cu.

Preparation of standard curve:

1. Dissolve 7.022gm of A.R Ammonium ferrous sulphate in 400ml of deionised water and add 5ml of concentrated H_2SO_4 and make up the volume to 1liter which gives 1000ppm Iron. From this, prepare 100ppm Iron solution by taking 10ml into 100ml volumetric flask and make up the volume. By preparing 5, 10, 15, 20 & 25ppm Iron solution and feeding to atomic absorption spectrophotometer, we can get standard curve in the instrument. Further by feeding sample digest, we can estimate the Iron content present in the sample.

$$\text{Fe in ppm} = R \times \frac{100}{\text{Wt. Of sample}} \times \text{dilution factor if any}$$

Note: We can also dissolve 1gm of pure Iron wire in 50ml of (1+1) AR grade HNO_3 and diluting to 1litre to get 1000ppm Fe.

Manganese estimation:

Dissolve 3.076gm of Manganese sulphate in double distilled water and make upto 1liter to get 1000ppm of Manganese. 10ml of this solution is diluted to100ml to get a solution of 100ppm of Manganese. From this we can prepare 5, 10, 15, 20, and 25ppm of Manganese solution for standard curve. After feeding the standards, we can feed the samples and get the readings.

$$\text{Mn in ppm} = R \times \frac{100}{\text{Wt. Of sample}}$$

Note: dissolve 1gm of Manganese metal in a minimum volume of (1+1) HNO_3 and dilute to 1liter with 1% HCl to get 1000ppm of Manganese.

Estimation of Zinc:

The procedure is similar to Iron and Manganese but standard solution should be prepared separately.

Standard solution: dissolve 4.398gm of AR Zinc sulphate in distilled water and make upto 1000ml which gives 1000ppm Zinc solution. 10ml of this solution is diluted to 100ml to get 100ppm solution of Zinc. From this solution we can prepare 5, 10, 15, 20 & 25ppm of Zinc solution for the standard curve. After feeding the standards, we can feed the samples for getting the readings.

Note: dissolve 0.5gm of Zinc metal in a minimum volume of (1+1) HCl and dilute to 1litre with 1% HCl for the purpose of standards if required.

Estimation of Copper:

Standard solution preparation: dissolve 3.929gm of AR Copper sulphate in distilled water and make upto 1litre which gives 1000ppm of Copper solution. 10ml of this solution is diluted to 100ml to get 100ppm of Copper solution. From this solution, you can prepare 5, 10, 15, 20 &25ppm of Copper solution for standard curve preparation. After that standards are fed and the readings are recorded.

Note: dissolve 1gm of Copper metal in minimum volume of (1+1) HNO_3 and dilute to 1litre with 1% HNO_3 for preparation of standard solution.

BASIC PRINCIPLES AND PRACTICE OF SOIL TESTING

In its simplest form soil testing means a measure of available nutrients during the immediate growing season in terms of the degree of sufficiency, deficiency or toxicity and of correction if any to grow a crop of desired yield and quality. However, soil testing is normally performed:

- When news crops are proposed to be grown on land
- To monitor soil fertility to grow crops at desired level of quantity and quality.
- To assess the extent of deterioration in terms of acidity, salinity, alkanity or development of any other forms of deficiency or toxicity.

The practice of soil testing involves four important steps:

- Soil fertility research.
- Soil sampling and sending to the soil testing laboratory or concerned extension agency
- Preparation of soil sample and its analysis.
- Interpretation of analysis and making in recommendations.

1. RESEARCH:

Any soil fertility evaluation programme can be no stronger than the research base on which it is built. Such a research must be problem – oriented, inter – disciplinary must take economics into consideration, must be of high quality and should provide maximum useful information.

2. SOIL SAMPLING:

2.1 Collection of a Representative Sample:

For soil testing to be meaningful, it is crucial that the soil samples should be a representative of the root zone of a field / plot for which the fertility evaluation is desired. Proper soil sampling saves time, energy and money. Soil testing also helps in the formulation of recommendations of the cost effective nutrient management practice among the farmers.

2.2 *Place of taking a Soil Sample*:

Surface soil layer upto 25 to 30 cm depth is the center of all biological activity. It provides the best medium for the growth of crops. Rapid changes in available nutrients occur in this layer depending upon the cultural practices and fertilizer / amendments applied. In contrast, sub – surface soil layers undergo little change and require testing only for diagnosing unusual problems like hard – pan, build –up of residues of mobile

nutrients and others. In general, soil sampling from 0 to 45cm (0 to 18'') depth is preferred for fruit crops white 0 to 22.5 cm (0 to 9'') depth is ideal for vegetable crops.

2.3 ***Information to be provided with the sample:***

The following information will not have to be provided along with sample, which is helpful in formulating the recommendations:

i. Name of the grower.
ii. Full postal address.
iii. Exact location of the field (Name/Landmark of the Block/Survey number).
iv. Irrigation – source, frequency and adequacy.
v. Drainage condition of the field (well – drained / water logged).
vi. Soil type (Soil / Clay / Loam).
vii. Previous cropping history and yield (name 2 – 3 crops and give approximate yield obtained).
viii. Manures and Fertilizers applied to previous crop / season.
ix. Date of Sampling.
x. Slope (level / gentle slope / sloppy / very steep).
xi. Salinity, if observed (whether salt encrustation; Kari or Alkaline patches visible on the surface).
xii. Area represented by the sample (acres / hectares).
xiii. Crop and variety to be grown during this season.
xiv. Cropping pattern to be followed during next two years.
xv. Other relevant information.

2.4 ***Procedure for soil sampling*:**

a) Use proper tools like soil tube, auger, spade, kurpi or pickaxe for sampling.

b) Tale a composite sample from each uniform area. Scrape away surface litter. Take the sample to the required depth randomly from at least 10 spots depending upon the sampled area. One sample may suffice for 6 ha if the land is uniform. In standing vegetable crops, the sample should be taken between the rows. For most standing fruit crops the zone of high root activity as identified using radio – tracer technique, lies between 80 and 135 cm radial distance from trunk. The soil sample may be taken midway between the trunk and the drip – circle / boundary of canopy. Avoid areas which are recently manured and fertilized, close to bunds, compost heaps or other non – representative spots. Collect soil samples prior to planting of the crop before any fertilizers are applied. For lands under cultivation testing once in 3 years may be adequate.

c) When a spade or Kurpi is used, make a V – shaped hole to the depth and then cut a 2cm thick slice of soil from top to bottom of exposed soil surface and collect it in a clean plastic bucket / container. Collect the entire sub – samples in the same bucket or container.

d) Pour the soil from the bucket on to a piece of clean paper, plastic sheet or cloth and mix thoroughly. Using quartering methods retain ½ kg soil and discard the rest. Mix the remaining portions and repeat quartering till ½ kg soil remains.

e) If the sample is wet or moist, dry in shade before putting in same bag.

f) Keep the information sheet inside the sample bag on a thick paper card such as luggage label. Keep a record of the areas sampled and a sketch map for reference. This is useful to adopt the recommendations correctly after the results are received from the laboratory.

g) To avoid contamination do not use the fertilizer bag for samples. Avoid storing or spreading of the soil samples near fertilizers. Use clean cloth or polythene bags to keep the samples

h) Send samples to the laboratory for analysis within 7 days of sampling and avoid delay.

i) In irrigated areas, quality of water must also be taken into account when formulating the recommendations for manures and fertilizers or soil amendment with reference to salinity and to chloride / boron toxicity. Collect about ½ litre of fresh water sample in clean / glass / plastic bottle and send for analysis to reach the laboratory with in 24 hours of collection. Label the sample clearly and keep its record for your reference.

3. PREPARATION OF SOIL SAMPLE AND ANALYSIS:

In soil testing, simple rapid chemical analytical procedures are designed to accurately measure the level of soil nutrients. It is imperative that such a test is adequately correlated with crop yield that give a range of crop response.

The extractants range from plain water to dilute acids / alkalies to complexing agents or a combination of the above that give an empirical measure of the available nutrients. While standardizing the extracts, soil: extractant ratio and reaction / shaking time is considered as also the analytical method. While the extractants and procedures differ from nutrient to nutrient, it is desirable to have a single extractant to test for many nutrients. The latter facilities the ease of testing in the laboratories.

A successful and efficient soil testing laboratory should be an operational system of properly designed space, appropriate equipment, carefully chosen methods for analysis, quality control and competent staff. The equipment may consist of tools for grinding samples, multiple unit dispenser, multiple unit stirrer, diluter and other devices that increase the ease and speed of soil testing. Most commonly used instruments are:

- Conductivity Bridge
- pH meter
- Photo – electric colorimeter of Spectrophotometer
- Flame photometer

If micronutrients are also to be tested, atomic absorption spectrophotometer is essential.

4. SOIL ANALYSIS:

A brief account of different analytical procedures is given here.

4.1 ***Nitrogen:***

The determination of soil organic carbon by the wet oxidation method is simple and is generally used in India for indicating Nitrogen status of soils.

4.2 ***Phosphorous:***

Olsen's Sodiumbicarbonate (Olsen et al., 1954) or Bray's No.1 (Bray and Kurtz, 1954) extractant is used commonly. The former is used universally in soils of neutral to alkaline reaction and the latter in soils of acid reaction.

4.3 ***Potassium***

The standard measure of available K in soils has been exchangeable – K extracted with neutral normal NH_4^oAC which is known to given an excellent index of Potassium availability to soils in many soils.

4.4 ***Micronutrients***

The introduction of atomic absorption spectrophotometer has made it possible to quickly and accurately analyze the extracts in respect of Fe, Mn, Zn and Cu.

A single DTPA extractant has further made the determinations easy and routine.

4.5 ***Soil acidity and Lime Requirement (LR):***

The use of soil pH alone does not suffice to characterize soil acidity or to provide a measure of LR. The use of buffered solutions as those proposed by SMP and others used elsewhere are not very practical under Indian conditions. The use of 1N KCl – extractable acidity multiplied by a factor of 1.5 – 2.0 appears to be more useful to overcome deficiency of Ca, Mg and Al to assess the further problem.

4.6 ***Salinity and Alkalinity*:**

When salt concentration exceeds $4dSm^{-1}$ salinity is a problem and if ESP exceeds 15% alkalinity or sodiumization causes problem. In the latter case, Gypsum requirement of the soil should be determined as proposed by Schoonover (1952).

4.7 ***Water Analysis:***

Water analysis is an important function of an Soil Testing Laboratory (STL) to determine routinely conductivity, pH, Na, Ca^{+}, Mg, K, Carbonates, Bicarbonates, SO_4^{-}, Cl^{-}. In specific cases B content also should be measured. These estimates are useful to identify potential salinity and Na hazards and specific toxicities of Na^{+}, Cl^{-}, B and others.

4.8 ***Plant Analysis:***

Plant analysis is a valuable tool especially in the case of fruit crops. A separate set up distinct from soil testing is required for this activity for handling, analysis, interpretation and recommendation which is being dealt with separately.

4.9 ***Quality Control:***

Performance of instruments in particular and of the laboratory is judged based on the sensitivity, precision and accuracy of the determinations. Since these cannot be taken for granted, checking the system on a regular basis in an integral part of the operations of STL's. A regular use of control of "check" (having known value) sample along with each lot of 10 unknown samples for verification serves the purpose. When the "check" samples are shared with different laboratories of a state / area, uniform test results and recommendations become possible. "Accreditation" of the laboratories is also helpful in quality control.

5. INTERPRETATION:

The ultimate objective of interpretation studies is to establish relationship between laboratory results and crop responses in the field. In the early stages pot – culture studies give useful information which can be verified under field conditions. The latter studies:

a) Confirm or adjust previously determined nutrient element critical levels or class separations or to establish critical levels where much information is not available.

b) To obtain fertilizer response required for economic interpretation. The results obtained are processed using Cate and Nelson technique to yield critical levels.

With this information it is possible to establish soil test rating as:

I. Low – where strong probability of good response below critical level exists;

II. High – where low probability of good response above critical levels exists;

III. Medium – where maintenance applications are desirable near or slightly above the critical level.

6. MAKING RECOMMENDATIONS:

Since fertilizer is the costliest item of expenditure for the farmer, particularly in a developing country like India utmost car4e should be exercised so that misleading recommendations are avoided at all costs.

A variety of information is required to aid a proper recommendation. As crop production is a complex process and soil test provides only a part of information. One has to obtain other information from that provided by the farmer along with the sample. Some important information's needed are:

a) Soil information: Soil test, soil moisture, slope, drainage and effective soil depth, soil physical properties, properties of soil below the plough zone, saline, alkali or acid conditions, deficiency of secondary or micronutrients and unusual conditions, if any.
b) Crop information: Cost species, variety and plant population, fertilizer response characteristics.
c) Economic information: Cost of the fertilizer, transportation or application.
d) Information about the farm and the farmer: Potential for production, farm management and farmer's financial situation.
e) Fertilizer application information: Which fertilizer, cost, time and method of application and appropriate form of fertilizer for particular crop and soil.

Recommendations process involves a generalized model considers:

- The Biological response.
- The nutrient level of the soil.
- Response versus Cost.

There are four points in the response as curve shown in **Graph – 1.**

Yield response

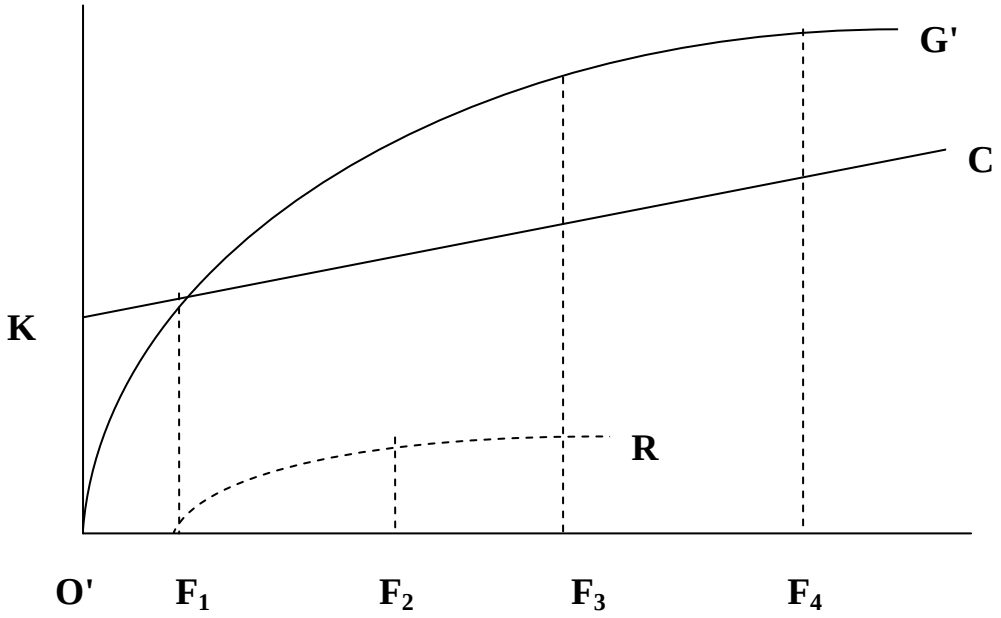


Figure 1: Application to consider to arrive at the best fertilizer recommendation for a particular farmer.

(O', G' – predicted type of response from the crop to be grown KC – the cost of fertilizer including application; F_1R – returns from the fertilizer applied).

1) Maximum yield that can be achieved by application of fertilizer at rate F_4. Since farmer's primary interest is profit rather than production, the final recommendation will be lower than F_4.
2) Minimum application required to break even represented by rate F_1. This is possible when the crop response is sufficient to cover the fixed costs of application and initial fertilizer increments. The farmer should be encouraged to apply that fertilizer provided he is willing to apply more than rate F_1.
3) Maximum rate of return achieved by the rate F_2 at which the farmer receives maximum return for each rupee he invests in fertilizer. This is ideal for a farmer who has limited credit at maximum risk, has no previous experience in using fertilizer and needs to be fully convinced of the value fertilizer application.
4)]maximum profit per unit for cropped area at rate F_3 as in case of progressive farmers who are fully convinced of the value of fertilizer and have adequate access to credit.

Obviously there is no single "best" fertilizer recommendation that will serve the needs of the farmers of an area. Therefore, it is proper to consider that

a) Whether a nutrient is needed to all
b) The optimum rate of fertilizer application to suit each farmer's situation
c) Time, method and form of fertilizer based on research information and local experience.

7. ANALYTICAL METHODS OF SOIL TESTING:

The procedures of some important soil tests are as follows:

7.1 *Soil Reaction (Soil pH):*

Apparatus: Glass electrode pH meter with flexible arm attachment; multiple dispensers, automatic pipette or pipetting machine, Multiple Stirrers.

Buffers required: pH 4.00, 7.00 and 9.20

Procedure: Place a rack having eleven 50ml beakers containing each 10g measured soil sample under multiple dispenser / pipetting machine. Add 20ml of distilled water and stir with multiple stirrer or stir intermittently with glass rod for 30 minutes. Determine pH of soil suspension with pH meter; stir the suspension in each beaker with glass rod of mechanical stirrer again just before taking the pH reading. Wash the electrodes with distilled water after each determination. Express pH to the nearest tenth of a pH unit.

Note: use freshly boiled and cooled distilled water for making buffer solution. Store buffer solution in polyethylene containers. Discard the solution if formation of mold is noticed. Use 2 or 3 buffer solutions – one for standardizing the pH meter and other one or two for checking proper functioning of the instrument.

7.2 *Soil Salinity:*

Apparatus: Conductivity meter and conductivity cell with known cell constant.

Reagents: Saturated solution of Calcium sulphate (Reagent Grade); 0.01 N KCl solution: Dissolve 0.7456 g of KCl in distilled water and dilute to 1 liter.

Procedure: Check the instrument with the saturated calcium sulphate solution (conductivity 2.2 dSm^{-1} at 25ºC) or 0.01N KCl solution (conductivity 1.41 dSm^{-1} at 25ºC) before proceeding with the samples.

The same suspension prepared for the determination of pH is also used for salinity determination. After recording the soil pH, allow the soil suspension in the beaker to settle for

30 minutes. Dip the conductivity cell into the supernatant and determine conductivity. Express conductivity in dSm^{-1} at 25ºC to the nearest first decimal.

7.3 ***Available Phosphorous:***

Two methods are presently recommended for determination of available Phosphorous in soil viz., Bray's No1 for acidic soils and Olsen's for neutral or alkaline soils.

Bray's Method No. 1:

Apparatus: Photoelectric colorimeter with automatic sample changer; multiple dispenser or automatic pipette, 50ml, 5ml; Multiple stirrers or Stirrer machines.

Reagents:

a) Bray's extractant No.1 (0.03N Ammonium fluoride NH_4F in 0.025N HCl)

Dissolve 2.22g of NH_4F in 200ml distilled water, filter and add to the filtrate 18Lof water containing 40ml of concentrated HCl. Make up the volume to 20 liters with distilled water.

b) Molybdate Reagent: Exactly 15g of AR Grade $(NH_4)_2MoO_4$ is dissolved in about 300ml of distilled water warmed to about 45ºC and the solution is filtered to remove sediment, if necessary. The Molybdate solution is cooled and 350ml of 1.0N HCl is added slowly with rapid stirring. When this solution has cooled again to room temperature, it is diluted with distilled water to exactly 1000 ml mixed thoroughly and stored in amber – colored glass stoppered bottle. This cannot be stored for more than 2 months.

c) Stannous Chloride stock solution: Dissolve 10g of $SnCl_2$ $2H_2O$ in 25 ML of concentrated HCl. Add a piece of pure metallic tin and store the solution in an amber – colored glass stoppered bottle.

Washing solution:

Dilute 0.5ml of the stock solution to 66.0ml with distilled water just before use. Prepare fresh dilute solution every working day.

Note: If HCl is not 10N, add calculated amount equivalent to 350ml of 10N HCl.

Preparation of Standard Curve:

Dissolve 0.1916g of pure dry KH_2PO_4 in 1 liter of distilled water. This solution contains 0.10mg of P_2O_5 per ml. preserve this (with a drop of Toluene) as a stock standard solution of Phosphate. Take 20ml of this solution and dilute it to 1000ml with distilled water. This solution contains 2µg of P_2O_5 per ml. take 0.5, 1, 2, 4, 6 and 8ml of this solution in separate 25ml volumetric flasks. Add to each 5 ml of extractant solution, 5ml of the Molybdate reagent and dilute with distilled water to about 20ml. Add 1ml of dilute $SnCl_2$ solution, shake again and dilute to the 25ml mark. After 10 minutes but before 20 minutes read the blue color of the

solution on photoelectric colorimeter using 660mμ (red filter in the case of Klett No.66). Plot the meter readings against μg of P_2O_5 and draw the curve. Repeat as many times as necessary, until all the points fall very nearly on a straight line.

Note: For a satisfactory Phosphorous procedure maintain constant conditions in the blank, standard and test solutions. Contamination should be scrupulously avoided. Inspite of all precautions, intensity of blue color changes slightly with each batch of Molybdate reagent or with every batch of extractant. It is imperative to check the standard curve every day by using 2 or 3 dilutions of standard phosphate solution. If the readings do not fall on the standard curve run the standard once again and draw a fresh curve.

Procedure: Add 50ml of Bray's extractant No.1 to 5g measured soil sample. Use multiple dispensers. Shake for 5 minutes and filter using Whatman No.1 Filter paper. Take 2.5ml aliquot and proceed as in the preparation of standard curve. Find the P_2O_5 content of the filtrate from the standard curve and calculate kilograms of P_2O_5 per hectare (2,000,000 kg soil) as:

A μg P_2O_5 is read from the curve then:

$$\frac{\mathbf{A}}{\mathbf{2{,}000{,}000}} \times \frac{\mathbf{50}}{\mathbf{5}} \times \frac{\mathbf{1{,}000{,}000}}{\mathbf{5}} \times \frac{\mathbf{1000}}{\mathbf{1000}} = \mathbf{1(kg\ P_2O_5 / hectare)}$$

Express P_2O_5 values to the nearest decimal.

Olsen's Method:

Apparatus: Same as Bray's Method No.1.

Reagents:

i. Bicarbonate Extractant: Dissolve 840g of Sodium Bicarbonate in 20 liters of distilled water and adjust the pH of the solution by addition of dilute NaOH or HCl solutions. Filter the solution if necessary.

ii. Activated Carbon: Darco G 60 or any suitable decolorizing carbon made free from suitable phosphorous by repeated washings with bicarbonate extracting solution.

iii. Molybdate reagent: Same as Bray's method No. 1 excepting that instead of 350ml of 10N HCl, add 400ml of 10N HCl.

iv. Stannous Chloride solution: Same as in Bray's Method No.1.

Preparation of Standard Curve:

As in Bray's method No.1

Procedure: Use multiple dispensers to add 50ml of the bicarbonate extractant to 2.5g measured soil sample.

Add 1g measured quality of decolorizing carbon, shake for 30 minutes on the mechanical shaker and filter. Develop color, read on colorimeter as described for Bray's Method No.1 and record concentration from standard curve. Calculate kilograms of P_2O_5 per acre by multiplying concentration in µg with 4.

7.4 ***Available Potassium:***

Flame Photometer Method

Apparatus:

1. Multiple dispenser or automatic pipette – 25ml
2. Multiple stirrers or shaking machine.
3. Flame Photometer.

Reagents:

- Normal neutral ammonium acetate solution: Dissolve 1540g of ammonium acetate (CH_3COONH_4) in 20 liters of water. Test with bromothymol blue or with a pH meter. If not neutral, add either Ammonium hydroxide or Acetic acid to neutralize it to pH 7. Alternatively, dilute 1080ml concentrated Ammonium hydroxide (specific gravity 0.88) to 10 liters and dilute 1150ml of glacial acetic acid (specific gravity 1.06) to 10 liters and mix the two. Adjust the pH to 7.
- Standard Potassium Solution: Dissolve 1.5851g pure KCl in one liter of distilled water. This solution contains 1mg K_2O per ml. preserve this as standard stock solution of potassium.

Preparation of Standard Curve:

Set up the Flame Photometer as per the instructions contained in the manual of the instrument. Atomize 0 and 40 per ml K_2O solutions and record the meter readings. Plot these readings against the respective potassium contents and draw the curve. Repeat as many times as necessary until all points fall very nearly on a straight line.

Procedure:

Add 25ml Ammonium acetate extractant to 5g measured soil sample. Use multiple dispenser for adding extractant. Shake or stir for 5 minutes and filter. Add 2 drops of Butyl alcohol to reach filtrate and determine potassium with Flame Photometer.

Find the Potash concentration of the filtrate from the standard curve and calculate kilograms of K_2O per acre by multiplying the concentration in µg with 5 as:

When A is µg K_2O read from the curve:

Then,

$$\frac{A \times 25}{2{,}000{,}000} \times \frac{1{,}000{,}000}{5} \times \frac{1000}{1000} = 2.5\ A\ (\text{kg } K_2O / \text{hectare})$$

Express the result to nearest integer.

7.5 ***Organic Carbon:***

Chromic Acid Method:

Apparatus:

8. Photoelectric Colorimeter.

2. Test tubes 50ml or 100ml flasks.
3. Automatic pipette or automatic multiple dispenser 10 to 20ml.

Reagents:

- Standard 1N $K_2Cr_2O_7$ solution: Dissolve 49.04g $K_2Cr_2O_7$ in distilled water and dilute to 1 liter.
- Concentrated Sulphuric acid AR containing 1.25 percent Ag_2SO_4 .
- Sucrose (AR, anhydrous).

Preparation of Standard Curve:

Weigh out accurately on a micron balance several different amounts of sucrose in the range of 1 to 30mg. transfer sucrose to 100ml conical flasks add 10ml of 1N $K_2Cr_2O_7$ solution from an automatic pipette and 20ml of concentrated H_2SO_4 .

The flask are swirled and allowed to cool for half an hour. A blank is similarly prepared without sucrose. Read the green color of the reaction liquid in photoelectric colorimeter using 660 µm red filter, adjusting the blank solution to zero. Plot the colorimeter readings against carbon values calculated from the weights of sucrose by multiplying with 0.42.

Procedure:

Add to each test tube or flask containing 1g measured soil sample, 10ml of 1N $K_2Cr_2O_7$ solution and 20ml of concentrated H_2SO_4 with automatic pipettes or with multiple dispensers. Stir the reaction mixture and allow to stand overnight. Alternatively, allow to stand for an hour and then centrifuge ten minute at 2000 rpm. Read the green chromous color of the clear supernatant liquid on the photoelectric colorimeter, using 660 µm red filter, after setting the blank prepared in the same manner to zero.

Read the carbon from the standard curve. Express it as percent of soil by multiplying the curve reading with 100, to the nearest second decimal place.

7.6 ***Lime requirement:***

Hunter method:

Apparatus:

1. Multiple dispensers.
2. Diluter.
3. Burette – 50ml.

Reagents:

- Saturated $CaSO_4$ solution: Shake about 5g of $CaSO_4$ with 1 liter of distilled water for 10 minutes on a mechanical shaker and filter.
- NH_4Cl and NH_4OH: Dissolve 67.5g of NH_4Cl in 570ml of NH_4OH (Sp.Gr.0.88) and dilute to 1 liter.
- Eriochrome Black – T (EBT) indicator: Dissolve 0.5g of EBT and 4.5g of Hydroxylamine Hydrochloride in 100ml of 95 % Ethanol.
- Standard $CaCl_2$ solution 0.01N: Dissolve 0.5g pure $CaCO_3$ in 10ML of dilute HCl and dilute to 1 liter.
- Standard versenate (EDTA) solution in 0.01N: Dissolve 2g of disodium Dihydrogen – ethylene diamine tetra – acetate and 0.05g of $MgCl_2$ $6H_2O$ in water and dilute to 1 liter. Standardize the solution against the standard $CaCl_2$ solution.

Procedure:

Measure 5g of prepared soil sample in 250ml conical flask and add 100ml of the saturated $CaSO_4$ solution. Shake for 5 minutes on the mechanical shaker and filter. Pipette 5ml of soil extract into a 100ml conical flask, dilute to about 25ml with distilled water. Add 0.5ml of the NH_4Cl + NH_4OH buffer, 3 to 4 drops of EBT indicator and titrated with the standard EDTA solution until the color changes from wine red to blue.

Titrate similarly 5ml of saturated $CaSO_4$ solution separately.

Micronutrients by Atomic Absorption:

Manganese , Copper, Zinc, and Iron can be determined directly from the filtrate of DTPA extractant. Standard curves can be prepared by using standard solution concentrations made up in the extracting solution. The DTPA extractant consists of 0.005M DTPA, 0.005M $CaCl_2$ and 0.1M TEA (Triethanolamine) buffered at pH 7.30. for the extraction, 10g of soil are shaken with 20ml of the extractant for 2 hours and filtered. Concentration of Zn, Fe, Mn and Cu in the extract is determined by atomic absorption spectrometry[-4]

The suggested critical levels of DTPA – extractable for sensitive crops are:

Table – 5

Nutrient	Deficient	Marginal	Adequate
Zn	<0.5*	0.5 – 1.0	>1.0
Fe	<2.5	2.5 – 4.5	>4.5
Mn	<1.0		>1.0
Cu	<0.2		>0.2

INSTRUMENTATION

Soil testing laboratories world over depends for analysis of soil, water, plant, organics and fertilizer on many instruments. Some of the advance countries are using the latest instruments like ICP, Ion chromatograph, elemental analyzer etc with online services. However for routine soil and plant analysis five basic instruments are used in all soil testing laboratories. They include pH meter, Conductivity meter, Colorimeter, Flame photometer and Atomic absorption spectrometer. In this lecture the basic principles involved in these instruments, their working and the analysis part is discussed.

NITROGEN ANALYSER – KJEDHAL DISTILLATION UNIT

Nitrogen determination has a long history in the area of analytical chemistry. **Johan Kjeldahl** first introduced the Kjeldahl nitrogen method in 1883 at a meeting of the Danish Chemical Society. As chairman of the chemistry department of the Carlsberg Laboratorium near Copenhagen, Kjeldahl was assigned to scientifically observe the processes involved in beer making. While studying proteins during malt production, he developed a method of determining nitrogen content that was faster and more accurate than any method available at the time. His method used simple equipment and could be performed by an inexperienced technician.

Applications

Since 1883, the Kjeldahl method has gained wide acceptance and is now used for a variety of applications. Kjeldahl nitrogen determinations are performed on food and beverages, meat, feed, grain, waste water, soil and many other samples. The method has been refined and tested for a wide variety of substances and approved by various scientific associations including: AOAC International (formerly the Association of Official Analytical Chemists) Association of American Cereal Chemists American Oil Chemists Society Environmental Protection Agency International Standards Organization United States Department of Agriculture

What is the Kjeldahl method?

The Kjeldahl method is a means of determining the nitrogen content of organic and inorganic substances. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles introduced by Johan Kjeldahl endure today. The Kjeldahl method may be broken down into three main steps:

Digestion - the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution.

Distillation - adding excess base to the acid digestion mixture to convert NH_4 + to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution.

Titration - to quantify the amount of ammonia in the receiving solution. The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.

The Digestion Process

A general equation for the digestion of an organic sample is shown below as one basic example: Organic N + H_2SO_4 → $(NH_4)2SO_4$ + H_2O + CO_2 + other sample matrix by-products A number of interrelated digestion conditions determine the rate of reaction and the completeness of the breakdown of nitrogen to ammonium sulfate. Among these are heat input to the acid digestion mixture, amount of inorganic salt added to elevate the acid boiling temperature, reflux rate of H_2SO_4 in the neck of the digestion flask, length of digestion, and catalyst addition. Adjusting any one of these factors has an influence on the others. Proper digestion conditions for a given sample matrix are achieved through establishing a balance of these factors in a controlled and repeatable fashion. In addition, if the sample contains nitrate or nitrite nitrogen, it is possible to chemically pretreat the digest to include or exclude this nitrogen source from the analysis as desired in a particular situation.

Acid Considerations

Sulfuric acid has been used alone for the digestion of organic samples. (As a convenience to reduce bumping of the digestion mixture, Alundum boiling chips or pumice are often added.) The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Remember that a Kjeldahl flask is essentially a bulb with a condenser neck off to the side for refluxing of the acid.

Heat Input and Digestion Length

Typically the heating elements used for Kjeldahl digestions have variable settings. Heat input is frequently specified as “that setting which brings 250 ml of water at 25° C to a rolling boil in 5 minutes.” Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO_2 evolves. Metallic ions might tint the clear digestion mixture. Note that solution clearing itself is not an indication that all organic nitrogen has been broken down. Digestion length must be determined by recovery studies on known materials of similar matrix if a new method is being developed. Recovery.

Salicylic acid followed by sodium thiosulfate has been used to pretreat the mixture to ensure complete reduction. Other reduction schemes have been devised. Or other pretreatments have been used to prevent nitrates from being reduced at all during the charring process, leaving a clear digest with no contribution from nitrate ions.

The Distillation Process

The acid digestion mixture is diluted and made strongly alkaline with NaOH, liberating NH_3 as follows:

Salt Additions

The problem with using sulfuric acid alone for digestion is very long digestion time's result with many samples due to the slow rate of organic decomposition. The addition of an inorganic salt to the digest elevates the boiling point of the H_2SO4. The solution temperature of concentrated sulfuric acid alone is about 330° C. Addition of a salt such as K_2SO_4 can elevate the solution temperature of the digestion mixture to 390° C or more, depending on the ratio of salt to acid. This significantly increases the rate of organic decomposition in the digestion mixture, shortening the length of time required for digestion. There are several precautions to keep in mind concerning salt addition. First, it is possible to raise the solution temperature of the digestion mixture too much. If the temperature goes much above 400° C during any phase of the digestion, volatile nitrogen compounds may be lost to the atmosphere. Remember that as acid is gradually consumed during the digestion process, for the various reasons mentioned above, the salt acid ratio of the digest gradually rises. This means that the hottest solution temperatures are attained at the end of the digestion. Heat input, consumption of acid by organic material and vaporization, salt/acid ratio, digestion length, and physical design of the Kjeldahl flask, are all interrelated. Each has an effect on the final solution temperature. A second precaution is that if the salt/acid ratio is too high, a considerable amount of material will "salt out" upon cooling of the digest. Concentrated acid pockets can be contained within the cake. These can react violently when concentrated base is added in the distillation process. A certain amount of salting out can be managed by diluting the digest with water while it is still somewhat warm, but not too hot.

Catalyst Additions

Several catalysts have been employed by Kjeldahl chemists over the years to increase the rate of organic breakdown during the acid digestion. Mercuric oxide has been the most effective and widely used. However, mercury forms a complex with ammonium ions during

digestion. The addition of sodium thiosulfate or sodium sulfide after digestion and before distillation will break the complex and precipitate mercuric sulfide. This is also important from a safety point of view, as mercury vapor might escape to the atmosphere during the distillation process. Because of environmental concerns over the handling and disposal of mercury, other catalysts are coming more into favor. Many methods employ copper sulfate. Titanium oxide and copper sulfate in combination have been found to be more effective than copper sulfate alone. Selenium is frequently used. Commercially prepared mixtures of potassium sulfate and a catalyst are available from laboratory chemical suppliers. Bulk custom mixtures are also available.

Nitrate and Nitrite Reduction

Kjeldahl digestions do not always recover all forms of nitrogen in a sample. Nitrate and nitrite ions in a sample must first be reduced prior to acid digestion for quantitative

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3\square + Na_2SO_4 + 2H_2O$$

Ammonium sulfate heat ammonia gas. The Kjeldahl flask is attached to a water condenser and is heated to boil off the NH_3 gas from the digest. The tip of the condenser is submerged in a flask of acidic receiving solution, either standard acid or boric acid solution, to again trap the distilled NH_3 in receiving solution.

Digestion Mixture Dilution

The acid digestion mixture is usually cooled and diluted with ammonia-free water. As mentioned above, with digestion mixtures containing high salt/acid ratios, dilution prevents or minimizes caking. Sometimes this is done while the digestion mixture is still quite warm (with caution!), and the K_2SO_4 has not yet salted out. With some digestion mixtures, a cake forms, but dissolves or breaks up if time is allowed following dilution. Alternatively, warming or sonicating after dilution may break up the cake. Cake material may cause low nitrogen recoveries in the distillation step. Also, entrapped acid in a cake may react violently with base during the distillation process. Dilution of the digestion mixture before making it alkaline and distilling also reduces the likelihood of bumping. Additional boiling chips added just before distillation also reduces bumping, especially towards the end of the distillation as the solution becomes more concentrated. Two or three drops of tributyl citrate may be added as an aid to reduce foaming.

NaOH Addition

Concentrated NaOH (usually 50% solution) is added slowly down the neck of the flask. Being heavier, it forms a layer underneath the diluted acid digestion mixture. Generally, for each 5 ml of concentrated sulfuric acid used in the digestion, 20 ml of 50% sodium hydroxide is required to make the digest strongly alkaline (pH of >11). The flask is connected to the condenser and mixed before heating and distillation begins. For samples not requiring a digestion step, such as direct ammonia determinations in water, the sample is buffered to a pH of 9.5 with a solution of sodium tetraborate and sodium hydroxide, to decrease hydrolysis of any complex organic nitrogen compounds present.

Distillation

The majority of the NH_3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen. Further extension of the distillation times and volumes collected simply results in more water being carried over to the receiving solution. Excess water does not change the titration results. Distillation times and distillate volumes collected should be standardized for all samples of a given methodology. The rate of distillation is affected by condenser cooling capacity and cooling water temperature, but primarily by heat input. Typically the heating elements used for distillation have variable temperature controllers. A distillation rate of about 7.5 ml/minute is most commonly cited in accepted methods. Connecting bulbs or expansion chambers between the digestion flask and the condenser is an important consideration to prevent carryover of the alkaline digestion mixture into the receiving flask. The slightest bit of contamination of the receiving solution can cause significant error in the titration step. When very low levels of nitrogen are being determined, it is advisable to “precondition” the distillation apparatus prior to distillation. This can be done by distilling a 1:1 mixture of ammonia-free water and 50% NaOH for 5 minutes just before sample distillation to reduce contamination from atmospheric ammonia.

Receiving Solutions

If the receiving solution is standardized HCl or H_2SO_4, it is desirable to have only a slight excess left after the NH_3 is distilled and trapped in the receiving solution to minimize the back titration. Based on the anticipated amount of nitrogen in the sample, a target amount of standard acid can be calculated from the following formula:

The Titration Process

There are two types of titration: back titration, commonly used in Macro Kjeldahl; and direct titration. Both methods indicate the ammonia present in the distillate with a color change and allow for calculation of unknown concentrations.

Nitrogen Determination by back titration

The ammonia is captured by a carefully measured excess of a standardized acid solution in the receiving flask. The excess of acid in the receiving solution keeps the pH low, and the indicator does not change.

[(% nitrogen expected in sample) x (sample aliquot used) x gram sample wt.] + 2 (normality of standard acid) x 1.4007 x digestion dilution volume

If boric acid is used, the exact concentration is not needed because the titration directly measures the amount of ammonia in the distillate by neutralizing the 1:1 complex formed by ammonia and boric acid. Large quantities of boric acid may be added to the receiving solution so complete absorption of the ammonia is assured. Receiving solution volumes may be increased by the addition of ammonia-free water so that the tip of the delivery tube is immersed. Delivery tubes should always be allowed to drain momentarily into the receiving flask before removal from the distillation apparatus. The receiving solution should remain below 45° C during distillation to prevent loss of ammonia. Ammonia standard sulfuric acid ammonium sulfate excess sulfuric acid

$$2\,NH_3 + 2\,H_2SO_4 \rightarrow (NH_4)2SO_4 + H_2SO_4$$

The excess acid solution is exactly neutralized by a carefully measured standardized alkaline base solution such as sodium hydroxide. A color change is produced at the end point of the titration. Ammonia sulfate measured excess acid measured sodium hydroxide ammonium sulfate

$$(NH_4)_2SO_4 + H_2SO_4 + 2NaOH \rightarrow (Na)_2SO_4 + (NH_4)_2SO_4 + 2H_2O$$

Nitrogen Determination by direct titration

If boric acid is used as the receiving solution instead of a standardized mineral acid, the chemical reaction is: ammonia gas boric acid ammonium-borate complex excess boric acid.

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ : H_2BO_3^- + H_3BO_3$$

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solution changes. Ammonium borate complex, sulfuric acid, ammonium sulfate, boric acid

$$2NH_4H\,2BO_3^- + H_2SO_4 \rightarrow (NH_4)\,2SO_4 + 2H_3BO_3$$

The addition of sulfuric acid exactly neutralizes the ammonium borate complex, and a reverse color change is produced. The boric acid method has two advantages: only one standard solution is necessary for the determination and the solution has a long shelf life.

Indicator Solutions

Many different indicators have been used to provide a "sharp end point" color change. The analyst's use of specific types of indicators can be a personal choice. The combination of methyl red and methylene blue indicators is frequently used in many methods. The color change of the indicator must fall within the equivalent point of the reaction. For standard acid/base titrations, methyl orange is usually the preferred indicator. If color change end points are difficult to detect, reference solutions made from a blank with an indicator can be very helpful.

Calculations

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. The equations given here are in long form. They are often simplified in the published standard methods. In the equations below, "N" represents normality. "ml blank" refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When standard acid is used as the receiving solution, the equation is:

Equipment and Apparatus

Very early Kjeldahl digestions and distillations were performed using stone fume hoods and gas mantles as a heat source. In the 1920's these were replaced by what is now known as classical macro-Kjeldahl digestion and distillation apparatus. Macro setups use Kjeldahl flasks from 500 to 800 ml volume and handle sample sizes from 0.5 to about 5.0 g. A smaller version of this apparatus is referred to as micro-Kjeldahl equipment. The equipment consists

of smaller benchtop heater units, and Kjeldahl flasks of 30 to 100 ml volume. A third more recent variation in equipment makes use of ceramic or aluminum heating blocks designed to accept a number of straight digestion tubes at once. "Block digestors" are often used in conjunction with benchtop distillation units with steam generators to shorten the distillation time. Each of these three types of apparatus is described in more detail below. In all cases, since the Kjeldahl process involves significant corrosive fumes, appropriate attention must be given to fume removal. Equipment must be suitably constructed of corrosion-resistant materials.

Classical Macro-Kjeldahl Apparatus

Many governmental and regulating agencies have developed methodologies that specify the classical macro-Kjeldahl apparatus. For example, standard methods for low level nitrogen determinations in water (0-10 mg/1) require a sample size of 250 to 500 ml, and therefore large Kjeldahl flasks. Methodologies in the agricultural industry often require larger sample sizes and involve matrices which can significantly foam. Equipment engineered to accommodate macro methods can be substantial. Apparatus designed to process 2 to 12 samples simultaneously is typical, and involves a bank of heaters and a fume manifold for digestion, and a second bank of heaters with condensers for distillation. The apparatus is available freestanding or contained within an integral hood. Freestanding installations require additional laboratory room modifications to handle fumes and heat. In either case the equipment is large, expensive, involves significant installation considerations, and ongoing utility and reagent costs. The digestion apparatus has separate heating mantles to retain each flask. Typically each mantle has an individual temperature controller. The long necks of the Kjeldahl flasks are positioned to release digestion fumes into a common manifold. Manifold aspiration can be provided either by a mechanical blower with exhaust through ductwork to the outside, or by a large water aspirator that provides water spray to dilute the fumes for disposal down a drain. Like the digestion apparatus, the distillation apparatus has separate heating mantles to retain each flask. With the flask in place on the mantle, the neck of each flask is attached to a "connecting bulb" or an expansion chamber that acts as a trap to keep any of the concentrated liquid digest from mechanically carrying over through the condensers and into the receiving solution.

[(ml standard acid x N of acid) - (ml blank x N of base)] - (ml std base x N of base) x 1.4007 weight of sample in grams

If the sample weight is in milligrams, the molecular weight of nitrogen should be changed to 1400.67. When boric acid is used as the receiving solution the equation is:

(ml standard acid - ml blank) x N of acid x 1.4007 weight of sample in grams

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix. Many protein factors have been developed for use with various types of samples. The list below represents just a few of the factors described in the standard methods of analysis published by the American Association of Cereal Chemists (AACC) and AOAC International. 6.38 milk and dairy 6.25 other grains 5.95 rice 5.70 wheat flour

Downstream from the connecting bulb is the condenser, which is a tube of stainless steel surrounded by a second water jacket tube. Typically multiple condenser/jacket units are ganged together in a single assembly. A glass delivery tube is attached to the end of the condenser. The delivery tube has a ball-shaped tip with small holes to help disperse large bubbles and eliminate pressure fluctuations during distillation. The tip is submerged in the flask of receiving solution to ensure that the distilling ammonia is completely captured.

Micro-Kjeldahl Apparatus

These are miniature versions of the macro-digestion apparatus which are movable and intended for use in a laboratory hood. They are designed to digest small samples in 30or 100 ml digestion flasks. Individually controlled heaters allow multiple flasks to be handled simultaneously. Some micro-digestors include a one-piece glass manifold for fume removal. The glass manifold is connected to a standard water aspirator to dilute and remove fumes that do not reflux in the flask necks. Whether or not a glass manifold is used, the digestion apparatus should be operated inside a laboratory hood. Digested samples can be diluted and an aliquot taken for distillation on a micro steam distillation unit. Micro steam distillation units are available to accept digested samples of up to 4 ml concentrated acid and volumes of about 55 ml. An electric immersion heater produces steam heat that causes the sample to boil and release ammonia gas. The vapors pass into a condenser where contact with water cooled glass condenses the vapors, which drip into the receiving solution via a delivery tube. Distillation time is approximately 5 minutes. Methodologies have been specifically developed for micro-Kjeldahl apparatus. These most often are methods that involve homogeneous samples, relatively high nitrogen levels in the sample, and small sample sizes normally less than 0.25 g. The installation considerations, initial expense, space requirements, and ongoing utility and reagent cost are much less with micro equipment.

Block Digestors

Block digestors are high temperature ceramic or aluminum blocks with wells to accept straight walled digestion tubes. They can accommodate from 2 to 25 tubes at once. Block digestors are heavy but movable self-contained units designed to be used in a laboratory hood. Digestion tubes range up to 300 ml capacity, suitable for sample sizes up to about 2 g or 75 ml. Since narrow, straight-walled digestion tubes are used instead of traditional Kjeldahl flasks with a bulb and neck, and since a number of tubes are grouped closely together in a vertical configuration in the block, acid reflux parameters are different. This means salt/acid ratios and heat inputs appropriate for macro-Kjeldahl methods do not exactly transfer to digestion in a block. Methods have been developed specifically for block digestors similar, but not exactly identical, to macro-Kjeldahl methods.

Typically block digestors have one controller that adjusts the temperature of the entire block. The controller can be automated, allowing for timed ramping and multiple temperature settings during the course of a digestion. Most often block digestors are used together with fume removal devices, even though they are operated in a hood. One such system consists of a glass manifold with individual bulb eductors in a rack designed to rest on top of a set of digestion tubes in the block. Another system uses individual exhaust caps with flexible tubing. In either case the manifold is connected to a water aspirator. Or, instead of a water aspirator, there are alternative units that employ mechanical aspiration and include bubbling the acid fumes through a neutralizing base solution. Block digestion followed by steam distillation is sometimes referred to as “rapid Kjeldahl” partly because a number of block digestion methodologies are shorter than classical macro-Kjeldahl digestions and benchtop steam distillation units in use are faster than classical distillations.

Rapid Steam Distillation Apparatus

The development of block digestors as an alternative to macro-Kjeldahl digestion has been paralleled by the development of benchtop steam distillation units. Basically, this equipment consists of a steam generator to inject steam into the alkaline digestion mixture and an evaporator-type condenser, where contact with water-cooled glass coils causes the vapors to condense and drip into the receiving solution via a delivery tube. Usually the unit is designed to accept straight digestion tubes from block digestors, though there is no reason in theory why any digestion techniques could not be combined with any distillation technique. Steam distillation is much more rapid than classical macro-distillation, typically taking from 3 to 8 minutes. On the other hand, only one distillation at a time can be done. Rapid steam distillation units are available with different features, ranging from relatively manual models

to highly automated models. Basic models dispense dilution water and base under control of a push button. Some models include a timer to control the length of distillation, shutting down automatically. Some units automate the entire distillation process once the digestion flask is in place, and others automatically titrate to end point after distillation, and calculate and display the results in a printed report.

Titrators

Of course, receiving solutions may be individually hand titrated using an indicator solution and burette, but several models of benchtop automatic titrator instruments are also available. Some units will titrate one receiving solution at a time to a set end point. Others will automatically titrate a number of receiving flasks sequentially. Still other models provide a printout of results along with digital readout. **(Fig – 40)**

SCRUBBER

Scrubber systems are a diverse group of air pollution control devices that can be used to remove some particulates and/or gases from industrial exhaust streams. Traditionally, the term "scrubber" has referred to pollution control devices that used liquid to "scrub" unwanted pollutants from a gas stream. Recently, the term is also used to describe systems that inject a dry reagent or slurry into a dirty exhaust stream to "scrub out" acid gases. Scrubbers are one of the primary devices that control gaseous emissions, especially acid gases.

Removal and neutralization

The exhaust gases of combustion may at times contain substances considered harmful to the environment, and it is the job of the scrubber to either remove those substances from the exhaust gas stream, or to neutralize those substances so that they cannot do any harm once emitted into the environment as part of the exhaust gas stream...

Wet scrubbing

A wet scrubber is used to clean air or other gases of various pollutants and dust particles. Wet scrubbing works via the contact of target compounds or particulate matter with the scrubbing solution. Solutions may simply be water (for dust) or complex solutions of reagents that specifically target certain compounds.

Removal efficiency of pollutants is improved by increasing residence time in the scrubber or by the increase of surface area of the scrubber solution by the use of a spray nozzle, packed towers or an aspirator. Wet scrubbers will often significantly increase the proportion of water in waste gases of industrial processes which can be seen in a stack plume. Compliance agencies typically place minimum DP thresholds on wet scrubber.

Dry scrubbing

A dry or semi-dry scrubbing system, unlike the wet scrubber, does not saturate the flue gas stream that is being treated with moisture. In some cases no moisture is added; while in other designs only the amount of moisture that can be evaporated in the flue gas without condensing is added. Therefore, dry scrubbers do not have a stack steam plume or wastewater handling/disposal requirements. Dry scrubbing systems are used to remove acid gases (such as SO_2 and HCl) primarily from combustion sources.

There are a number of dry types scrubbing system designs. However, all consist of two main sections or devices: a device to introduce the acid gas sorbent material into the gas stream and

a particulate matter control device to remove reaction products, excess sorbent material as well as any particulate matter already in the flue gas.

Dry scrubbing systems can be categorized as dry sorbent injectors (DSIs) or as spray dryer absorbers (SDAs). Spray dryer absorbers are also called semi-dry scrubbers or spray dryers.

Dry sorbent injection involves the addition of an alkaline material (usually hydrated lime or soda ash) into the gas stream to react with the acid gases. The sorbent can be injected directly into several different locations: the combustion process, the flue gas duct (ahead of the particulate control device), or an open reaction chamber (if one exists). The acid gases react with the alkaline sorbets to form solid salts which are removed in the particulate control device. These simple systems can achieve only limited acid gas (SO_2 and HCl) removal efficiencies. Higher collection efficiencies can be achieved by increasing the flue gas humidity (i.e., cooling using water spray). These devices have been used on medical waste incinerators and a few municipal waste combustors.

In **spray dryer absorbers**, the flue gases are introduced into an absorbing tower (dryer) where the gases are contacted with finely atomized alkaline slurry. Acid gases are absorbed by the slurry mixture and react to form solid salts which are removed by the particulate control device. The heat of the flue gas is used to evaporate all the water droplets, leaving a non-saturated flue gas to exit the absorber tower. Spray dryers are capable of achieving high (80 + %) acid gas removal efficiencies. These devices have been used on industrial and utility boilers and municipal waste combustors.

Mercury removal

Mercury has no known beneficial uses in nature, but it is a common substance found in coal that must also be removed. Wet scrubbers are only effective for mercury removal under certain conditions. Mercury vapor in its elemental form, Hg^0, is insoluble in the scrubber slurry and not removed. Oxidized mercury, Hg^{2+}, compounds are more soluble in the scrubber slurry and can be captured. The type of coal burned as well as the presence of a selective catalytic reduction unit both affect the ratio of elemental to oxidized mercury in the flue gas and thus the degree to which the mercury is removed.

Scrubber waste products

One side effect of scrubbing is that the process only moves the unwanted substance from the exhaust gases into a solid paste or powder form. If there is no useful purpose for this solid waste, it must be either contained or buried to prevent environmental contamination.

Limestone-based scrubbers can produce a synthetic gypsum of sufficient quality that can be used to manufacture drywall and other industrial products.

Mercury removal results in a waste product that either needs further processing to extract the raw mercury, or must be buried in a special hazardous wastes landfill that prevents the mercury from seeping out into the environment.

Bacteria spread

Until recently, scrubbers have not been associated with health risks involving bacteria spread as a result of inadequate cleaning, unlike other devices such as cooling towers. However, a 2005 outbreak of Legionnaires' disease in Norway was proven to emanate from a scrubber, causing ten deaths and more than fifty cases of infection as it spread the bacteria through the air during a period of only two scrubbers being the source of such bacteria. **(Fig – 41)**

SPECTROPHOTOMETER

In physics, **spectrophotometry** is the quantitative study of electromagnetic spectra. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Also, the term does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A **spectrophotometer** is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range.

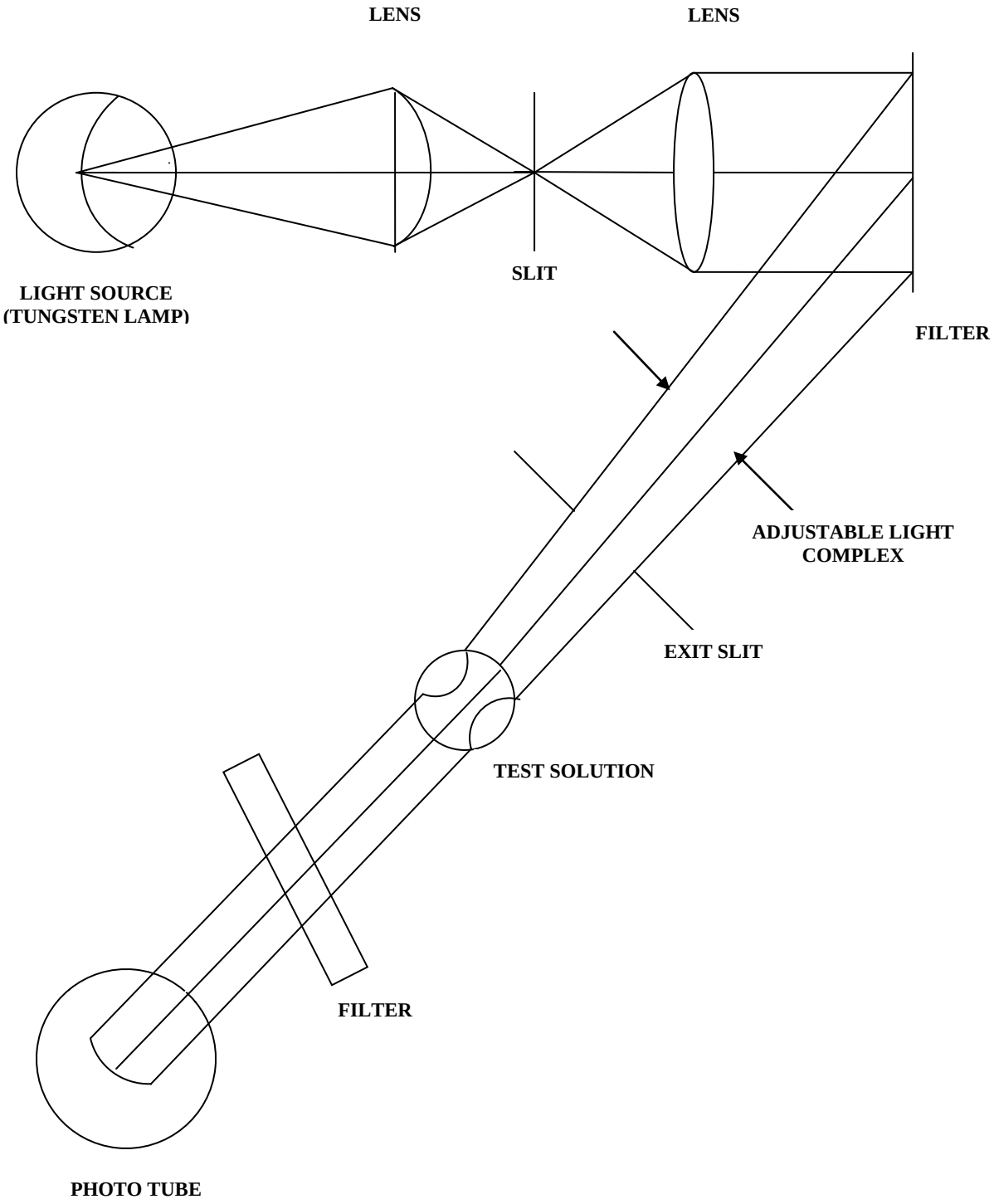
Perhaps the most common application of spectrophotometers is the measurement of light absorption, but they can be designed to measure diffuse or specular reflectance. Strictly, even the emission half of a luminescence instrument is a kind of spectrophotometer.

There are two major classes of spectrophotometers; single beam and double beam. A double beam spectrophotometer measures the ratio of the light intensity on two different light paths, and a single beam spectrophotometer measures the absolute light intensity. Although ratio measurements are easier, and generally stabler, single beam instruments have advantages; for instance, they can have a larger dynamic range.

Visible-region spectrophotometers

Visible region 400-700nm spectrophotometry is used extensively in colorimetry science. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They usually take readings every 20 nanometers along the visible region, and produce a spectral reflectance curve. These curves can be used to test a new batch of colorant to check if it makes a match to specifications. Traditional visual region spectrophotometers cannot detect if a colorant has fluorescence. This can make it impossible to manage color issues if one or more of the printing inks are fluorescent. Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45. The names are due to the geometry of the light source, observer and interior of the measurement chamber.

PARTS OF SPECTROPHOTOMETER



(Fig – 42)

Scientists use this machine to measure the amount of compounds in a sample. If the compound is more concentrated more light will be absorbed by the sample.

Components: 1. The light source shines through the sample. 2. The sample absorbs light. 3. The detector detects how much light the sample has absorbed. 4. The detector then converts how much light the sample absorbed into a number.

Spectroradiometers

Spectroradiometers, which operate almost like the visible region spectrophotometers, are designed to measure the spectral density of illuminants in order to evaluate and categorize lighting for sales by the manufacturer, or for the customers to confirm the lamp they decided to purchase is within their specifications.

Components:

1. The light source shines onto or through the sample.

2. The sample transmits or reflects light.

3. The detector detects how much light was reflected from or transmitted through the sample.

4. The detector then converts how much light the sample transmitted or reflected into a number.

UV and IR spectrophotometers

The most common spectrophotometers are used in the UV and visible regions of the spectrum and some of these instruments also operate into the near-infrared region as well. **Spectrophotometers** designed for the main infrared region are quite different because of the technical requirements of measurement in that region. One major factor is the type of photosensors that are available for different spectral regions, but infrared measurement is also challenging because virtually **everything** emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Historically, spectrophotometers use a monochromator to analyze the spectrum, but there are also spectrophotometers that use arrays of photosensors and, especially in the IR, there are spectrophotometers that use a Fourier transform technique to acquire the spectral information in a technique called Fourier Transform Infrared.

The spectrophotometer measures quantitatively the fraction of light that passes through a given solution. In a spectrophotometer, a light from a lamp in a near-IR/VIS/UV spectrophotometer (typically a deuterium gas discharge lamp) is guided through a monochromator, which picks light of one particular wavelength out of the continuous spectrum. This light passes through the sample that is being measured. After the sample, the intensity of the remaining light is measured with a photodiode or other light sensor, and the transmittance for this wavelength is then calculated.

FLAME PHOTOMETER

PRINCIPLE: The atoms or ions present in the solution gets energy in a flame, they get exited and results in the emission of srectrum. The energy absorbed by electrons, shifts them to positions more distant from the atomic nucleus. As the electrons regain their state, the previously absorbed energy is remitted as electromagnetic radiations. The wavwlengths of which correspond to the quantity of energy involved in the respective electron shifts and the quantity of radiation is directly proportional to the amount of the element emitting the rays. This is then measured with a galvanometer connected in a suitable circuit. The emission spectrophotometric analysis is most useful where a large number of soil or plant samples have to be analysed on a routine basis.

Flame photometer has the following components and is schematically shown in the figure.

i. ***Atomizer – Burner Assembly:*** It consists of
   - A suction capillary through which the sample is sucked.
   - An air pipe which helps to spray the sample into the flame and is attached to the air – compreser.
   - A fuel gas tube attached to the gas cylinder (Acetylene or LPG)
   - A drain tube through which heavy particles of the samples are drained out.
   - A burner in which the fuel gas burns and the flame is produced.. in this flame, the element which has been sprayed is exited.

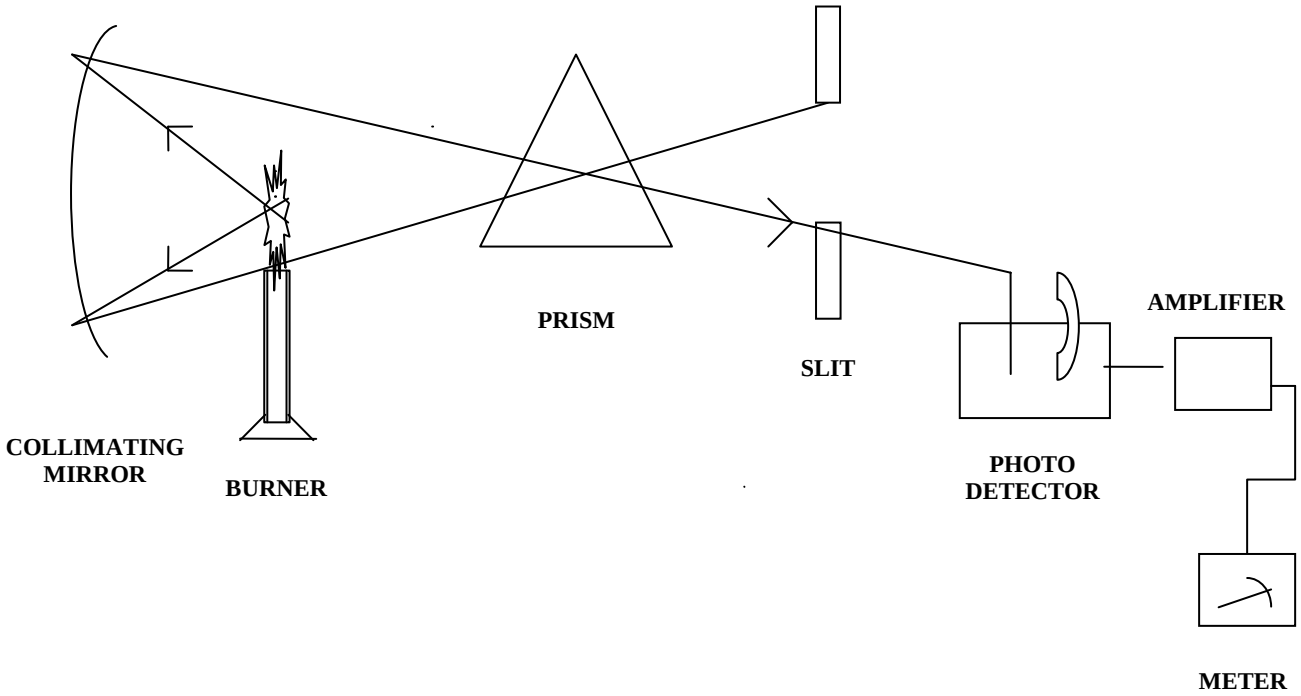
ii. ***Filter:*** It selects the radiation of the required wavelength.

iii. ***Galvanometer:*** It measures the light energy that has been converted to eledtrical energy.

Steps to be followed in the use of Flame photometer:

1) Connect the instrument to the mains after ensuring correct voltage.
2) Set the air – compressor in operation and check the pressure gauge so that the air pressure is adjusted to 0.4 to 0.6 $kgcm^{-2}$.
3) Starts gently the gas supply from the gas line fitted to the gas cylinder and light the burner carefully through the inspection window.
4) Again adjust the air and the gas pressure to give a sooth free blue flame and about 4cm in height.

A SCHEMATIC DIAGRAM OF FLAME PHOTOMETER



(Fig - 43)

5) Close the inspection window and set the galvanometer reading to zero by feeding distilled water into the atomizer.
6) Feed the standard solution of maximum concentration and adjust the galvanometer to read 100, feed other standard solutions and note down the reading.
7) Feed the test sample and note down the reading.
8) Turn off the gas tap first, while switching off the instrument.

Procedure:

a. Weigh 5g of soil into a 250ml conical flask.
b. Add 25ml of normal Ammonium acetate solution.
c. Shake the contents of the flask on an electrical shaker for 5 minutes and filter.
d. Feed the filtrate into the atomizer of the flame photometer which has been adjusted to 100 with 40 ppm (in case of Systronics Flame Photometer) or 10 ppm (in case of Elico Flame Photometer) standard solution of Potassium and note down the reading.
e. Locate this reading on the standard curve and calculate the amount of Potassium in the soil as shown below.

Precautions:

i. The filtrate should be clear to avoid clogging the capillary.
ii. The air – pressure should be with in 0.4 – 0.6 $kgcm^{-2}$. It should not deviate too much.
iii. The gas inlet should be opened after opening the air inlet and closed before shutting off the air supply.
iv. The flame should be soot free and blue.
v. The instrument should be allowed to warm up for 10 – 15 minutes before using it.
vi. The light filter should be of the same element, which we want to determine.
vii. For soils having less than 2000 ppm Potassium, calibrate the instrument with 0 – 10 ppm standards of Potassium content in the range of 200 – 500 ppm, calibrate using 1 – 20 ppm range. If the Potassium content is more than 500 ppm, the soil to solution ratio can be widened to 1 : 40 to 1 : 50.

viii. When exchangeable Potassium is relatively high, it is preferable to estimate Potassium (K) on field moisture samples and then calculate on air dry basis as Potassium is likely to be fixed during drying.

pH METER

The pH meter of a soil is one of the most important chemical properties of soils. It influences the availability of nutrients, microbial property, physical property and suitability of a soil for a crop. It also throws light on the need for gypsum or lime application. The term pH is defined as "the negative logarithm of the activity of hydrogen ion in soil".

$$\mathbf{pH = - \log H^{+}}$$

The pH scale ranges from 0 to 14. the mid point scale i.e. 7.0 is neutral. pH > 7.0 indicates alkalinity and the pH < 7.0 indicates acidity. The pH of a solution can be measured by colorimetric indicator method or by potentiometric method. In soil testing laboratories potentiometric method is used to determine the pH of soil using pH meter.

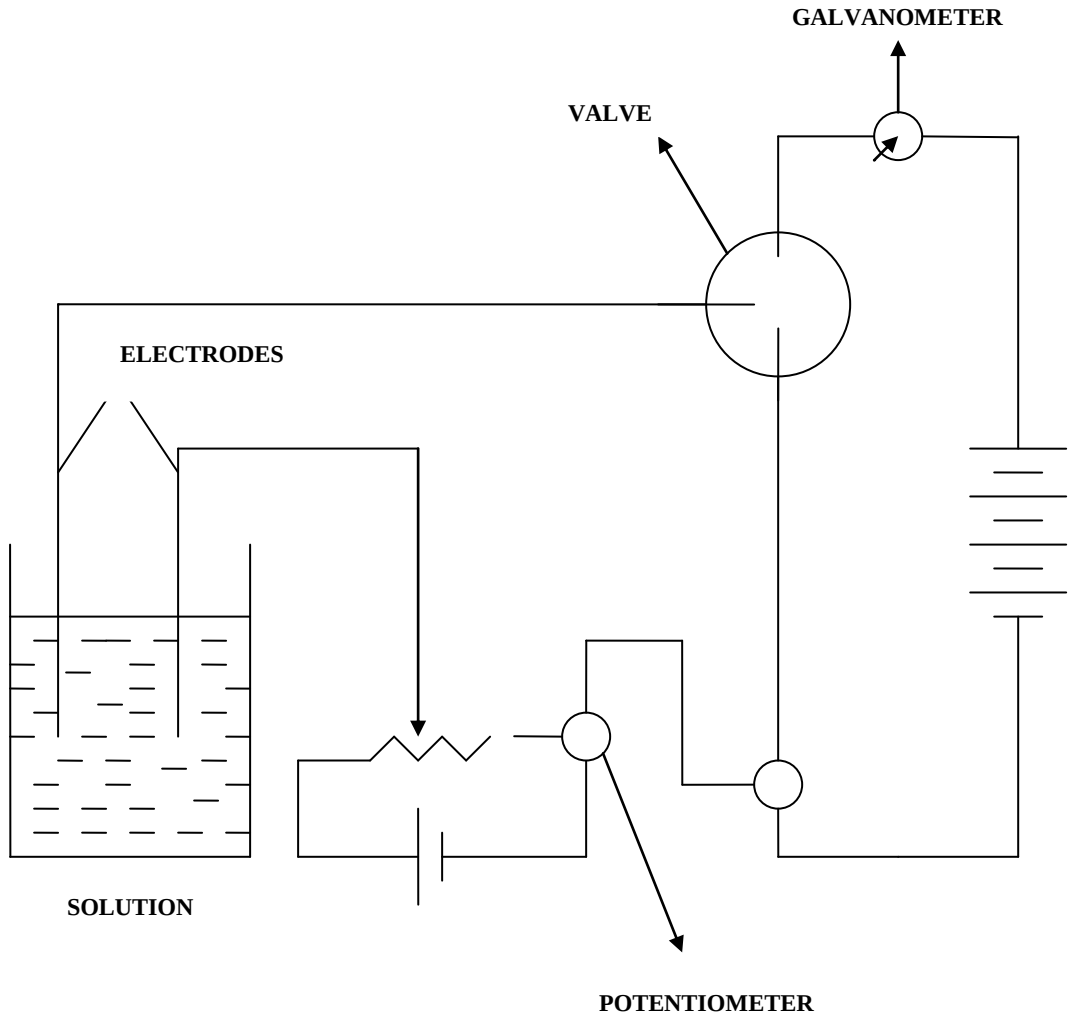
A pH meter is an instrument, which measures the voltage developed by the combination of glass electrode and a reference electrode. The scale is graduated in terms of both pH and mill volts (mV).

Glass Electrode:

It consists of a thin walled bulb made of chemically pure and soft soda glass. A suitable electrolyte, usually 0.1 N HCl is present in the bulb and a silver wire coated with AgCl, is dipping in the acid present inside the bulb. The potential developed at the glass membrane due to the difference in the connection of hydrogen of the outside and inside solutions is measured by connecting it to a reference (Calomel) electrode. The potential developed is measured in the terms of volts (generally in mV as the potential developed is very small).

An ordinary voltmeter cannot be used for measuring the voltage of the glass electrode – reference of electrode assembly for the reason that the glass electrode being a non – conductor allow an extremely small current which would not activate a galvanometer. In a pH meter, this small current is amplified through electronic tubes so that it can be measured easily.

PARTS OF GLASS ELECTRODE pH METER



(Fig – 44)

Measurement of Soil pH:

pH meter with glass – calomel combined electrode is most commonly used.

Reagents:

i. ***pH 4.0 buffer solution:*** Dissolve 10.21g of Potassium hydrogen phthalate 90.05 M in water and dilute it to one liter. This solution should not be used after 5 – 6 weeks or earlier if fungal growth is seen.

ii. ***pH 7.0 buffer solution:*** Dissolve 0.948g of KH_20_4 and 1.57g of Na_2HPO_4 in water and dilute it to one liter.

iii. ***pH 9.2 buffer solution (0.01M Borax):*** Dissolve 3.81g of $Na_2B_4O_7.10H_2O$ in carbon dioxide free water and dilute to one liter. This solution should be protected from atmospheric carbon dioxide and should be placed once in a month.

The buffer tablets of powder are available commercially for pH 4.0, 7.0 and 9.2. these buffer solutions can be prepared by dissolving the powder / tablet in required quantity of boiled cooled distilled water (100 ml).

Procedure (for Elico pH meter):

- Take 20g of soil in a 50ml beaker and add 25ml of distilled water and stir at least five times over a 30 minutes period to allow for soil and water to each equilibrium.
- In the meantime switch on the instrument , set temperature knob to room temperature and set range selector to zero.
- Set the galvanometer to zero with set zero knob after 15 minutes.
- Dip the electrodes into a buffer of known pH, turn the range selector and adjust the pH meter to pH of the buffer solution by set buffer knob.
- Turn the selector to zero, press the key stand by hand and take out the electrode. Wash the electrode with distilled water and dry it with a piece of filter paper.
- Shake the soil suspension and insert the electrodes into it. Turn the selector in the proper pH range and read the pH on the dial of the pH meter. Turn back the selector to zero and take out the electrode.
- Rinse the electrode with distilled water and lower them back into a beaker containing distilled water.

The pH value is erratic and unreliable when determinations are made to lower soil moisture due to

a) Poor contact between soil and the glass electrode
b) The high mechanical resistance of the soil

On the other hand, too high a soil – water ratio increases the pH value owing to dilution. In general, more dilute the soil suspension, the higher the soil pH values found, whether the soil is acidic or alkaline. The rise in the soil pH by 0.2 to 0.5 units as moisture content increases from the sticky point. For uniform results ratio of 1: 2.5 of soil to water is used.

Precautions in the use of pH meter:

1. The electrodes should not be allowed to remain in suspension or test solution longer than necessary.
2. The electrode should be washed with a gentle stream of distilled water immediately.
3. The electrodes should be suspended in distilled water when it is not in use. The drying of the electrodes should be avoided.
4. If soil has more soluble salts, soil suspension may be prepared, using either 0.1M $CaCl_2$ of KCl solution instead of water to overcome the effect of soluble salts on pH.

Interpretation:

Table - 6

pH	Interpretation	Management required
< 6.5	Acidic	Requires liming
6.5 – 8.5	Neutral	No amendments, optimum for crops
> 8.5	Alkaline	Requires Gypsum for reclamation

CONDUCTIVITY METER

Electrical Conductivity (EC) is an important soil chemical parameter. It tells us about the quality of dissolved salts present in the soil. This property affects the ability of a crop to grown in a given soil. Soil containing excess salts is saline and need to be reclaimed for growing normal crops.

Principle:

Solutions offer resistance to the passage of electricity through them depending on the concentration of soluble salts. Higher the salt content, lower the resistance to the flow of current. The conductivity is the reciprocal of resistance and it depends on:

a) Length of the solution (1) or distance between the electrodes.
b) Area of the electrodes (a).
c) Nature of the salts present and
d) Temperature of the solution.

Conductance (C) = a /1 = (K.a) / 1

$$\text{Hence, } K = \frac{(C \times 1)}{A}$$

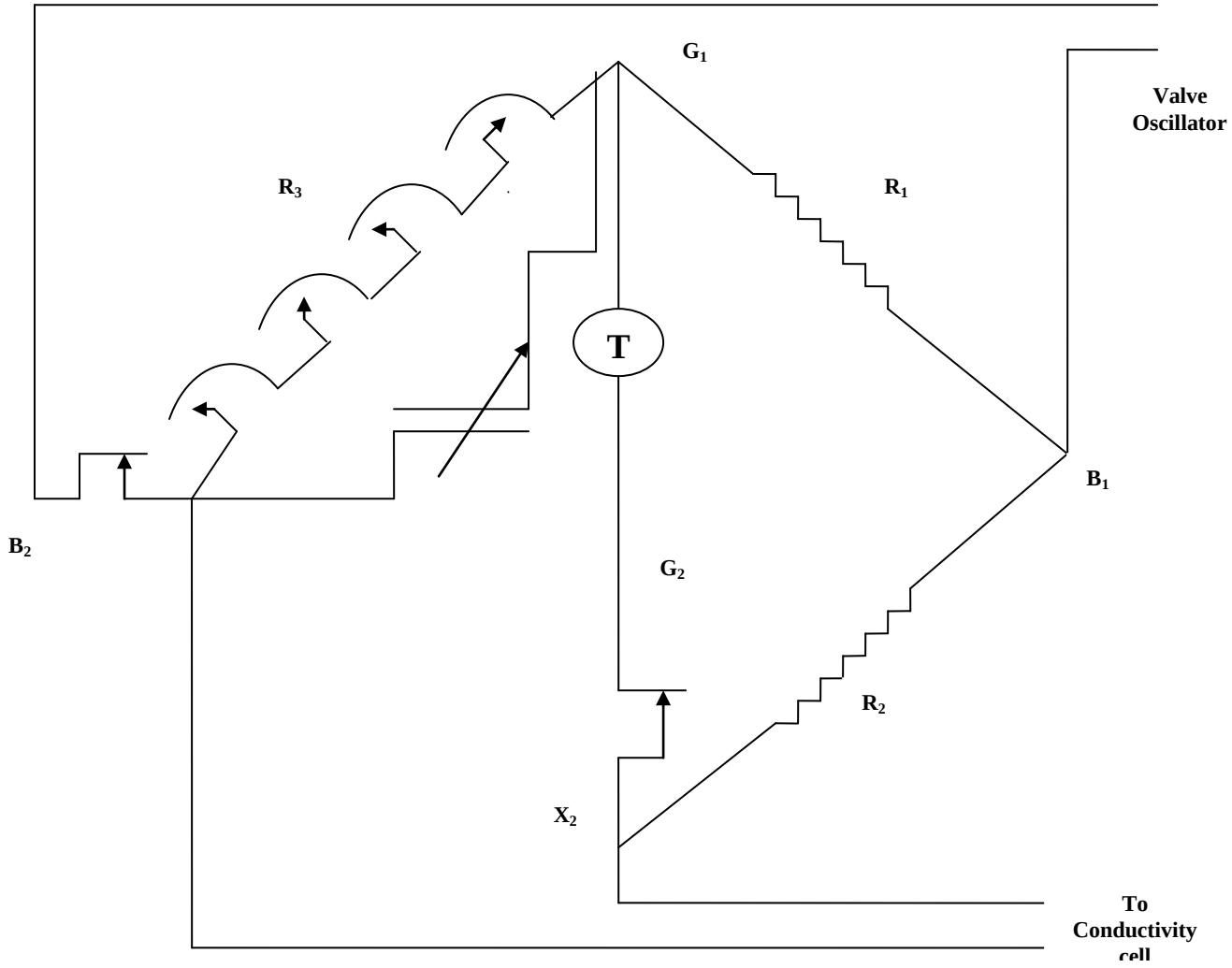
Apparatus: Conductivity meter

Reagent used: 0.01 M KCl.

Procedure: Determination of Cell Constant (K)

The cell constant is the ratio of the distance (1) between the electrodes to the area (a) of the electrode. Cell constant of a given cell is determined by measuring the conductivity of 0.01M KCl solution whose specific conductivity (1.412 dSm^{-1} at 25ºC) is taken as a reference standard.

THE ARRANGEMENT OF THE APPARATUS FOR THE DETERMINATION OF THE CONDUCTIVITY OF SOIL SUSPENSIONS



$$\text{Cell Constant} = \frac{\text{Calculated Conductance of KCl}}{\text{Observed Conductance of KCl}} \times \frac{1.412}{X}$$

(Fig – 45)

Determination of Electric Conductivity:

Principle: The EC of a soil is measured with a conductivity meter known as 'Solute Bridge'. It is based on the principle of Wheat – Stone Bridge in which an alternating current is used instead of direct current in order to eliminate the polarization of electrodes. The balance point is indicated by a 'magic eye' of a telephone. The schematic diagram of a soluble bridge is given in the figure below.

A conductivity cell consists of two platinum electrodes in the form of rectangular pieces fused on glass on one side and covered with black spongy platium on the other. Two wire heads connect it to proper terminals on the Soluble Brodge. A figure known as cell constant is marked on the cell.

Procedure: (for Elco Soluble – Bridge):

1. Take 10g of soil into a beaker and add 25ml of distilled water.

2. Stir the suspension intermittently and leave it overnight in order to obtain a clear supernatant solution.
3. Switch on the instruments (by setting it at room temperature for 15 minutes before taking reading for stabilization.
4. Dip the conductivity ell into the supernatent solution.
5. Move the pointer on the dial ti get maximum area in shade in the magic eye.
6. Note the reading on the Solute Bridge and Calculate EC as given below.

Calculation and Observation:

Cell constant of the Conductivity Cell = X

Solute Bridge reading = Y m. mhos.

EC = X x Ym.mhos cm^{-1}

Interpretation:

Table - 7

Electrical Conductivity (m. mhos cm^{-1})	**Nature of Soil**
< 0.8	Normal
0.8 – 1.6	Critical for tolerant crops
1.6 – 2.5	Critical for salt tolerant crops
> 2.5	Injurious for many crops

Precautions:

a. Keep the soil – water ratio in the suspension at 1: 2: 5 (EC decteases with dilution).
b. Allow the soil suspension to stand for a sufficient time to obtain clear supernatant.
c. Adjust the temperature of the Solute Bridge while measuring EC.
d. Dip the electrodes of the cell completely into the solution to get accurate reading.
e. Keep the electrodes dipped in distilled water when not in use(to get more accurate reading).

ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

Principle: The absorption energy by ground state atoms in the gaseous state forms the basis of Atomic Absorption Spectroscopy (AAS). When a solution containing metallic species is introduced into a flame, the vapour of mettallic species will be obtained. Some of the metal atoms may be raised to an energy level ssufficiently high to emit the characteristics radiation of the metal – a phenomenon that is utilized in the familiar technique of emission lame photometry. But a large percentage of metal atoms will remain in the non – emitting ground state. These ground state atoms of a particular element are receptive of light radiation of their own specific resonance wavelength (in general the same wavwlength as they would emit if they are exited). Thus, when a light of this wavelength is alloowed to pass through the flame, having atoms of metallic species, a part of that light will be absorbed and the adsorption will be proportional to the density of the atoms in the flame. Thus, in AAS, one determines the amount of light absorbed. Once the value of absorption is known the concentration of metallic element can be known because of the absorption is proportional to the density of the atoms in the flame.

Instrumentation:

Light of certain wavelength (produced by a special kind of lamp), which is able to emit the spectral lines corresponding to the energy required for an electronic transition from the ground state to the exited state is allowed to pass through the flame. Meanwhile, the sample solution is aspirated into the flame. Before it enters the flame, the solution gets dispersed into a mist of a very small droplets which evaporate in the flame to give the dry salt and the vapoir of the salt. At least, a part of this vapour will be dissociated into atoms of the element to be measured, thus, the flame processes free un – exited atoms which are capable for absorbing radiation. Froman external source when the radiation corresponds exactly to the energy required for transition element from the ground electronic state to an upper exited level. Then the unabsorbed radiation from the flame is allowed to passa through a monochromator which isolates the exited spectral lines of light is then registered by a photodetector, the output of which is amplified and measured on a recorder. Absorption is measured by the differences in the transmitted signal in the presence and absence of the test element.

Components of Atomic Absorption Spectrophotometer (AAS):

i. **Radiation source*:*** The radiation source of AAS should emit stable intense radiation of the elemeny to be determined.

Hollow Cathode Lamp:

The hollow cathode lamp consists of anode and cathode (which is terminated in the lamp as a hollow cup). The anode is generally a tungsten wire and the cathode cup is made up of such a element whole spectrum is desired or it may be constructed from an inert element into which the desired element or the salt of the desired element is kept. The hollow cathode lamp is generally filled with neon gas at low pressure. When a voltage of 300 – 500 is put across the anode and the cathode, the atom of the filter gas undergo ionozation at the anode and are rapidly attracted by the cathode and physically displaces the surface metal atoms of the cathode into neon (or argon gas). Further collision of the vapourised metal atoms from the cathode atoms which emit characteristic spectrum of the metal used to construct the cathode. Thus the emission spectrum produced by the hollow cathode lamp is the sharp linef spectrum of the cathode material and the filled gas. The neon or helium gas filled in the hollow cathode lamp performs three functions:

- It is the main souce of the current carrying capacity in the hollow cathode lamp.
- It dislodges the atoms from the surface of the cathode.
- It is primarly responsible for the exitation of the ground metal atoms.

ii. **Chopper:**

A rotation wheel is interposed between the hollow cathode lamp and the flame. This rotation wheel is known as chopper and is interposed to break the steady light from the lamps into an intermittent or pulsating light. This gives a pulsating current into the photocell and only the pulsating or alternating current is amplified and recorded and thus, the absorption of light will be measured witjout interference from the light emmited by flame itself.

iii. **Production of Atomic Vapour:**

In order to achieve absorption by atoms, it becomes necessary to reduce sample to the atomic state. The most common way is to use a flame which is used for converting the liquid sample to a gaseous state and also for cinversion of the molecule entities into an

atomic vapour. This is achieved by use of total consumption burner of pre – mixed burmer.

iv. Nebulisation of the Liquid sample:

Before the liquid sample enter the burner, it is first converted into small droplets.This method of formation of small drops from the liquid sample is called nebulisation. A common method method of nebulisation is by use of gas moving at high velocity called pneumatic nebulization.

iv. Monochromators:

In AAS the most common monochromator is prism and gratings. The function of monochromator is to select the fiven absorption lines emmited from the hollow cathode lamp.**(Fig – 46)**

v. Detectors:

For AAS the Photo – multiplier tube (PMT) is most suitable. PMY is an evacuated envelopewhich contains a photo – cathode, a series of electrodes called dynodes and an anode. As soon as the photon strikes the photo – cathode, an electron is dislodged and the proton is accelerated to anode to 1, resulting in the liberation of two or more electrons fromthis dynode. Similarly the electron from this dynode 1 is accelerated to dynode 2 and resulting in the liberation of more electron. Thus, the current multiplied at each dynode and the resultant electron current is received by the anode to produce EMF across RL which goes to the external ampliflier and read – out system.

vi. Ampliflier:

The electric current from the PMT is fed to the ampliflier which ampliflies the electronic current many times. Generally a “Lock in” amplifiers are preferred which provide a narrow frequency and band pass and help to achieve an excellent signal noise ratio.

vii. Read – out system:

The results are fed directly to the computer terminal which can be either seen on the screen or stored in the memory.

COOLING CENTRIFUGE

A **centrifuge** is a piece of equipment, generally driven by a motor that puts an object in rotation around a fixed axis, applying force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density. There are many different kinds of centrifuges, including those for very specialized purposes.

Theory

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions per minute. The acceleration is often quoted in multiples of g, the acceleration due to gravity at the Earth's surface. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations. The acceleration can be calculated as the product of the radius and the square of the angular velocity.

History and predecessors

English military engineer Laval (1707-1751) invented a whirling arm apparatus to determine drag, and **Antonin Prandl** invented the first centrifuge in order to separate cream from milk to make churning butter much easier.

Types and uses

There are basically four types of centrifuge:

- Tabletop/clinical/desktop centrifuge or micro centrifuge
- High-speed centrifuge
- Cooling centrifuge
- Ultra-centrifuge

Biology and biochemistry

Simple centrifuges are used in biology and biochemistry for isolating and separating biomolecules, cell organelles, or whole cells. They vary widely in speed and capacity. They usually comprise a rotor containing two, four, six, or many more numbered wells within which centrifuge tips may be placed.

The rotor is covered by a plastic cover. The cover is usually interlocked to prevent the motor from turning the rotor when it is open, and from allowing the cover to be opened before the rotor stops for several minutes. The cover protects the user from being injured by touching a rapidly spinning rotor. It also protects the user from fragments in case the rotor fails catastrophically.

To ensure that the rotor does not turn unbalanced, it must be balanced by placing samples or blanks of equal mass opposite each other. Since most of the mass is derived from the solvent, it is usually sufficient to place blanks or other samples of equal volume. As a safety feature, some centrifuges may stop turning when wobbling is detected.

The centrifuge tips are usually made of plastic or glass; they may vary in capacity from tens of milliliters, to much smaller capacities used in micro centrifuges used extensively in molecular biology laboratories. These micro centrifuges typically accommodate micro centrifuge tubes with capacities from 250 µl to 2.0 ml.

The ultracentrifuge was invented in 1925 by **Theodor Svedberg**, which by use of very high acceleration, and allowing the observation of sedimentation rates for macromolecules, allowed for the determination of their approximate molecular weights. Svedberg's 1926 Nobel Prize in Chemistry was earned for work made possible using his ultracentrifuge. Other centrifuges, the first being the Zippe-type centrifuge, are used to separate isotopes, and these kinds of centrifuges are in use in nuclear power and nuclear weapon programs.

Gas centrifuges are used in uranium enrichment. The heavier isotope of Uranium (Uranium-238) in the uranium hexafluoride gas tend to concentrate at the walls of the centrifuge as it spins, while the desired Uranium-235 isotope is extracted and concentrated with a scoop selectively placed inside the centrifuge. It takes many thousands of centrifuges to enrich uranium enough for use in a nuclear reactor (around 3.5% enrichment), and many thousands more to enrich it to atomic bomb-grade (around 90% enrichment).

Commercial applications

- Standalone centrifuges for drying (hand-washed) clothes - usually with a water outlet.
- Centrifuges are used in the attraction Mission: SPACE, located at Epcot in Walt Disney World, which propels riders using a combination of a centrifuge and a motion simulator to simulate the feeling of going into space.
- In soil mechanics, centrifuges utilize centrifugal acceleration to match soil stresses in a scale model to those found in reality.

- Large industrial centrifuges are commonly used in water and wastewater treatment to dry sludge. The resulting dry product is often termed **cake**, and the water leaving a centrifuge after most of the solids have been removed is called **centrate**.

Use and safety

The load in a laboratory centrifuge must be carefully balanced. Small differences in mass of the load can result in a large force imbalance when the rotor is at high speed. This force imbalance strains the spindle and may result in damage to centrifuge or personal injury.

Centrifuge rotors should never be touched while moving, because a spinning rotor can cause serious injury. Modern centrifuges generally have features that prevent accidental contact with a moving rotor.Because of the kinetic energy stored in the rotor head during high speed rotation, those who have experienced the loss of a rotor inside of an ultracentrifuge compare the experience to having a bomb explode nearby.

Calculating relative centrifugal force (RCF)

Relative centrifugal force is the measurement of the force applied to a sample within a centrifuge. This can be calculated from the speed (RPM) and the rotational radius (cm) using the following calculation.

$$\mathbf{g = RCF = 0.00001118 \times r \times N^2}$$

Where,

g = Relative centrifuge force

r = rotational radius (centimeter, cm)

N = rotating speed (revolutions per minute, r/min).

(Fig – 47)

HOT AIR OVEN

Hot air ovens are electrical devices used in sterilization. The oven utilizes dry heat to sterilize articles. Generally, they can be operated between 50 deg. C to 250/300 deg. There is a thermostat controlling the temperature. These are digitally controlled to maintain the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat. These are fitted with the adjustable wire mesh plated trays or aluminium trays and may have an on/off rocker switch, as well as indicators and controls for temperature and holding time. The capacities of these ovens vary. Power supply needs vary from country to country, depending on the voltage and frequency (Hertz) used. Temperature sensitive tapes or other devices like those utilizing bacterial spores can be used to work as controls, to test for the efficacy of the device in every cycle.

Advantages and disadvantages

They do not require water and there is not much pressure build up within the oven, unlike an autoclave, making them safer to work with. This also makes them more suitable to be used in a laboratory environment. They are much smaller than autoclaves but still as efficacious. They can be more rapid than an autoclave and higher temperatures can be reached compared to other means. As they use dry heat instead of moist heat, some organisms like prions, may not be killed by them every time.

Usage

A complete cycle involves heating the oven to the required temperature, maintaining that temperature for the proper time interval for that temperature, turning the machine off and cooling the articles in the closed oven till they reach room temperature. The standard settings for a hot air oven are:

- 1.5 to 2 hours at 160 °C (320 °F)
- 6 to 12 mins at 190 °C (374 °F)

plus the time required to preheat the chamber before beginning the sterilization cycle. If the door is opened before time, heat escapes and the process becomes incomplete. Thus the cycle must be properly repeated all over. These are widely used to sterilize articles that can

withstand high temperatures and not get burnt, like glassware, powders, paraffin. Linen gets burnt and surgical sharps lose their sharpness.

Construction: It is a double walled steel chamber with a slout door with insulation om other side. The interior contains one or more perforated metal shelves or shelves made up of wire mesh and the oven is fitted with a thermoregulator and a thermometer. The top or side contains a ventilator which is left open during sterilization to disperse any medium, moisture and volatile matter. Hot air passes between the wall and is usually heated by electricity through some order models are heated by gas. The electric heaters are placed at the bottom between the two walls.

Place the articleds to be sterilized tubes, flasks, pipettes wrapped in paper or placed in lens on the shelves. Make sure all articles are perfectly dried. Close the doors firm and turn on the source of heat. Take the time for oven reaches the required temperature. Note the time that heat was turned on. The time of sterilization should be 1 hour at 167ºC or 1.5 hours at 150ºC. turn off heat and allow the ovwn to become quite cold before opening the door. Otherwise glass articles may crack on sudden exposure to the cooler room air.

(Fig – 48)

COLORIMETER

Colorimetry is based on the principle that colored solutions preferentially absorb some wavelengths and the color seen is charactristic of the wavelength not abvsorbed. The colour on the wavelength band of the incident light used for a colorimetric determination is selected to coincide with that most absorbed by the test solution and this is done by the passage of light through light filter.

According to Beer's law, equal successive increments of concentration dC of a true solution containing a coloured constituent absorb equal fractions, di/l of the incident light.

Mathematically,

$$T = \frac{dt}{L_o} \times 100$$

If the colorimeteer gives transmittance readings, these can be converted to absorbance by the following relationship:

Absorbance = 2 – log (% T)

(Fig – 15)

CYCLOMIXER

A variable speed mixer to eliminate time consuming hand mixing. Holding tube against vibrating rubber cup does rapid mixing of contents. Speed regulator controls the degree of vibration. A unique touch feature operates the unit when tube is pressed on the rubber cup.

(**Fig – 14)**

MICROSCOPE

Microbiology is the branch of biology which deals with the microscopic oganisms which are too small to be seen with naked eye. The word microscope is derived from two greek words micro – small and scope – view. A microscope is a instrument use dfor the visual examination of small objects which cannot be examined properly with naked eyes. A simple microscope condists of a biconvex lens along with a stage to keep the specimen for examination and a light reflecting mirror. The compound microscope employs two separate lens system objective lens and an occular lens or an eye piece in order to achieve greater magnification. The objective lens focuses the light rays from the specimen to form a real image with in the body tube of the microscope. The real image is further magnified by the occular lens system which is sutuated at the top of the draw tube.The microscope consist of a mechanical portion whose function is to hold the two separate lens system in position and to fold the object being examined. An optical portion serves to magnify the objective, the mechanical portion consists of a 'U' shaped base stand from which arises a short vertical pillar at the upper end of which arises a 'c' shaped side arm which is joined to the pillar by an inclination joined which allows the arms to be tilted at a convinient angle at the top of pillar is the horizontal platform or stage with an opening called the stage aperature which can be brought into line with the long axis of the body tube. There are two stage clips which help to hold the microscopic slides carrying the object for examination.At the top of the arm is attached to a body tube which carries the objective lens at the lower end an occular lens at the upper end. The body tube so can be raised and lowered by means of the coarse adjustment screw. The fine adjustment screw is to bring the object exactly into focus the body tube sometimes contains within it a closely fitting under inner tube is called the draw tube which can be drawn out and that increases the distance between the two lens systems. There is a revolving nose piece to which the special objective can be screwed low power objective with high power objecive (Dry objective and oil immersion).

OPTICAL PORTIONS:

***Low power objective*:** it ususlly has a total distance of 16mm. It is sometimes called 10mm objective. It gives a magnification of 10 times and is marked as 10X.

HIGH POWER DRY OBJECTIVE:

Usually it has a focal distance of 4mm objective it gives a magnification of 40 times and is marked as 40X.

OIL IMMERSION:

This objective is so named because it is necessary to place a drop of oil between the lower end of the objective and the objective being examined in order to get good resolution and a clear image. The focal distance is about 1.5mm and it is called as the 1.8mm objective. The magnification is usually 90 – 100 and the objective is marked as 90X, 95X and 100X. these objectives are served into the revolving nose piece and can be brought into use one by one until the particular objective lens has its long axis in line with the long axis of the body tube. The objectives are the most important and most expensive parts of microscope.

OCCULAR EYEPIECE:

If the microscope has only one eye piece. It is called mono – locular microscope. If it has two occular.then it is called as Bilocular microscope. A few microscopes have a third occular and is called tri – locular microscope to which is attached to a camera for taking photograph. The eye piece consists of two lenses at either ends of a smsll tube. The magnification given by the eye piece will depend on the lenses and the distance between the two lenses. The most commomly used eye piece magnifies the object sometimes and is called as 10X. Devices for containing light to get the best image an optimum amount of light is required and the microscopes have certain devices to regulate the light they are

Condensor:

This consists of one or more lenses which helps to focus the light rays to the object being examined on the stage. The condensor can be raised or lowered and the change of position of the condensor will change the light getting through it.

Iris Diaphragm:

This can be opened or shut to allow the full light to pass or to restrict the amount of light.

Source of light:

Some microscopes especially bilocular models have an electric bulb immediately under the condensor, the mirror has two surfaces from the source of light are parallel. The concave surface is used when the rays from the light source are divertred which occur when light bulb is the source.

(Fig – 49)

RESULTS

IMPROVED CROP RESPONSE IN GREEN GRAM (Phaseolus aureus) GROWN ONLY WITH SOIL AND SOIL + GOT IN OUR RESEARCH FIELD

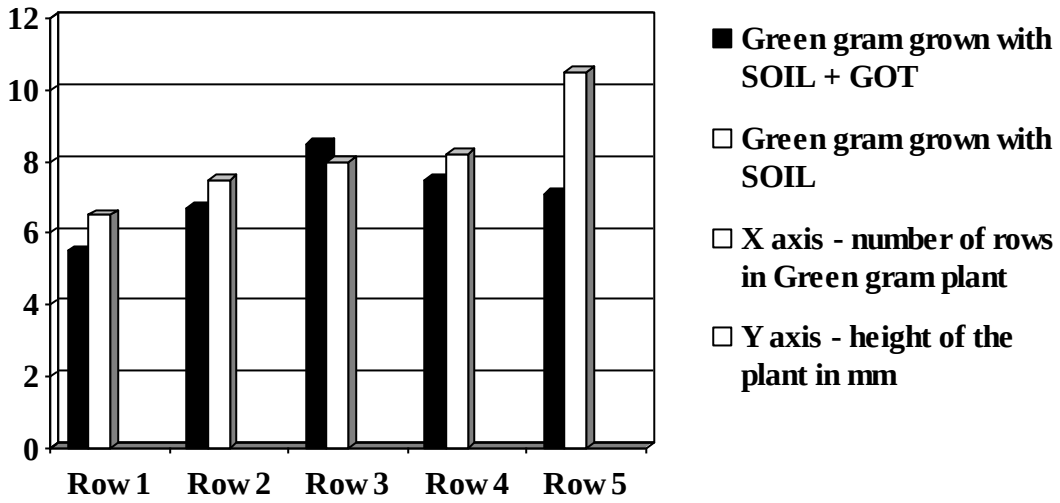
Chemically treated seeds of Green gram (Phaseolus aureus) were sown on 4.11.2007 at 1:40 pm in the plots with and without GOT.

READING – 1: Initial Stage – (Fig – 3)

Table – 8

Date (8.12.2007)	The measurement taken from stem to shoot tip in Green gram grown with SOIL + GOT (mm)	The measurement taken from stem to shoot tip in Green gram grown only with SOIL(mm)
1.	5.5	6.5
2.	6.7	7.5
3.	8.5	8
4.	7.5	8.2
5.	7.1	10.5
Average	7.06	8.14

Graph - 2

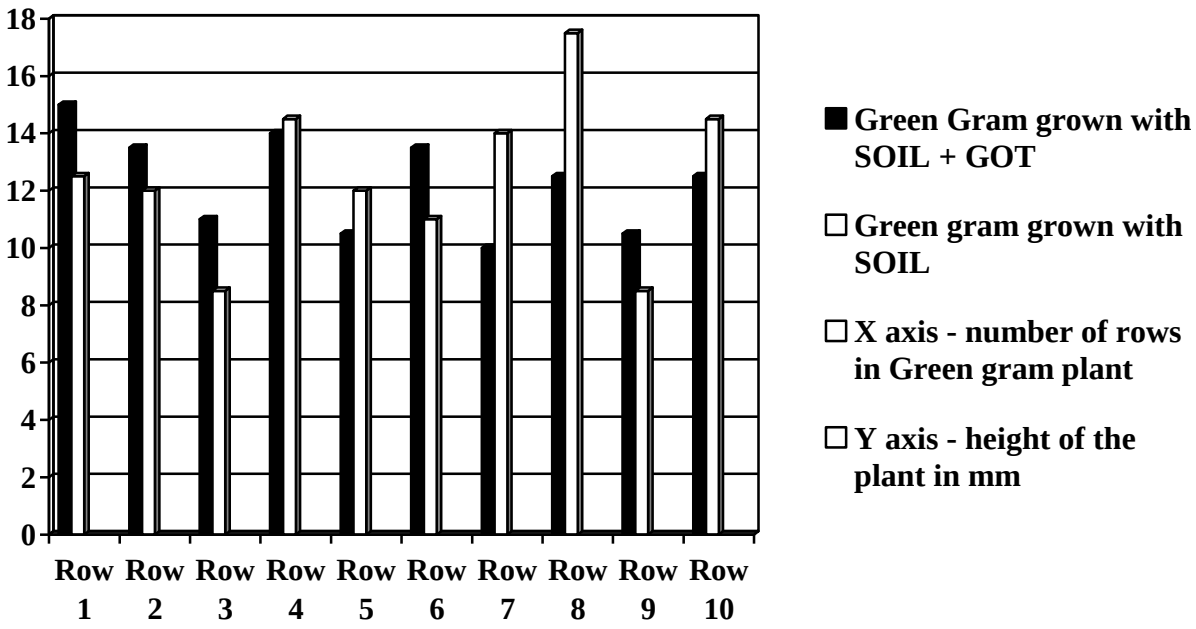


READING – 2:

Table - 9

Date (27.12.2007)	The measurement taken from stem to shoot tip in Green gram grown with SOIL + GOT (mm)	The measurement taken from stem to shoot tip in Green gram grown only with SOIL(mm)
1.	15	12.5
2.	13.5	12
3.	11	8.5
4.	14	14.5
5.	10.5	12
6.	13.5	11
7.	10	14
8.	12.5	17.5
9.	10.5	8.5
10.	12.5	14.5
Average	12.3	12.5

Graph - 3

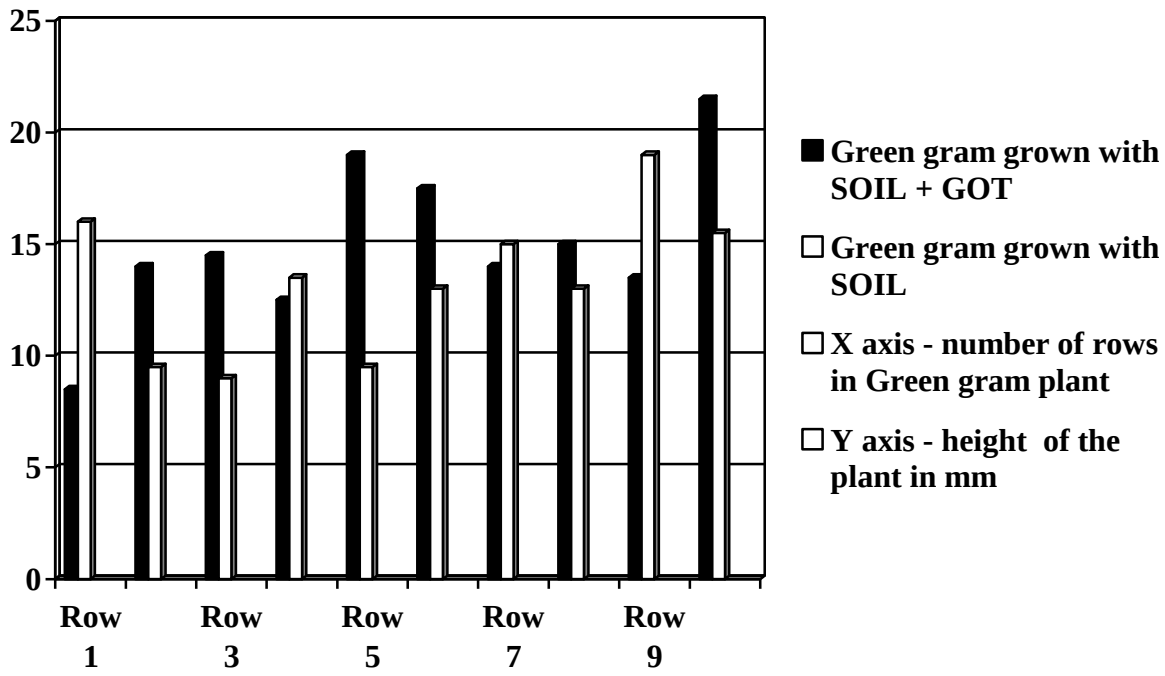


READING – 3

Table - 10

Date (21.1.2008)	The measurement taken from stem to shoot tip in Green gram grown with SOIL + GOT (mm)	The measurement taken from stem to shoot tip in Green gram grown only with SOIL(mm)
1.	8.5	16
2.	14	9.5
3.	14.5	9.0
4.	12.5	13.5
5.	19.0	9.5
6.	17.5	13.0
7.	14.0	15.0
8.	15.0	13.0
9.	13.5	19.0
10.	21.5	15.5
Average	15	13.3

Graph - 4

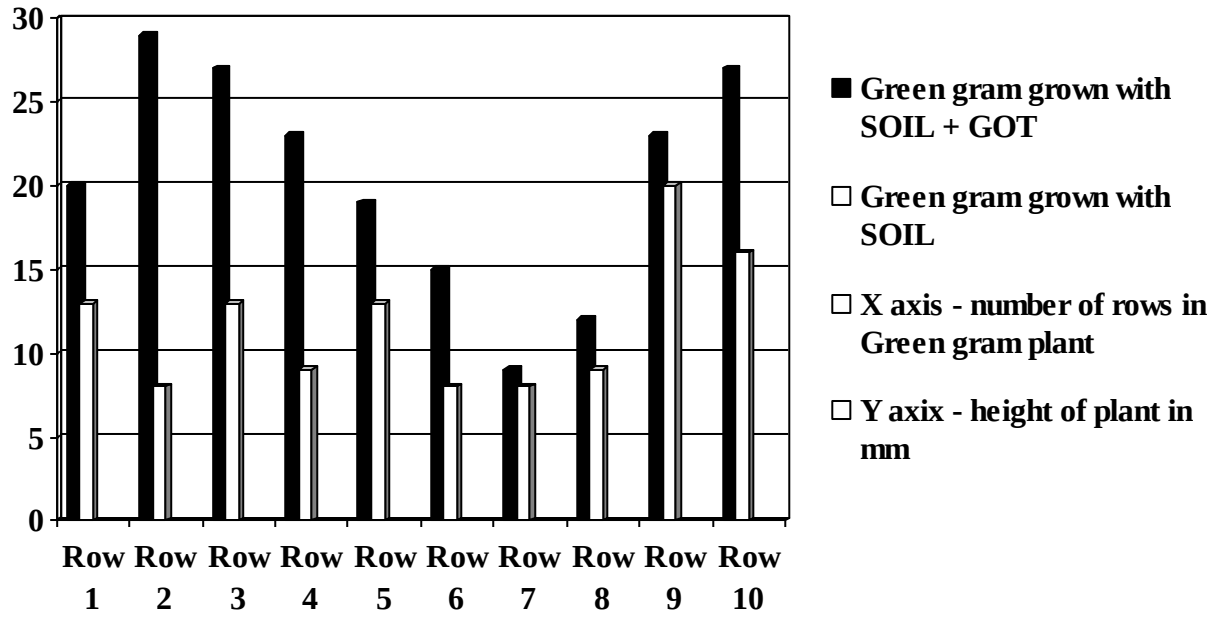


READING – 4: Number of pods present in each plant – (Fig – 4)

Table - 11

Date (21.1.2008)	Number of pods present in Green gram grown with SOIL + GOT	Number of pods present in Green gram grown only with SOIL
1.	20	13
2.	29	8
3.	27	13
4.	23	9
5.	19	13
6.	15	8
7.	9	8
8.	12	9
9.	23	20
10.	27	16
Average	20.4	11.7

Graph - 5



- **Water holding capacity of Green gram plant (Phaseolus aureus):**

The water holding capacity is 2 days more in plants grown in GOT when compared to the plants grown in pure soil.

- **Study of Root Nodules in Green gram plant (Phaseolus aureus):**

Table - 12

Sl.no	Green gram grown with SOIL + SOIL	Green gram grown only with SOIL
1.	The growth and number of formation of root nodules are good when compared to green gram grown with soil.	The growth and number of formation of root nodules are poor.

(Fig – 5)

- After the harvesting is done the pods of Green gram grown with and without GOT in a research field was subjected for drying. The weights were as follows:

Table - 13

Sl no.	Weight of Green gram grown with SOIL + GOT	Weight of Green gram grown with SOIL
1.	910 grams	660 grams.

(Fig – 9)

ESTIMATION OF PROTEINS IN GREENGRAM BY LOWRYS METHOD

Experiment was conducted on: 16.02.2008

Green gram grown with Soil + GOT

Table – 14: (Fig – 13)

Serial no.	Volume of standard protein (ml)	Volume of distilled water (ml)	Concentration of standard protein (µg)	Volume of Alkaline Copper reagent (ml)	Volume of FC reagent	OD at 660 nm
1.	0.0	1.0	0	5ml	0.5ml	0.0
2.	0.2	0.8	40	5ml	0.5ml	0.09
3.	0.4	0.6	80	5ml	0.5ml	0.21
4.	0.6	0.4	120	5ml	0.5ml	0.28
5.	0.8	0.2	160	5ml	0.5ml	0.42
6.	1.0	0	200	5ml	0.5ml	0.47
7.	0.05	9.95	92	5ml	0.5ml	0.21

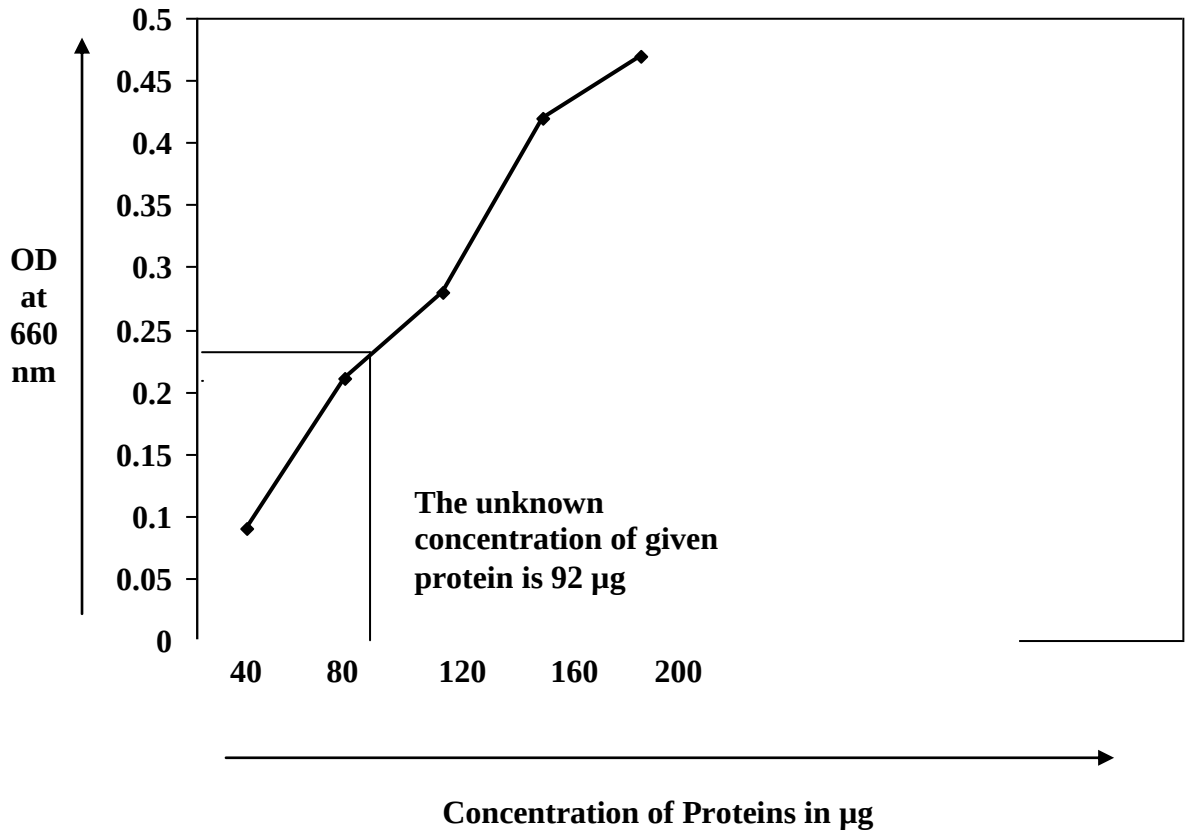
NOTE: After adding 5ml of Alkaline Copper reagent, incubate the test tubes at room temperature for about 10 minutes. Again after addition of FC reagent incubate the test tubes at room temperature for 30 minutes and check the Optical density.

* The concentration of protein is maximum in green gram plants grown in SOIL +GOT.

* The weight of Green gram after harvesting yielded **910 grams**

GRAPH PLOTTED FOR GREEN GRAM GROWN WITH SOIL + GOT

Graph - 6



CALCULATION:

- The concentration of unknown protein for 30% saturation of unknown sample contain **92 µg** of proteins
- 0.05ml of solution contains **92 µg** of proteins.
- $15 \times \frac{92}{0.05} = \mathbf{27{,}600\mu g}$

Therefore, 15 ml of solution contains **27,600** µg of proteins

- $4550 \times \frac{92}{0.05} = \mathbf{83{,}72000\mu g}$

Therefore, the total Green gram crop grown with Soil + GOT yields 4550 ml of solution contains **83, 72000** µg of proteins.

ESTIMATION OF PROTEINS IN GREENGRAM BY LOWRYS METHOD

Experiment was conducted on: 16.02.2008

Green gram grown only with Soil

Table – 15: (Fig – 12)

Serial no.	Volume of standard protein (ml)	Volume of distilled water (ml)	Concentration of standard protein (µg)	Volume of Alkaline Copper reagent (ml)	Volume of FC reagent	OD at 660 nm
1.	0.0	1.0	0	5ml	0.5ml	0.0
2.	0.2	0.8	40	5ml	0.5ml	0.09
3.	0.4	0.6	80	5ml	0.5ml	0.21
4.	0.6	0.4	120	5ml	0.5ml	0.28
5.	0.8	0.2	160	5ml	0.5ml	0.42
6.	1.0	0	200	5ml	0.5ml	0.47
7.	0.05	9.95	86	5ml	0.5ml	0.20

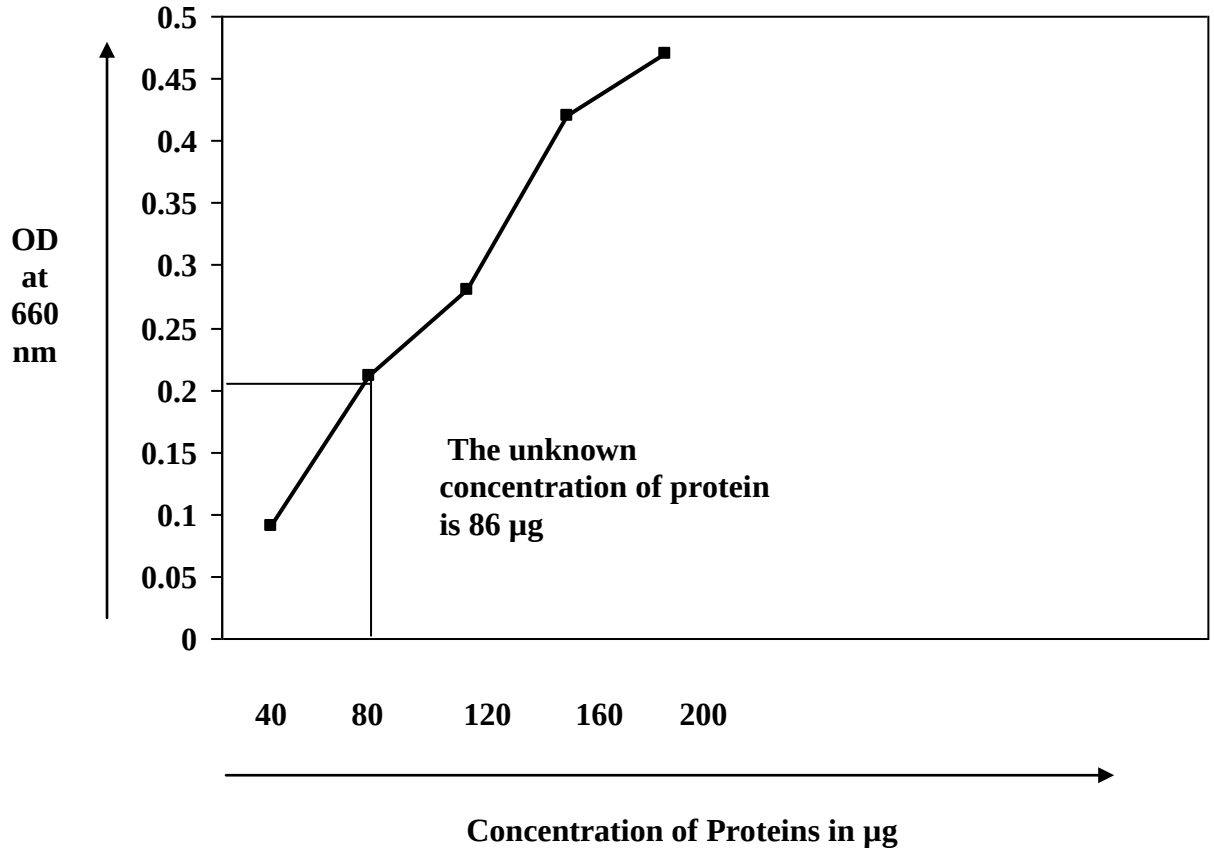
NOTE: After adding 5ml of Alkaline Copper reagent, incubate the test tubes at room temperature for about 10 minutes. Again after addition of FC reagent incubate the test tubes at room temperature for 30 minutes and check the Optical density.

* The concentration of protein is minimum in green gram plants grown only in SOIL.

* The weight of Green gram after harvesting yielded **660 grams**

GRAPH PLOTTED FOR GREEN GRAM GROWN ONLY WITH SOIL

Graph - 7



CALCULATION:

- The concentration of unknown protein for 30% saturation of unknown sample contain **86 µg** of proteins
- 0.05ml of solution contains **86 µg** of proteins.
- $15 \times \frac{86}{0.05} = \mathbf{25{,}800\mu g}$

  Therefore, 15 ml of solution contains **25,800** µg of proteins
- $3300 \times \frac{86}{0.05} = \mathbf{56{,}76000\mu g}$

Therefore, the total Green gram crop grown only with Soil yields 3300 ml of solution contains **56, 76000** µg of proteins.

IMPROVED CROP RESPONSE IN CAULIFLOWER (Brassica oleracea botrytis) GROWN ONLY WITH SOIL AND SOIL + GOT IN OUR RESEARCH FIELD

ISI Certified seeds of Cauliflower (Brassica oleracea botrytis) **(Fig - 16)** were sown on 8.12.2007 at 3:15 pm in the plots with and without GOT. **(Fig -18)**

HEIGHT OF SEEDLINGS

Table – 16 **(Fig – 19)**

Date	Brassica oleracea botrytis (Cauliflower)
8.12.2007	2 mm

READING - 1

Area of the cauliflower leaf (from the petiole) is taken randomly in a plot

Cauliflower grown with SOIL + GOT

Table - 17

Date 27.12.2007	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length xWidth)
1.	6.7	4	26.8
2.	8	3.8	30.4
3.	7.1	3.3	23.43
4.	5.8	3	17.4
5.	7.2	3.9	28.08
Average	6.96	3.6	25.22

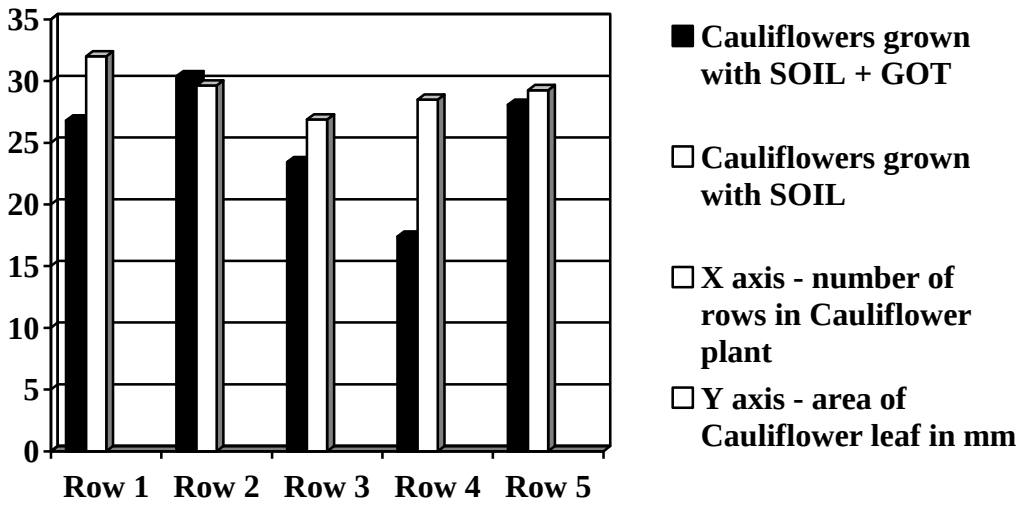
Area of the cauliflower leaf (from the petiole) is taken randomly in a plot

Cauliflower grown only with SOIL

Table - 18

Date 27.12.2007	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	8	4	32
2.	7.8	3.8	29.64
3.	8.4	3.2	26.88
4.	7.5	3.8	28.5
5.	8.1	3.6	29.16
Average	7.96	3.68	29.23

Graph – 8



READING - 2

Area of the cauliflower leaf (from the petiole) is taken randomly in a plot

Cauliflower grown with SOIL + GOT

Table - 19

Date 21.1.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	14.5	6	87
2.	16.75	6.9	115.57
3.	18.0	9.0	162
4.	19.0	6.5	123.5
5.	19.5	9.0	175.5
Average	17.55	7.48	131.27

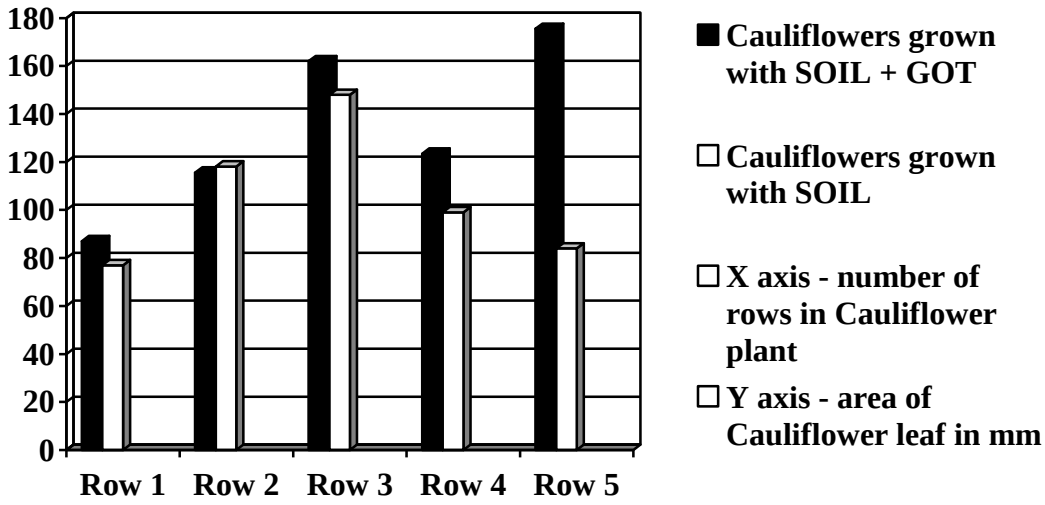
Area of the cauliflower leaf (from the petiole) is taken randomly in a plot

Cauliflower grown only with SOIL

Table - 20

Date 21.1.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	14.0	5.5	77
2.	17.5	6.75	118.12
3.	18.5	8.0	148
4.	16.5	6.0	99
5.	14.0	6.0	84
Average	16.1	6.45	103.84

Graph - 9



READING - 3

Area of the cauliflower leaf (from the petiole) & diameter of the flower is taken randomly in a plot

Cauliflower grown with SOIL + GOT

Table - 21

Date 10.2.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)	Flower (mm)
1.	16	7.4	118.4	7
2.	20.5	6.8	139.4	8
3.	18.2	5.5	100.1	7.5
4.	16.3	7.0	114.1	7.5
5.	15.8	7.0	110.6	8
Average	17.36	6.74	117.006	7.6

(Fig – 20)

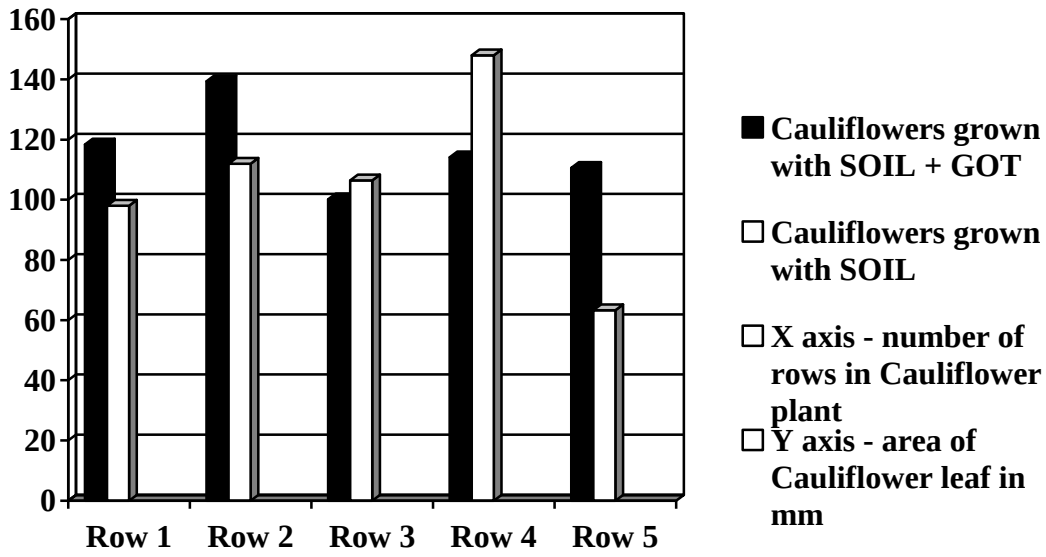
Area of the cauliflower leaf (from the petiole) & diameter of the flower is taken randomly in a plot

Cauliflower grown only with SOIL

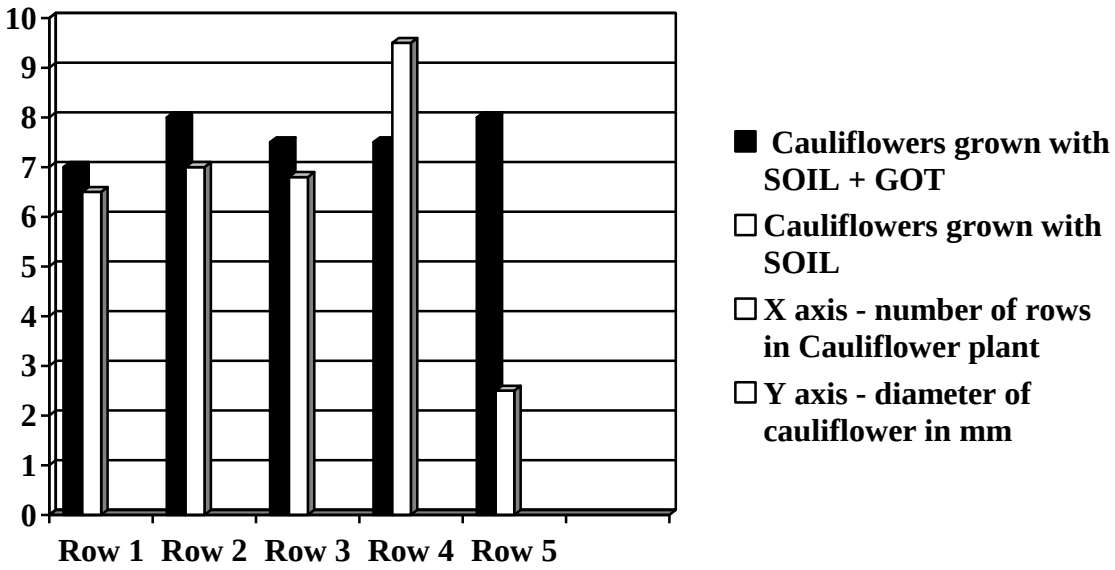
Table - 22

Date 10.2.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)	Flower (mm)
1.	14.0	7	98	6.5
2.	16	7	112	7
3.	15.2	7	106.4	6.8
4.	18.5	8.0	148	9.5
5.	11.5	5.5	63.25	2.5
Average	15.04	6.9	103.57	6.46

Graph – 10



Graph - 11



WEIGHING THE HARVESTED CAULIFLOWER

Table - 23

Date 17.2.2008	Weight of cauliflower grown in SOIL + GOT	Weight of cauliflower grown only with SOIL
1.	900 grams	600 grams
2.	975 grams	450 grams

(Fig - 20 and 21)

IMPROVED CROP RESPONSE IN CABBAGE (Brassica oleracea capitata) GROWN ONLY WITH SOIL AND SOIL + GOT IN OUR RESEARCH FIELD

ISI Certified seeds of Cabbage (Brassica oleracea capitata) **(Fig – 24)** were sown on 8.12.2007 at 3:30 pm in the plots with and without GOT. **(Fig – 25)**

HEIGHT OF SEEDLINGS

Table - 24

Date	Brassica oleracea capitata (Cauliflower)
8.12.2007	2.25 mm

READING – 1

Area of the cabbage leaf (from the petiole) is taken randomly in a plot

Cabbage grown with SOIL + GOT

Table – 25

Date 27.12.2007	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	5.9	3.5	20.65
2.	6.6	3.5	23.1
3.	4.8	2.8	13.44
4.	6.9	4.9	33.81
5.	7.0	4.1	28.7
Average	6.24	3.76	23.94

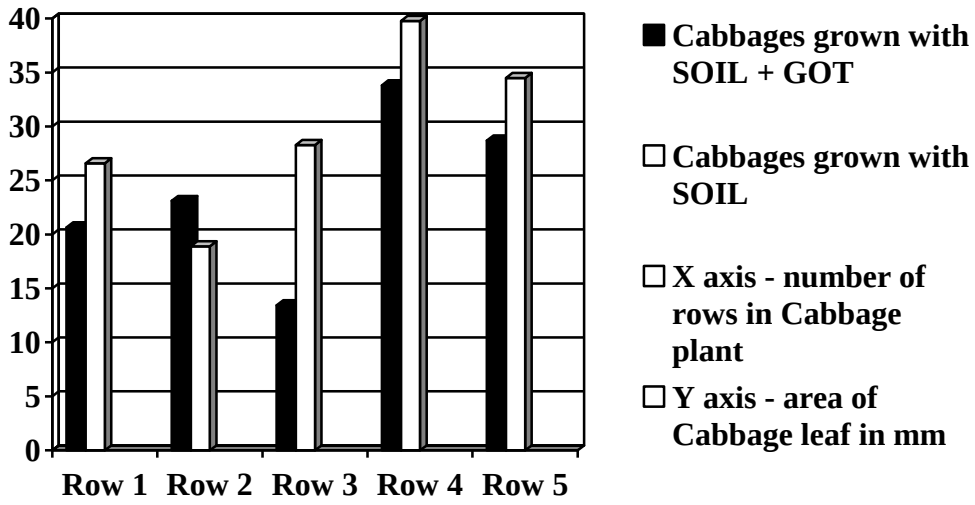
Area of the cabbage leaf (from the petiole) is taken randomly in a plot

Cabbage grown only with SOIL

Table – 26

Date 27.12.2007	The measurement is taken from the petiole of the leaf to apex Length(mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	7	3.8	26.6
2.	5.4	3.5	18.9
3.	6.9	4.1	28.29
4.	7.8	5.1	39.78
5.	7.5	4.6	34.5
Average	6.92	4.22	29.61

Graph - 12



READING - 2

Area of the cabbage leaf (from the petiole) is taken randomly in a plot

Cabbage grown with SOIL + GOT

Table - 27

Date 21.1.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)
1.	10.0	8.5	85
2.	13.5	11.5	155.25
3.	12.0	10.0	120
4.	11.0	8.0	88
5.	10.0	12.0	120
Average	11.3	10	113

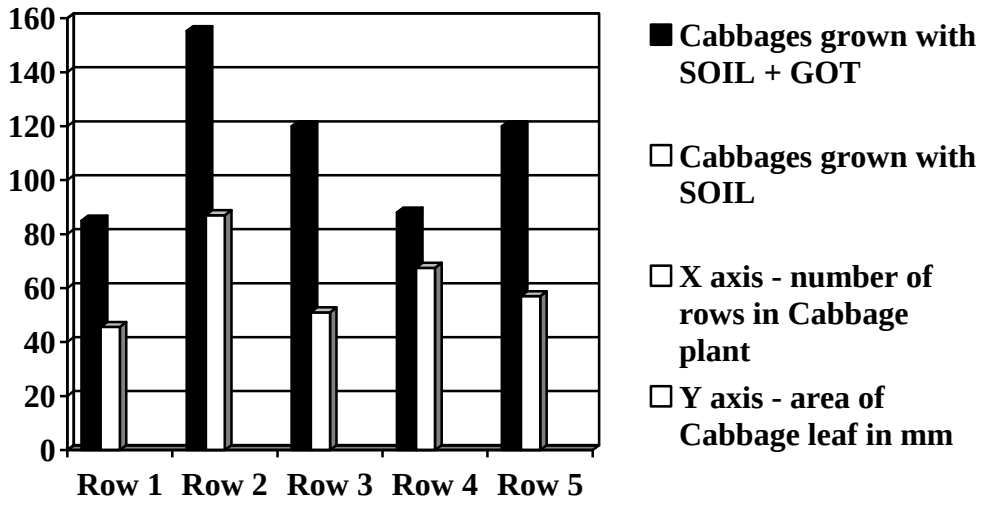
Area of the cabbage leaf (from the petiole) is taken randomly in a plot

Cabbage grown only with SOIL

Table - 28

Date 21.1.2008	The measurement is taken from the petiole of the leaf to apex Length	The measurement is taken from the petiole of the leaf to apex Width	Area (mm) (Length x Width)
1.	8.0	5.7	45.6
2.	10.0	8.7	87
3.	8.5	6.0	51
4.	9.	7.5	67.5
5.	9.5	6.0	57
Average	9	6.78	61.02

Graph – 13



READING - 3

Area of the cabbage leaf (from the petiole) & diameter of the flower is taken randomly in a plot

Cabbage grown with SOIL + GOT

Table - 29

Date 10.2.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)	Flower (mm)
1.	12	10	120	4
2.	11	10.5	115.5	4
3.	13	12	156	5
4.	14	11	154	7
5.	11	9.0	99	6
Average	12.2	10.4	127.5	4.8

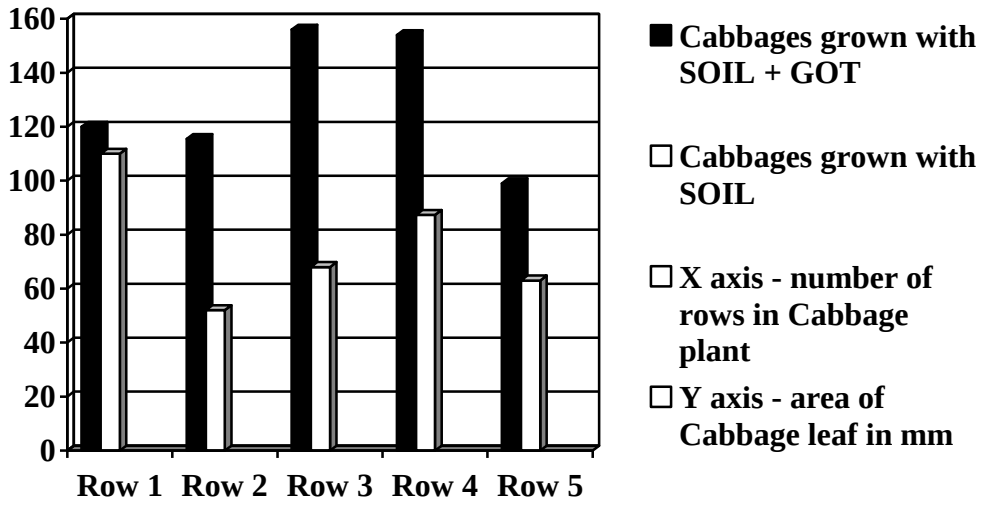
Area of the cabbage leaf (from the petiole) & diameter of the flower is taken randomly in a plot

Cabbage grown only with SOIL

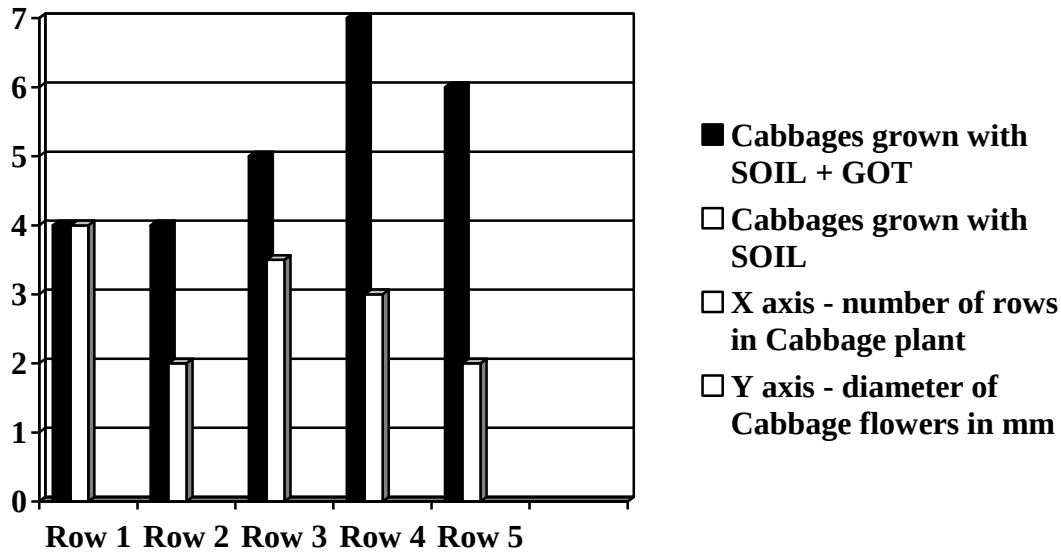
Table - 30

Date 10.2.2008	The measurement is taken from the petiole of the leaf to apex Length (mm)	The measurement is taken from the petiole of the leaf to apex Width (mm)	Area (mm) (Length x Width)	Flower (mm)
1.	11	10	110	4
2.	6.5	8	52	2
3.	8	8.5	68	3.5
4.	9.7	9	87.3	3
5.	7	9	63	2
Average	5.5	8.44	48.97	6

Graph – 14



Graph - 15



READING - 4

Diameter of the flower is taken randomly in a plot

Cabbage grown with SOIL + GOT

Table – 31

Date 10.2.2008	Cabbage grown with Soil + GOT Flower (mm)
1.	8.0
2.	7.5
3.	7.75
4.	7.0
5.	8.0
Average	7.65

(Fig – 29)

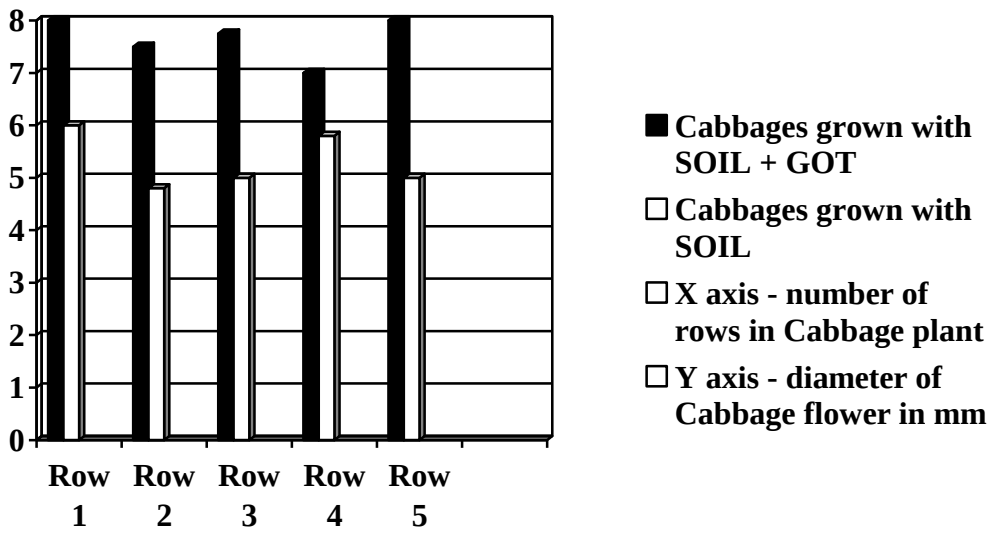
Diameter of the flower is taken randomly in a plot

Cabbage grown only with SOIL

Table - 32

Date 10.2.2008	Cabbage grown with Soil Flower (mm)
1.	6.0
2.	4.8
3.	5.0
4.	5.8
5.	5.0
Average	5.32

Graph – 16



- The cabbage plants grown without GOT are attacked by pests and fungus.

WEIGHING THE HARVESTED CABBAGE

Table - 33

Date 17.2.2008	Weight of cabbage grown with SOIL + GOT	Weight of cabbage grown only with SOIL
1.	1,300 grams	1 kg
2.	950 grams	800 grams

(Fig - 27 and 28)

WATER ANALYSIS BY MET – CHEM LABORATORY

The water sample was collected from the borewell of the research field and was sent to analysis at Met – Chem Laboratories (ISO: 9001: 2000 Certified Lab).

Table - 34

Sl. no	Parameters	Drinking water limits as per ISO:10599/1991	Results
1.	Color	<05.00 Hazen	01.25
2.	Turbidity	< 05.00 NTU	02.51
3.	pH value	06.50 – 08.50	07.29
4.	Total dissolved salts	< 1000.00 ppm	450.00
5.	Phenolphthalein alkanity as $CaCO_3$	< 200.00 ppm	03.58
6.	Total alkalinity as $CaCO_3$	< 200.00 ppm	160.80
7.	Total hardness as $CaCO_3$	< 300.00 ppm	221.98
8.	Calcium	< 75.00 ppm	24.25
9.	Magnesium	< 30.00 ppm	06.14
10.	Sodium	N.S	43.58
11.	Iron	< 00.30 ppm	00.030
12.	Chlorides	< 500.00 ppm	48.19
13.	Sulphates	< 200.00 ppm	19.20
14.	Nitrates	< 45.00 ppm	07.38
15.	Ammonium	< 04.80 ppm	01.74
16.	Fluoride	< 01.50 ppm	00.31
17.	Silica		15.90
18.	Conductivity in Micro mhos	N.S.	790.00

Result: The water sample is portable.

LIST OF SOIL SAMPLES & VEGERABLES SENT TO IIHR FOR ANALYTICAL STUDY

- Dried cauliflower flowers grown in SOIL + GOT
- Dried cauliflower flowers grown in PURE SOIL
- One cauliflower market sample
- Dried cabbage leaves grown in SOIL + GOT
- Dried cabbage leaves grown in SOIL
- One cabbage market sample
- GOT sample
- Pure soil sample
- Pure soil of green gram
- Soil + GOT of green gram
- Pure soil of cauliflower
- Soil + GOT of cauliflower
- Pure soil of cabbage
- Soil + GOT of cabbage

(Fig – 32, 33, 34 and 35)

OPTIMUM NUTRIENT RANGES IN SOIL RECOMMENDED BY IIHR

Table - 35

NUTRIENTS	UNIT	RANGE
pH	dsm-1	6.30 – 7.50
Electrical Conductivity	%	< 1.00
Organic Carbon	ppm	0.75 – 1.00
Available N	ppm	125 – 250
Available P	ppm	30 – 50
Available K	ppm	150 – 300
Available Ca	ppm	1000 -1500
Available Mg	ppm	180 – 250
Available S	ppm	15 – 25
Available Fe	ppm	5 -10
Available Mn	ppm	3 -10
Available Zn	ppm	1.0 – 1.50
Available Cu	ppm	0.5 – 1.0

ANALYTICAL STUDY BY IIHR

Table - 36

ANALYTICAL RESULTS OF THE SAMPLES					
RED SOIL SAMPLE (Before sowing crops)			**GOT SAMPLE**		
Nutrients	**Unit**	**Analytical Result**	**Nutrients**	**Unit**	**Analytical Result**
pH		**6.4 1**	**pH**		**7.57**
EC	dsm-1	**0.545**	**EC**	dsm-1	**2.895**
Organic Carbon	%	**1.08**	**Organic Carbon**	%	**0.42**
Available N	ppm	**175**	**Available N**	ppm	**68**
Available P	ppm	**247**	**Available P**	ppm	**77**
Available K	ppm	**94**	**Available K**	ppm	**897**
Available Ca	ppm	**1140**	**Available Ca**	ppm	**2682**
Available Mg	ppm	**280**	**Available Mg**	ppm	**163**
Available S	ppm	**43.7**	**Available S**	ppm	**180.0**
Available Fe	ppm	**15.5**	**Available Fe**	ppm	**13.6**
Available Mn	ppm	**1.66**	**Available Mn**	ppm	**8.2**
Available Zn	ppm	**2.5**	**Available Zn**	ppm	**6.6**
Available Cu	ppm	**0.87**	Available Cu	ppm	**6.42**

CHEMICAL PROPERTIES OF GOLD ORE TAILINGS (GOT)

Table - 37

Analytical tests	Control (GOT)	Standard recommended by IIHR
pH	7.57	6.30 – 7.50
EC (dsm^{-1})	2.895	< 1.00
Organic Carbon (%)	0.42	0.75 – 1.00
Nitrogen (ppm)	68	125 – 250
Phosphorus (ppm)	77	30 – 50
Potassium (ppm)	897	150 – 300
Calcium (ppm)	2682	1000 -1500
Magnesium (ppm)	163	180 – 250
Sulphur (ppm)	180.0	15 – 25
Iron (ppm)	13.6	5 -10
Manganese (ppm)	8.2	3 -10
Zinc (ppm)	6.6	1.0 – 1.50
Copper (ppm)	6.42	0.5 – 1.0

(Fig -30)

ANALYTICAL RESULTS OF GOT SAMPLE GIVEN BY IIHR:

- The pH of the sample depicts that the sample is calcareous.
- High electrical conductivity denotes total soluble salts in the sample.
- Low organic carbon content.
- Nitrogen present at low level.
- Phosphorous is at optimum level.
- Potassium is above optimum level and can sustain crop growth.
- Calcium is at very high level (property of calcareous soil).
- Magnesium is at medium level.
- Sulphur as essential as nitrogen is present at higher level.
- The micro nutrients Fe is at optimum level and Mn, Zn, Cu is at very high level.

ANALYTICAL RESULTS OF SOIL SAMPLES GIVEN BY IIHR **Table - 38**

SL. NO	DETAILS	pH	EC dsm^{-1}	OC %	N ppm	P ppm	K ppm	Ca ppm	Mg ppm	S ppm	Fe ppm	Mn ppm	Zn ppm	Cu ppm
1	**Pure Soil of land 20.06.2008**	6.41	0.545	1.08	175	247	94	1140	280	43.7	15.5	1.66	2.5	0.87
2	**Green gram (Soil) 31.12.2007**	6.41	0.339	1.20	194	240	175	984	238	33.7	21.3	13.4	2.9	1.07
3	**Green gram (Soil + GOT) 31.12.2007**	6.86	0.832	1.02	165	130	156	1485	223	142.5	14.2	6.6	4.0	1.32
4	**Cabbage (Soil) 31.12.2007**	6.22	0.245	0.78	126	181	106	861	196	32.5	30.5	15.5	2.1	1.12
5	**Cabbage (Soil + GOT) 31.12.2007**	6.05	0.429	0.63	102	171	112	712	172	23.7	24.0	1.7	1.8	1.02
6	**Cabbage (Soil) 17.02.2008**	6.45	0.424	0.81	131	210	112	1106	257	32.5	24.8	2.2	2.3	1.12
7	**Cabbage (Soil + GOT) 17.02.2008**	5.98	0.413	0.60	97	152	106	775	201	26.2	27.1	1.6	1.7	1.02
8	**Cauliflower (Soil) 31.12.2007**	6.50	0.442	1.11	180	166	175	1080	223	38.7	21.6	13.5	2.9	1.42
9	**Cauliflower (Soil + GOT) 31.12.2007**	6.72	0.661	1.02	165	112	162	1593	227	147.5	16.9	10.7	2.9	1.37
10	**Cauliflower (Soil) 17.02.2008**	6.87	0.397	1.14	185	231	94	1041	232	36.2	19.9	23.2	3.2	1.50
11	**Cauliflower (Soil + GOT) 17.02.2008**	7.01	0.809	1.08	175	251	125	1725	222	197.5	14.0	18.7	4.1	1.57
12	**GOLD ORE TAILINGS (GOT)**	7.57	2.895	0.42	68	77	387	2682	163	180.0	13.6	8.2	6.6	6.42

ANALYTICAL RESULTS OF PLANT SAMPLES GIVEN BY IIHR

Table - 39

SL. NO	DETAILS	N %	P %	K %	Ca %	Mg %	S %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
1	**Cabbage (Soil) 20.06.2008**	2.24	0.127	2.20	0.92	0.27	0.375	59	14	16.9	4.2
2	**Cabbage (Soil + GOT) 20.06.2008**	2.41	0.127	2.40	0.84	0.26	0.382	54	13	17.5	5.0
3	**Cauliflower(Soil) 20.06.2008**	2.94	0.181	3.00	0.68	0.31	0.377	71	19	32.0	5.9
4	**Cauliflower (Soil + GOT) 20.06.2008**	2.83	0.207	2.80	0.48	0.22	0.370	98	21	31.0	6.5

INTERPRETATION

ANALYTICAL STUDY IN GREENGRAM

Table - 40

Analytical tests	Soil 31.12.07	Soil + GOT 31.12.07	Control (SOIL) before sowing crops	Standard recommended by IIHR	Amount of nutrient absorbed by the crop Soil 31.12.07	 Soil + GOT 31.12.07
pH	6.41	6.86	**6.4 1**	6.30 – 7.50	**0**	**-0.45**
EC (dsm^{-1})	0.339	0.832	**0.545**	< 1.00	**0.206**	**-0.287**
***Organic Carbon (%)**	1.20	1.02	**1.08**	0.75 – 1.00	**-0.12**	**0.06**
***Nitrogen (ppm)**	194	165	**175**	125 – 250	**-19**	**10**
***Phosphorus(ppm)**	240	130	**247**	30 – 50	**7**	**117**
***Potassium (ppm)**	175	156	**94**	150 – 300	**-81**	**-62**
Calcium (ppm)	984	1485	**1140**	1000 -1500	**156**	**-345**
***Magnesium (ppm)**	238	223	**280**	180 – 250	**42**	**57**
Sulphur (ppm)	33.7	142.5	**43.7**	15 – 25	**10**	**-98.8**
***Iron (ppm)**	21.3	14.2	**15.5**	5 -10	**-5.8**	**1.3**
***Manganese (ppm)**	13.4	6.6	**1.66**	3 -10	**22.244**	**-4.94**
Zinc (ppm)	2.9	4.0	**2.5**	1.0 – 1.50	**-0.4**	**-1.5**
Copper (ppm)	1.07	1.32	**0.87**	0.5 – 1.0	**-0.2**	**-0.45**

In reference to above table, when the comparative study done between the Soil sample and the crop i.e. Green gram grown with soil + GOT shows maximum growth by absorbing nutrients like Nitrogen, Phosphorus, Magnesium, Potassium, Manganese, Iron rather than Calcium, Zinc & Copper.

ANALYTICAL STUDY IN CAULIFLOWER

Table - 41

Analytical tests	Soil		Soil +GOT		Control (SOIL) before sowing crops	Standard recommended by IIHR	Amount of nutrient absorbed by the crop			
							Soil		Soil + GOT	
	31.12.07	17.2.08	31.12.07	17.2.08			31.12.07	17.02.08	31.12.07	17.02.08
pH	6.50	6.82	6.72	7.01	**6.4 1**	6.30 –7.50	**-0.09**	**-0.41**	**-0.31**	**-0.6**
EC (dsm^{-1})	0.442	0.397	0.661	0.809	**0.545**	< 1.00	**0.103**	**0.148**	**-0.116**	**-0.264**
Organic Carbon (%)	1.11	1.14	1.02	1.08	**1.08**	0.75 –1.00	**-0.03**	**-0.06**	**0.06**	**0**
Nitrogen (ppm)	180	185	165	175	**175**	125 – 250	**-5**	**-10**	**10**	**0**
Phosphorus (ppm)	166	231	112	251	**247**	30 – 50	**81**	**16**	**135**	**-4**
Potassium (ppm)	175	94	162	123	**94**	150 – 300	**-81**	**0**	**-68**	**-29**
Calcium (ppm)	1080	1041	1593	1725	**1140**	1000 -1500	**60**	**99**	**-456**	**-585**
***Magnesium (ppm)**	223	232	227	222	**280**	180 – 250	**57**	**48**	**53**	**58**
Sulphur (ppm)	38.7	36.2	147.5	197.5	**43.7**	15 – 25	**5**	**7.5**	**-103.8**	**-153.8**
***Iron (ppm)**	21.6	19.9	16.9	14.0	**15.5**	5 -10	**-6.1**	**-4.4**	**-1.4**	**1.5**
Manganese (ppm)	13.5	23.2	10.7	18.7	**1.66**	3 -10	**-11.84**	**-21.54**	**-9.04**	**-17.04**
Zinc (ppm)	2.9	3.2	2.9	4.1	**2.5**	1.0 – 1.50	**-0.4**	**-0.7**	**-0.4**	**-1.6**
Copper (ppm)	1.42	1.50	1.37	1.57	**0.87**	0.5 – 1.0	**-0.55**	**-0.63**	**-0.5**	**-0.15**

In reference to above table, when the comparative study done between the Soil sample and the crop i.e. Cauliflower grown with soil + GOT shows maximum growth by absorbing nutrients like Magnesium, Iron, rather than Nitrogen, Phosphorus, Calcium, Sulphur, Zinc, Manganese, Potassium, Copper.

ANALYTICAL STUDY IN CABBAGE

Table - 42

Analytical tests	Soil		Soil +GOT		Control (SOIL) before sowing crops	Standard recommended by IIHR	Amount of nutrient absorbed by the crop			
							Soil		Soil + GOT	
	31.12.07	17.2.08	31.12.07	17.2.08			31.12.07	17.02.08	31.12.07	17.02.08
pH	6.22	6.45	6.05	5.98	**6.4 1**	6.30 – 7.50	**0.19**	**-0.04**	**0.36**	**0.19**
***EC (dsm^{-1})**	0.245	0.424	0.429	0.413	**0.545**	< 1.00	**0.3**	**0.121**	**0.116**	**0.132**
***Organic Carbon (%)**	0.78	0.81	0.63	0.60	**1.08**	0.75 – 1.00	**0.3**	**0.27**	**0.45**	**0.48**
***Nitrogen (ppm)**	126	131	102	97	**175**	125 – 250	**49**	**44**	**73**	**78**
***Phosphorus (ppm)**	181	210	171	152	**247**	30 – 50	**66**	**37**	**76**	**95**
Potassium (ppm)	106	112	112	106	**94**	150 – 300	**-12**	**-18**	**-18**	**-12**
***Calcium (ppm)**	861	1106	712	775	**1140**	1000 -1500	**279**	**34**	**428**	**365**
***Magnesium (ppm)**	196	257	172	201	**280**	180 – 250	**84**	**23**	**108**	**79**
***Sulphur (ppm)**	32.5	32.5	23.7	26.2	**43.7**	15 – 25	**11.2**	**11.2**	**20**	**17.5**
Iron (ppm)	30.5	24.8	24.0	27.1	**15.5**	5 -10	**-15**	**-9.3**	**-8.5**	**-11.6**
***Manganese (ppm)**	15.5	2.2	1.7	1.6	**1.66**	3 -10	**-13.84**	**-0.54**	**-0.04**	**0.06**
***Zinc (ppm)**	2.1	2.3	1.8	1.7	**2.5**	1.0 – 1.50	**0.4**	**0.2**	**0.7**	**0.8**
Copper (ppm)	1.12	1.12	1.02	1.12	**0.87**	0.5 – 1.0	**-0.25**	**-0.25**	**-0.15**	**-0.15**

In reference to above table, when the comparative study done between the Soil sample and the crop i.e. Cabbage grown with soil + GOT shows maximum growth by absorbing nutrients like Nitrogen, Phosphorus, Calcium, Magnesium, Sulphur, Manganese & Zinc rather than Potassium, Iron and Copper.

INTERPRETATION OF RESULTS SHOWING THE DIFFERENCE BETWEEN THE MINERAL CONTENT IN CONTROL AND TEST SAMPLES OF CABBAGE

Table – 43

SL. NO.	DETAILS	N %	P %	K %	Ca %	Mg%	S %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
1.	**Cabbage(Soil+GOT)**	2.41	0.127	2.40	0.84	0.26	0.382	54	13	17.5	5.0
2.	**Cabbage (Soil)**	2.24	0.127	2.20	0.92	0.27	0.375	59	14	16.9	4.2
3.	**Difference between Soil & Soil + GOT**	0.17	0.0	0.20	0.8	0.01	0.007	5.0	1.0	0.6	0.8

From the table when a comparison made between the cabbages grown only in Soil and that of GOT mixed soil revealed the fact that minerals such as Nitrogen, Potassium, Sulphur, Zinc and Copper showed an increase trend in cabbage grown in GOT mixed soil, but the influence of GOT was negligible in case of minerals such as Phosphorus, Calcium and Magnesium. GOT has shown suppressive effect in case of Iron and Manganese up taken by plant.

INTERPRETATION OF RESULTS SHOWING THE DIFFERENCE BETWEEN THE MINERAL CONTENT IN CONTROL AND TEST SAMPLES OF CAULIFLOWER

Table - 44

SL. NO.	DETAILS	N %	P %	K %	Ca %	Mg %	S %	Fe ppm	Mn ppm	Zn ppm	Cu ppm
1.	**Cauliflower (Soil+GOT)**	2.83	0.207	2.80	0.48	0.22	0.370	98	21	31.0	6.5
2.	**Cauliflower (Soil)**	2.98	0.181	3.00	0.68	0.31	0.377	71	19	32.0	5.9
3.	**Difference between Soil & Soil + GOT**	0.15	0.026	0.20	0.20	0.09	0.007	21	2.0	1.0	0.6

From the table above when a comparison was done between cauliflower grown only in Soil and that in GOT mixed soil revealed that Nitrogen, Potassium, Calcium, Magnesium and Sulphur showed a decreasing trend in cauliflower grown in GOT mixed soil. But minerals such as Phosphorus, Iron and Manganese showed an increasing trend in case of cauliflower grown in GOT mixed soil. Where as the influence of GOT was negligible in case of minerals such as zinc and Copper when compared to pure soil.

DISCUSSION

The Green gram grown in research field was subjected to protein estimation by Lowry's method where the Green gram grown with Soil + GOT shows maximum protein concentration when compared with Green gram grown only with Soil. Root nodules of Green gram grown with Soil + GOT has shown higher percentage of Nitrogen Fixation as the presence of Rhizobium bacteria is more when compared to the Green gram grown with Soil in absence of GOT.

A comparative study between the Soil sample and the crop i.e. Green gram grown with Soil + GOT shows maximum growth by absorbing nutrients like Nitrogen, Phosphorus, Magnesium, Potassium, Manganese, Iron rather than Calcium, Zinc & Copper. From this study we came to know that there was 30% increase in the yield with respect to Green gram

The comparative study done between the Soil sample and the crop i.e. Cauliflower grown with Soil + GOT shows maximum growth by absorbing nutrients like Phosphorus, Magnesium, Iron, rather than Nitrogen, Calcium, Sulphur, Zinc, Manganese, Potassium, Copper.

A comparative study between the Soil sample and the crop i.e. Cabbage grown with Soil + GOT shows maximum growth by absorbing nutrients like Nitrogen, Phosphorus, Calcium, Magnesium, Sulphur, Manganese & Zinc rather than Potassium, Iron and Copper.

when a comparison made between the Cabbages grown only in Soil and that of GOT mixed soil revealed the fact that minerals such as Nitrogen, Potassium, Sulphur, Zinc and Copper showed an increase trend in cabbage grown in GOT mixed soil, but the influence of GOT was negligible in case of minerals such as Phosphorus, Calcium and Magnesium. GOT has shown suppressive effect in case of Iron and Manganese up taken by plant.

When a comparison was done between cauliflower grown only in Soil and that in GOT mixed soil revealed that Nitrogen, Potassium, Calcium, Magnesium and Sulphur showed a decreasing trend in cauliflower grown in GOT mixed soil. But minerals such as Phosphorus, Iron and Manganese showed an increasing trend in case of cauliflower grown in GOT mixed soil. Where as the influence of GOT was negligible in case of minerals such as zinc and Copper when compared to pure soil.

This results shows that the crops grown with Soil + GOT shows the maximum growth and maximum absorption of minerals from both soil and GOT.

CONCLUSIONS

- GOT can be used to blend either the manure or the fertilizer in Agriculture.
- Saves investment on the use of fertilizers.
- Increases the yield and decreases the duration of the harvesting time.
- Provides the best solution to dispose enormous quantities of readily available mining waste (GOT) dumped unused for decades.
- The vast land used to dump such mining waste can be reclaimed.
- Poor farmers, who cannot afford expensive Phosphatic fertilizers, can use such mining waste as a source of Phosphate.

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