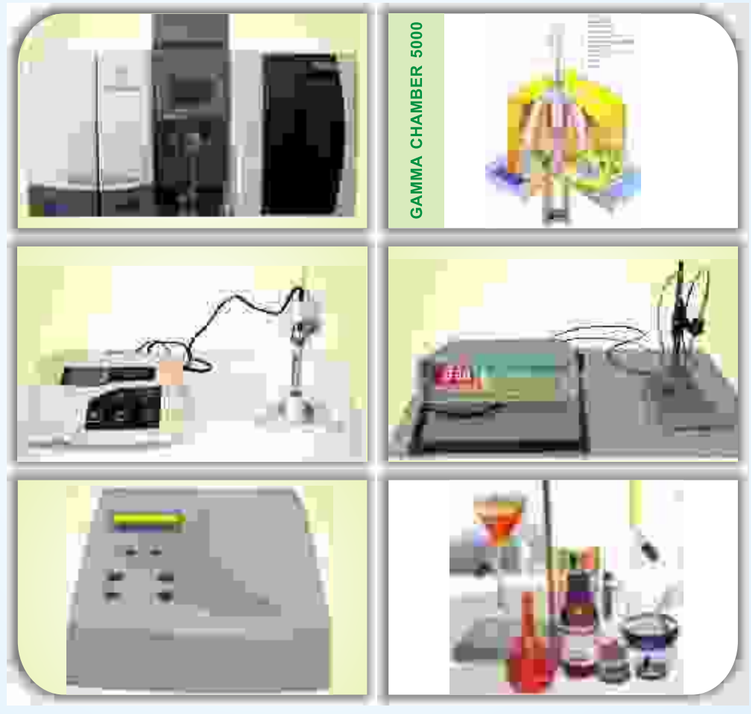
Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.)

TRAINING ON

Biotic and Abiotic Resources Management for Eco-friendly and Sustainable Agriculture

3-23 October, 2011

LABORATORY MANUAL



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CENTRE OF ADVANCED FACULTY TRAINING
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Laboratory Manual

Course Programme on

Biotic and Abiotic Resources Management for Eco-friendly and Sustainable Agriculture

(03-23 Oct. 2011)

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Collection of Representative Soil Sample, its Processing and Handling in Laboratory

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Soil testing is a chemical method for estimation of nutrient supplying power of a soil/ soil fertility evaluation.

Soil fertility may be defined as the capacity of soil to furnish available plant nutrients to the plants in proper amount and appropriate balance, under ideal condition of plant growth. Whereas, Soil productivity is the capacity of soil to produce under specific condition of crop production.

Advantages of soil testing :

- More rapid method as compare to biological or deficiency symptoms/ plant analysis.
- One may determine the need of the soil before the planting of crop.

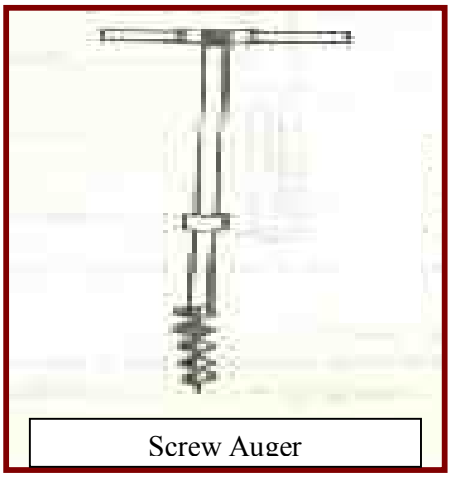
Objectives of soil testing :

- To study/maintain fertility status of a field.
- To predict the probability of obtaining a profitable response of lime & fertilizers.
- To provide basis for recommendation of fertilizers.
- To evaluate fertility status of soil of an area/state/country for developed of plans for research and education work.
- To study the acidity, alkalinity and salinity problems.
- To determine the suitability of the soil for laying gardens.
- Lime problems.
- Soil survey.

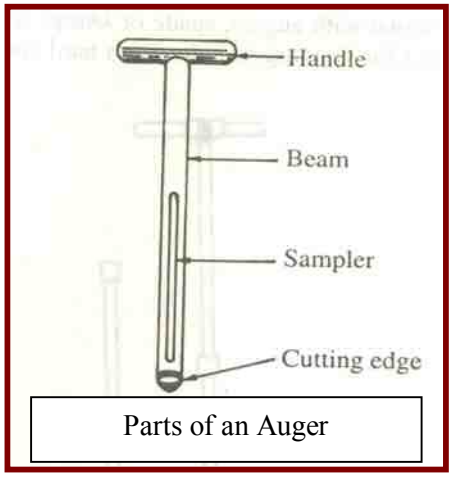
The error in soil sampling in a field is generally greater than the error in laboratory analysis. The most recommendation call for soil testing of each field is about every 3 years with more frequent testing on lighter soils. Therefore, it is necessary that the soil sample should be representative of the area. Further, the subsequent handling operation in the laboratory should be carefully performed because a minute quantity (1 – 10g) of the large soil mass of the field is actually used for the analysis in the laboratory. Unless one is sure of representative and proper sampling, the results obtained in the laboratory analysis will be of no use under the field conditions.

Apparatus and materials :

⇒ *Khurpi*
⇒ Spade
⇒ Augers
⇒ Plastic bowl
⇒ Scale
⇒ Rack
⇒ Wooden roller
⇒ Mortar and pestle
⇒ Sieve
⇒ Polythene/paper/cloth bags
⇒ Labels
⇒ Card board cartons
⇒ Aluminium boxes



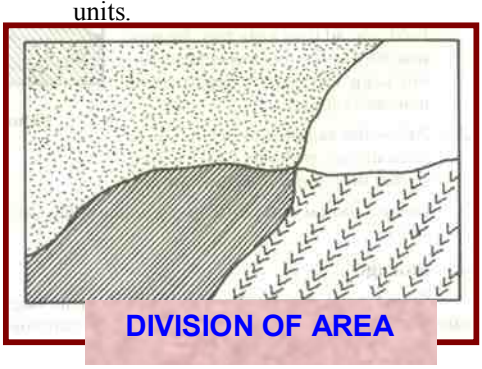
Screw Auger



Parts of an Auger

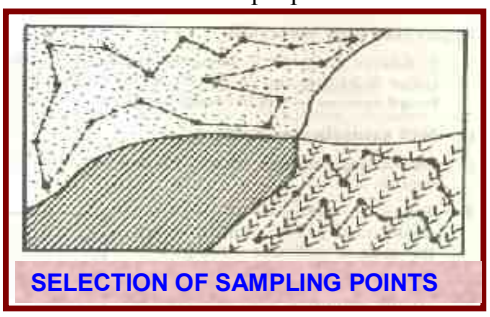
Collection of representative soil sample :

- Depending on field conditions and the objective of sampling, select proper sampling tool (s).
- Based on difference in soil type, colour, crop growth or slope, divide the area in different homogenous units.



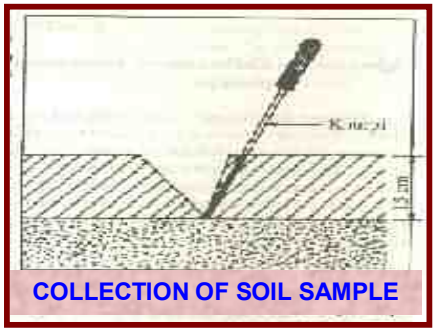
DIVISION OF AREA

- In the uniform field, demarket the sampling points in a zig zag fashion or randomly in such a way that the whole field should be covered i.e. about 30-35 sample per ha.



SELECTION OF SAMPLING POINTS

- At the sampling site, remove the surface litter with *Khurpi* or spade. With the help of the sampling tool (Auger) collect a sample in a plastic bowl.
- If the soils is hard, make a 'V' shape cut upto 15 cm depth. Remove the soil of the pit. Now scrap or remove 1 cm / 1" soil from the surface upto 15 cm depth from both the side with the help of khurpi. This scraped soil is collected in a plastic bowl. This sample is known as 'primary' sample. Such primary samples should be approximately the same weight.



COLLECTION OF SOIL SAMPLE

- After collecting at least 30 – 35 primary samples, mix all the samples in plastic bowl thoroughly and draw about ½ to 1 kg composite sample by quartering method. Label the sample in the bowl and divide the sample approximately 4 equal part. Discard the 2 opposite portions of the samples and remaining 2 portions are again thoroughly mixed and again divided in to 4 equal parts and 2 opposite parts again discarded. This procedure is continue until ½ to 1 kg sample remain in the bowl. This is known as composite sample which is true representative of the area.
- The most suitable containers for soil samples are polythene bags 6x9'' made of film about 0.13 mm thick, which may be sealed by twisting or tying the neck or by mean of rubber bands or adhesive tape.
- If the soil is to be kept in moist condition for moisture determination, bacterial count and nitrate estimation etc. air tight containers are preferred.

If the soil to be used for the estimation of micronutrients like Zn, Cu, Fe, Mn. Use of metallic tools should be avoided. Use sharp stick or stainless steel. Brass sieve should be avoided. Nylon net or aluminum sieve should be used.

In majority of cases, large stones and pieces of gravel (7.6 – 2mm) can be discarded. Soil is broken up and spread out in a thin layer on strong paper or polythene film, preferably on a rack of wire mesh to allow air to circulate. The drying area protected from direct sun and wind.

Soil samples should be labelled/ numbered by field staff with water proof ink or paint. These bag numbers being entered in the sampler's record books, as each sample is taken together with other information. He needs to identify and describe the samples.

If labels are used, they should either printed numbers on them already or water proof ink should be used to write information on them (this excludes pencil, washable ink pens and ball point pens). Duplicate labels should be placed between the two bags, never in the bag with the soil. The information on a label should be kept to a minimum preferably a number which may be either a 'bag number' or 'sample number'. Depth may be given usefully for soil profile samples.

When labels are hand written special care should be taken to prevent ambiguity. For arabic numbers "6" and "9" and combination like "69" and "96" should be under line, and '1' and '7' should be clearly distinguished. Where letters & numbers are used '5' may be easily confused with 'S'. This is why printed labels are always better.

Field samplers have their own numbering system and may have record book containing

printed forms for entry of information serially numbered. These 'sample number' are main identification of soil for most purposes but the relevant form should also have a record of the 'bag number' and subsequent 'laboratory number'.

When a box of samples is dispatched to the laboratory, it should contain a packing note giving the total number of sample, 'sample number' of each sample and its corresponding 'bag number', the depth of soil sample from profile pit and other information needed by the laboratory staff for registration purposes, particularly on the analysis required. A duplicate packing note should be sent separately so that missing boxes can be investigated.

On arrival of the soil samples at the laboratory, the content of a box should be checked against the packing note if any discrepancies should be reported to the sampler. The samples are register in laboratory giving each sample a 'laboratory number' for particular analysis.

Small laboratory simply the numbered serially as they arrive. Larger laboratory may have 2 or more numbering system, using a prefixed letter (group of letter) to distinguish them. This procedure helping to channel samples into various analytical stream. Larger laboratory may need to 'punched card system' or other means of storing complete information on all samples. So that can be recovered quickly.

It is essential to keep a record of the date of arrival and the source of all samples. A table can be drawn up for each month.

Preparation of soil sample for analysis :

- Ensure the sample is uniform size, free of stone, contain no soil aggregates bigger than 1 cm and air dry. These only need grinding and sieving.
- If the sample arrived at the laboratory as it has been taken from the sampling tool in the field, it is spread out on a tray of metal (Al) or plastic or strong brown paper, stones one removed and large soil aggregate broken up, pieces of un decomposed plant should be removed.
- If the sample is large, it is reduced by quartering method. A sample splitter may be used. The remainder of the sample is returned to its tray.

The sample is labelled with "laboratory number" which should be printed on a piece of plastic material since it remain permanently with the sample until it is no longer required. Use of different colour plastic labels to distinguished different numbering system. Thick cards may be used if plastic cards is not available. Labelling the tray is inconvenient since it is used in rotation for many different samples.

Ensure that the plastic or card labels are not accidentally transferred from one sample to another or lost.

Wet samples are air dried. To hasten 'air drying' elevated temperature not exceeding 40^0 C in air conditioner. During the air drying, samples should be kept in well ventilated condition so that the water vapor can escape easily.

Shelves of open wire mesh are convenient. If air conditioning is not installed, fan should circulate the air gently. Samples may be mixed during drying to expose fresh survaces.

Sample for minor metal analysis should be crushed in a motor of porcelain or stoneware and sieve through a stainless steel or nylon sieve. Crushing should always be gentle to avoid breaking up gravels.

Care must be taken with sample containing soft chalk or limestone where the degree of grinding can affect the result of calcium carbonate.

Crushing with roller of flat hardwood may be employed. Special soil grinding machine which allow crushed material to pass through a 2 mm sieve during operation. Machine which grind the whole sample including gravel must not be used.

Certain analysis required sample passing 0.5 mm sieve, a sub sample of 25-50g ground until all the soil passes through a 0.5mm sieve and transferred to a small tray to await analysis.

The safest way to ensure this is to spread out the well mixed sample on a flat tray and take small portion at random with a spatula at full depth of soil layer not just from surface until the required weight is obtained.

It is inaccurate to take a 2mm sample for analysis straight from a bottle or carton, the content of which may not be uniformally mixed.

Weighing for analysis :

When the weight of 5 g or more air dry soil require, a 2 mm sample is suitable. For weight < 5g, it is advisable to use 0.5 mm sample to reduce sampling error because a 2 mm soil sample contain different size particle. The removal of small portion for analysis must be done in such a way that each portion contain, the same proportion of different sized particle as for as possible as in the main sample.

Only one tray at a time is placed during weighing, thus avoiding contamination of samples by accidental spillage.

For routine analysis, sampling by volume may be used in place of sampling by weight. A spoon of required volume (5, 10 and 20g) suitable for pH & EC determination. The result clearly indicate the volume of soil or its ratio to the volume of water or soil extractant is used.

The soil sample taken by volume is tipped from the measuring spoon into the required vessel and any soil remaining in the spoon is dislodges by gentle tapping, the spoon may be brushed out or clean before proceeding to the next sample.

All measurements of soil sample should be done in a separate room because it is impossible to avoid contamination of the air with the fine particles of soil during sampling

The measured samples may be treated with extracting solution or water in the same room or transferred to other rooms for analysis after the vessels have been covered to avoid accidental addition of a second sample or foreign material.

STORAGE :-

The registered and labelled samples in laboratory are finally placed in a cardboard carton. Label the carton properly with the details of soil sample and store in a separate room. The room should be away from direct sunlight/wind or dampness.

The room exposed to heat or cold or dampness in not advisable.

Precautions in collecting a soil sample :

- Avoid sampling from low – lying spots, manure dumping sites, near trees and from fertilizer placed zones.
- Use clean bags for sample collection. Do not use bags which had earlier contained fertilizer, manure or plant protection chemicals etc.

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Simultaneous Measurement of Bulk Density and Water Content by Nuclear Methods

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Recent advances is nuclear technology which have evolved methods for the non destructive determination of the soil water content. Two methods have been used with success. One the neutran scattering method and the other is the y-radiation method.

Neutron method : In this method measurement is made of the number of hydrogen nuclear that are present per unit volume of soil and therefore, water content by volume θ, is measured. Neutrons are uncharged particles having almost the same mass as that of protons or that of hydrogen nuclei. A radium-beryllium mixture in form of pellets is used as source of fast neutrons. The measurement of soil water by the neutron method is essentially a measure of the density of the slowed neutron cloud developing when Ra-Ba source is inserted in the soil, the density of the slowed neutrons being a measure of the soil water content by volume θ.

Simultaneous determination of bulk density and water content :

With the increased interest in the behaviour of water in swelling soils and resulting theoretical studies (Smile and Rosenthal, 1968; Philip, 1969) on the subject, a method was required for measuring changes in both the water content and soil density in order to experimentally evaluate these theoretical analyses. Numerous authors have proposed measuring the attenuation of gamma rays of two different energies to non destructively determine both water content and soil density in the same sample. Soane (1967) illustrated the effectiveness of the dual gamma method for measuring bulk density and water content of three soils. The samples were placed between a cesium source and detector and then between americium to using a combined source but neither Soanenor other workers (Gardner and Calissendoff, 1967; Gardner, Campbell and Calissendaff, 1969) have attempted to combine the sources in a single collimator because the higher energy gamma photons produce Compton scatter through interaction with the sample and some of this Compton scatter will be counted as gamma rays from the lower energy source. The error can be eliminated by with equipment and method evolved by Corey, Peterson and Wakat, 1971). This method is feasible for measuring the water content and soil density of soil columns simultaneously when two sources are combined in a single collimator. Measurement of attenuation of ^{137}cesium and ^{241}americium is done in this method. Radiation intensity (counts/min) after passage through the soil, container and container alone is calculated by the equation (Corey *et al*.) given in method. To obtain values of intensity the equipment is required as shown in Fig. 1.

^{241}Am emits a large number of gamma rays of various energies but the major energy is 59.6 KeV ^{241}Am has a half life of 458 years eliminating the need for decay corrections ^{137}CS decays with a gamma ray of 662 KeV and has a half life of 30 years.

Detector and pulse height analyzers are used in this system.

Correction for comption scatter

Two methods were compared and dual source method evaluated. Known water content and soil densities were measured. The two soils were Houston black, a soil containing monitorillonitic clay and cecil a soil containing kaolinitic clay. The soil containing different amount

of water was packed to varying densities into plastic boxes 7.5 x 4.46 x 4.95 cm. These boxes were placed between sources and detector with long axis parallel to beam. Comparisons between the known, soil density.

Water content and values determined by two methods for four cecil soil samples and five Housten Black soil samples are given in table 1.

Table 1: Water content and values determined by twi methods.

Water content g/cm^3			Soil density g/cm^3		
Known	Calculated		Known	Calculated	
Cecil soil					
0.001	0.000	0.00	1.831	1.830	1.830
0.044	0.081	0.073	1.712	1.711	1.720
0.120	0.124	0.143	1.999	2.001	1.980
0.175	0.118	0.151	2.050	2.067	2.081
Houston Black Soil					
0.010	0.000	0.000	1.504	1.504	1.504
0.141	0.147	0.139	1.297	1.281	1.290
0.270	0.284	0.281	1.343	1.367	1.371
0.438	0.472	0.446	1.369	1.349	1.378
0.495	0.532	0.514	1.297	1.234	1.255

There is little difference between the results obtained by the two correction techniques and method best suited will be determined by the instrumentation available.

Following satisfactory evaluation of the dual source method for determining the known water content and soil density of soils, the method was used to determine water content and soil densities of Houstan Black and Cecil soil columns following infiltration. More water was added to Houston soil because its greater water holding capacity. Twenty four hours following the addition of water the transmission measurements were repeated and the water content and density was calculated water remained ponded on Houston Black soil at the end of 24 hour period but had infiltrated completely with the Cecil soil. The two soils responded quite differently to the addition of water. Neither the soil density nor lengths of Cecil soil column changed. The column of Hoston black soil increased 2 cm in length and the soil density decreased in top 4 cm.

The combined source method is applicable to the study of swelling soils, the phenomena of freezing and thawing and measurement of water content and density of the soil inside core barrels.

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Estimation of Soil Moisture by Different Methods

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Direct method (Gravimetric method) :

This is the simplest and most widely used method for measuring soil moisture.

Equipments :

Sampling tube/auger
Moisture cans (numbered)
Balance with weights/automatic
Drying oven
Desicator

Procedure :

Collect soil samples by tube or auger from a number of points with in the experimental site and mix thoroughly. Place composite sub samples of about 50 gm to 100 gm in soil moisture cans with tight fitting lids. Take atleast three sub-samples. The moist samples are weighed immediately, dried to constant weight in an oven at 105°C (for about 24 hrs) and reweighed after cooling in a desicator. Determine the tare weight of moisture cans. Calculate the soil moisture content by determining the loss in weight on drying and the weight of the oven dry soil as follows :

$$\text{Soil moisture content by weight (\%)} = \frac{\text{(wt. of wet soil + tare) - (wt. of dry soil + tare)}}{\text{(wt. of dry soil + tare) - (tare)}}$$

$$\text{Mw (\%)} = \frac{\text{Loss of wt. on drying}}{\text{Wt. of oven dry soil}} \text{ X } 100$$

Percentage of moisture on volume basis = Percentage of moisture on weight basis x bulk density.

Gravimetry with drying by burning Alcohol or spirit (Bouyoucos, 1937) :

In this method, the soil moisture is evaporated by igniting the soil mass with alcohol or spirit. The process is repeated to get constant weight of ignited soil.

Equipments and reagents :

(1) Moisture cans
(2) Glass rod
(3) Ethyl, methyl or propyl alcohol
(4) Match box
(5) Balance with weights
(6) measuring cylinder
(7) Desicator.

Procedure :

Weigh about 20-25 gm of wet soil in a moisture can add about 5ml alcohol or spirit drop to fully saturate the soil and ignite. Cool the cans in a desicator and record the weight. Repeat the process several times till a constant weight of the ignited soil is recorded. Compute the percentage moisture on the basis of the constant dry weight of the burnt soil.

This method is rapid, reproducible and more suited for field soil moisture determinations where laboratory facilities are not adequate.

Burning of the soil results in loss of organic matter. This method needs to be calibrated against oven dry method for each soil type.

In direct method (By use of neutron moisture probe) :

Rather than taking a sample of soil, it is often desired to measure the soil moisture status in situ without disturbing the system. The neutron moisture meter is such a device which is much used in the field for measuring water content (Belcher, 1952, Gardner and Kirkhana, 1952, Holmes, 1956 and Van Bavel *et al*., 1956). The neutron method is a measure of the number of hydrogen nuclei that are present per unit volume of soil. Therefore, it is a means of directly observing the moisture content by volume.

Principle :

Hydrogen nuclei have a marked property for scattering and slowing neutrons. This property is exploited in the neutron method for measuring soil water content. High energy neutrons (0.1 to 10 MeV) emitted from a radioactive substance such as radium beryllium (5Mc) or americium-beryllium (30Mc) neutron source are called fast neutrons and travel with high speed of the order of 100 miles per second.

When such a fast neutron source is placed in a moist soils, the emitted neutrons interact with the surrounding medium. They collide with the nuclei of the soil in a billiard ball fashion, changing direction as a result and loosing energy. With the energy loses, the speed diminishes until it approaches the speed characteristics for molecules at the prevailing temperature. Such neutrons are called thermal or slow neutrons. The slow neutrons are finally absorbed by other nuclei + and thus their existence ends. In a material containing appreciable hydrogen a neutron after the first the collision with a hydrogen nucleus is not likely to travel much farther consequently if the neutron source is enclosed in a material that is rich in hydrogen, it will be enveloped in a dense spherical cloud of slow neutrons. The density of this cloud represents an equilibrium between the rate of thermalization and absorbed by the medium and the rate of production by the source. This equilibrium is reached in a fraction of micro second after the insertion of source. If the medium surrounding the source contains less hydrogen, the cloud of slow neutrons will be less dense and extend farther from the source. This is so because a fast neutron has to travel further, on an average, to in counter a hydrogen nucleus and start becoming thermalized. Most of the nitrogen in soil is associated with water and lesser amounts with organic matter. Thus, the measurement of soil water by neutron method is essentially a measurement of the density of the slow neutron cloud developing when the Ra-Be or Americium-Beryllium source is inserted into the soil.

Equipments and material :

1. Small fast neutron source such as radium-beryllium (Mc) or americium-beryllium (30 MC)
2. Shield for storage of the neutron source. Shielding commonly used consists of lead, paraffin or polyethylene in a cylindrical shaped unit with a cylindrical hole through the axis to accommodate the probe.
3. Detector of slow neutron - most commonly used for soil moisture measurement is a Bf_3-enriched proportional counter mounted in a cylindrical arrangement with transistorised preamplifier mounted in the cable end.
4. Counting device (scaler) - the density of slow neutrons in the soiil and in the counting tube is evident in the form of a given counting rate. This counting rate may be determined by a rate meter or by a scaler. The first indicates the count rate directly and the second register the total number of counts over a given time period. In either case identical results are obtained.
5. Access tubing and soil auger sted or aluminum tubing is most commonly used, but other materials such as plastic have been used. Two sizes of access tubes are in common use. 20-gauge steel or aluminum tubings 1.625 inches (4.13) or 2 inches (5cm) outer diameter or 1.9 inches (4.83 cm) of inner diameter of aluminum irrigation tubing. Aluminum is practically transparent for fast and slow neutrons and does not corrode seriously. Tube once installed may give years of service.

   A soil auger slightly smaller the tubing should be available for drilling the access holes. Moisture probe should be calibrated for particular material and size of access tubing to be used.

6. Cable - A 10 meter electric cable connects detector and scaler. All possible care should be taken to keep the connections and firm and the connectors free of dirt and moisture.

Procedure :

The access tube is first inserted into the soil after drilling a hole with the help of auger taking care that no bend in the access tube is created. The access tube is kept few inches above the soil and covered with an inverted can to prevent entrance of trash.

The neutron probe must be inserted in the access tube and held at the desired depth. Holding the probe may be done by various means, but a simple and effective method is to use an ordinary marking tape, coloured cloth adhesive tape or surgical tape. While making a measurement turn on the scaler a few minutes earlier to warmup (transistorized units require no warmup). Make several standardization counts with the probe each time. The normal counting time is minute. The background "thus obtained should not be much more than 100 counts per minute. As measurements are being taken, the standard count is determined again from time to time. The frequency can depend on convenience, plolayout and experience. It is often convenient to make a standard count at the start and end a series of readings in each access tube. Keep a record of standard count to provide an index of equipment condition. After determining the standard count, take the reading at successive depth intervals starting at least 18 to 25 cm from the soil surface. Approximately, 9-15 cm soil layer is characterized by a single measurement.

Divide readings by standard to obtain a count ratio (referred to as relative count ratio to standard or per cent of standard etc.) and refer to instrument calibration curve to obtain water content by volume at various depths. The calibration curve supplied with instrument usually may be used. But, since wide differences of soils are know to exist, the calibration checks, should be made to each soil type as guided by experience in area. It is very important to realize that the potential precision of the neutron method is greater than that of any other method under field condition. Therefore, calibration should be carried out with large homogeneous masses of soil of which the bulk density and moisture content (by gravimetric method) are accurately known.

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Determination of Soil pH and Electrical Conductivity

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Determination of pH is actually a measurement of hydrogen ions activity in soil – water system. It is defined as negative logarithm of the hydrogen ion activity. Mathematically, it is expressed as:

$$pH = - \log a H^+$$

The pH value of a soil is an indication of soil reaction i.e. acidic, neutral or alkaline. The nutrient availability is governed by soil reaction. It is maximum at neutral pH and decreases with increase in acidity or alkalinity. Thus, pH value gives an idea about the availability of nutrients to plants.

Principle :

The pH is usually measured by pH meter, in which the potential of hydrogen ion indicating electrode (glass electrode) is measured potentiometrically against calomel saturated reference electrode which also serves as salt bridge. Now a days, most of the pH meters have single combined electrode. Before measuring the pH of the soil, the instrument has to be calibrated with standard buffer solution of known pH. Since, the pH is also affected by the temperature, hence, the pH meter should be adjusted to the temperature of the solution by temperature correction knob.

Reagents :

Standard buffer solutions: These may be of pH 4.0, 7.0 or 9.2 and are prepared by dissolving one standard buffer tablet in 100 ml distilled water, It is necessary to prepare fresh buffer solution after few days. In absence of buffer tablet, a 0.05 M potassium hydrogen phthalate solution can be used which gives a pH of 4.0 (Dissolve 10.21 g. of A.R. grade potassium hydrogen phthalate in distilled water and dilute to 1 litre. Add 1 ml of chloroform or a crystal of thymol per litre as a preservative).

Procedure :

(a) Soil to water ratio of 1:2 (pH_2)

Take 20 g soil in 100 ml beaker and add 40 ml. of distilled water to it. The suspension is stirred at a regular interval for 30 minutes. Determine the pH by immersing electrodes in suspension. For soils containing high salts, the pH should be determined by using 0.01M calcium chloride solution. (Dissolve 0.110 g of $CaCl_2$ in water and dilute to 1 litre).

(b) Saturates soil paste (pHs)

Add small amount of distilled water to 250g of air dried soil. Stir the mixture with a spatula. At saturation, the soil paste glistens and flows slightly when the container is tapped it slides freely and ensures cleanly off the spatula. After mixing, allow the sample to stand for an hour. If the paste has stiffened markedly or lost its glistening, add more water or if free water has collected on the surface of the paste, add an additional weighed quantity of dry soil and mix it again. Then insert the electrode carefully in the paste and measure the pH.

(c) Saturation extract (pHe)

The soil is extracted using vacuum extractor and the pH is measured in the saturation extract.

Conductivity :

The knowledge of total soluble salts is essential in crop production, specially during the process of salinization. Since, there is a direct relationship between the quantity of soluble salts and the electrical conductance. Hence soluble salts in soils are measured indirectly by measuring the electrical conductance of the soil.

Principle :

The electrical conductivity is measured with the help of solubridge. The instrument is calibrated and cell constant is determined with the help of 0.1 N KCl solution. This solution gives an electrical conductance of 1.41 mmhos/cm or dSm^{-1} at 25^0C.

Reagents :

Potassium chloride: Dissolve 0.7456g dry potassium chloride (AR) in distilled water and make up the volume to one litre.

Procedure :

Take 20 g of soil in 100 ml beaker, add 40 ml of distilled water and shake intermitantly for 30 minutes. Determine the conductivity of the supernatant liquid with the help of conductivity meter. The electrical conductivity of saturation extract (E.C.e) is also determined for salinity ratings.

Determination of Soil Organic Carbon

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The majority of mineral surface soils range from 1.2 to 3.5% organic carbon. Since soil organic matter averages about 58% carbon, it follows that soils generally range from about 2 to 6 % organic matter (% O.M. = %C x 1,724. The factor 1.724 = 100/58). There is also a close relationship between carbon and nitrogen in soils. Most organic matter average about 5% nitrogen so that the N : C ratio is 1:11.6. Therefore by multiplying the soil organic matter percentage by 0.05 an approximate value for the soil nitrogen, percentage is obtained.

In soil the chief source of some of the nutrients essential for plant growth is organic matter, such nutrients are N, S and boron is also largely derived form organic matter.

Principle :

A suitable quantity of the soil is digested with chromic acid and Sulphuric acid making the use of heat of dilution of Sulphuric acid soil is digested and organic matter of the soil is oxidized. Excess of chromic acid left over unreduced by the organic matter of the soil is determined by a titration with standard Ferrous Ammonium sulphate solution using diphenylamine as indicator.

In this exercise, chromic acid in the presence of excess H_2SO_4 is to be used as an oxidizing agent for oxidizable organic matter of the soil. The heat of dilution of H_2SO_4 works as a standardized ferrous sulphate solution.

$$4\,Cr^{6+} + 3C \longrightarrow 4Cr^{+++} + 3C^{4+}$$
$$2H_2Cr_2O_7 + 3C + 6H_2SO_4 \longrightarrow 2Cr_2(SO_4)_3 + 3CO_2 + 8H_2O$$
$$K_2Cr_2O_7 + 4\,H_2SO_4 \longrightarrow K_2SO_4 + 2Cr_2(SO_4)_3 + H_2O + 3O$$
$$2FeSO_4 + H_2SO_4 + O \longrightarrow Fe_2(SO_4)_3 + H_2O$$

Apparatus and Reagents :

1. 500 ml conical flasks.
2. Pipette
3. Burette
4. Phosphoric acid 85%.
5. Sodium fluoride 2%.
6. Sulphuric acid 96 % containing 1.25 % Ag_2SO_4.
7. Standard 1N $K_2Cr_2O_7$ – 49.04 g/liter.
8. Standard 0.5 N Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ 196 g in 800 ml water containing 20 cc H_2SO_4 and diluted to1 litre.
9. Diphenylamine – 0.5g in 20cc water and add 100 ml conc. H_2SO_4.

Procedure :

1. Weigh 1g soil sample in 500 ml conical flask. Add 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml conc. H_2SO_4 (containing Ag_2SO_4). Mix thoroughly and allow reaction to proceed for 30 minutes.
2. Dilute the reaction mixture with 200 ml water and 10 H_3PO_4 add 10 ml of NaF solution and 2 ml of diphenylamine.
3. Titrate the solution with standard FAS to a brilliant green colour. A blank without soil should be run simultaneously.

Observations & Results :

Weight of sample - 1 g
Normality of $K_2Cr_2O_7$ used - 1 N
Vol. of $K_2Cr_2O_7$ - 10 ml
Normality of FAS - 0.5 N

$$OC\ (\%) = \frac{10}{Blank}\ (Blank - Reading)\ X\ \frac{0.003 \times 100}{Wt.\ of\ soil}$$

Limits :

Low	:	< 0.5%
Medium	:	0.5 – 0.75%
High	:	> 0.75%

Determination of Available (Mineralization) Nitrogen in Soil by Alkaline Permanganate Method

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Preparation of soil sample :

The soil samples from definite depth are randomly collected from the field with the help of screw auger. All the possible technical precautions as prescribed for standard soil sampling are also taken. Samples are then brought to the laboratory, air-dried in the shade and grounded by wooden roller, thereafter sieved through 2 mm stainless steel sieve and stored in polythene bags and used for chemical assay.

Principle :

A known weight of the soil is mixed with alkaline potassium permanganate ($KMnO_4$) solution and distilled. The organic matter present in soil is oxidized by the nascent oxygen, liberated by potassium permanganate, in the presence of sodium hydroxide and the released ammonia is condensed and absorbed in known volume of a boric acid with mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

Reactions involved:

I. Distillation :

$$2\,KMnO_4 + H_2O \xrightarrow[\text{medium}]{\text{Alkaline}} 2\,MnO_4 + 2KOH + 3O^- \quad \text{(Nascent oxygen)}$$

$$R.CHNH_2COOH + O^- \xrightarrow{\text{Oxidation}} R.CO.COOH + NH_3$$

Organic- N fraction (Ammonia)

$$NH_3 + H_2O \xrightarrow{\text{Distillation}} NH_4OH$$

$$3NH_4OH + H_3BO_3 \xrightarrow{\text{Absorption}} (NH_4)_3BO_3 + 3H_2O$$

(Green colour)

II. Titration

$$2(NH_4)_3BO_3 + 3H_2SO_4 \longrightarrow 2(NH_4)_3SO_4 + 2\,H_3BO_3$$

(Pink colour and original)

Equipment and apparatus :

1. KEL PLUS Automatic Nitrogen Estimation System

The said instrument is used for determination of available nitrogen in soil. It consists of the following:

- **Automatic Distillation System (Model Classic DX):** It is fully automatic distillation system with programmable auto run digital features, with automatic dilution and addition of boric acid, NaOH and $KMnO_4$. Both modes (auto and

manual) are available for distillation reagents addition.

- **Refrigerated Water Cooling System for Condenser (Model Kel Freeze):** It is refrigerated water cooling system for distillation and condensing system with inbuilt compressor and recirculator pump.

2. Electronic balance
3. Burette
4. Conical flask
5. Distilled water

Reagents :

1. 0.32 % potassium permanganate ($KMnO_4$) solution.
2. 2.5 % sodium hydroxide (NaOH).
3. 2 % boric acid solution containing 20 - 25 ml of mixed indicator / liter.
4. Mixed indicator: 0.066g methyl red + 0.099g bromocresol green dissolve in 100 ml of 95 % alcohol.
5. 0.02 N sulphuric acid (H_2SO_4).

Procedure :

- Weigh 5 g of prepared soil sample and transfer it to the digestion tube.
- Load the tube in distillation unit and other sides of hose keep 20 ml of 2 % boric acid with mixed indicator in 250 ml conical flask.
- 25 ml each of potassium permanganate (0.32 %) and sodium hydroxide (2.5 %) solution is automatically added by distillation unit programme.
- The sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 2 % boric acid containing mixed indicator solution kept in a 250 ml conical flask.
- With the absorption of ammonia, the pinkish colour turns to green.
- Nearly 150 ml of distillate is collected in about 10 minutes.
- The green colour distillate is titrating with 0.02N sulphuric acid and the colour changes to original shade (pinkish color).
- Simultaneously, blank sample (without soil) is to be run.
- Note the blank & sample titer reading (ml) and calculate the available nitrogen in soil.

Calculations :

$$\text{Available N (kg ha}^{-1}\text{)} = \frac{\text{R (Titer reading - Blank reading)} \times \text{Normality of acid} \times \text{Atomic weight of nitrogen} \times \text{Weight of one hectare of soil}}{\text{Sample weight (g)} \times 1000}$$

$$= \frac{R \times 0.02 \times 14 \times 2.24 \times 10^6}{5 \times 1000}$$

Factor = R x 125.44

Interpretation of results :

Available N (kg ha^{-1})		Soil rating
< 280	:	Low
280-560	:	Medium
> 560	:	High

References :

Subbiah, B.V. and Asija, G. L. (1956). A rapid procedure for the estimation of nitrogen in soils. *Curr. Sci*., **25**: 259-260.

Determination of Total Nitrogen in Soil and Plant

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Total nitrogen is estimated by the micro-Kjeldahl method as per procedure suggested by AOAC (1995).

Preparation of plant and soil samples :

The plant analysis has been considered as a superior diagnostic technique for mineral content. Whole plant is dried in open air for few days after that it was further dried in hot air oven at about 60 ± 2° C for eight to ten hours per day to achieve complete drying. After drying, whole plant is powdered with the help of a grinder, passed through 2 mm stainless steel sieve and used for chemical assay. The soil sample from definite depth was randomly collected from the field with the help of screw auger. All the possible technical precautions as prescribed for standard soil sampling were also taken. Samples were brought to the laboratory, air-dried in the shade and grounded by wooden roller, thereafter sieved through 2 mm stainless steel sieve and stored in polythene bags and used for chemical assay.

Principle :

Nitrogen in samples like plant and soil exists in a very complicated bonding structure. During digestion, a known weight of the plant/soil samples in the presence of sulphuric acid with catalyst mixture under high temperature is digested where complicated structures are broken to simple structure, thereby releasing nitrogen in the form of ammonium radical (NH_4^+). During distillation in presence of sodium hydroxide, the released ammonia is condensed and absorbed in known volume of a boric acid with mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

The micro-Kjeldahl method consists of the three steps;

1. Digestion
2. Distillation and
3. Titration.

Equipment and apparatus :

1. KEL PLUS Automatic Nitrogen Estimation System :

The said instrument is used for determination of nitrogen. It consists of the following;

- **Macro Block Digestion System (Model KES 12L):** This digestion system is suitable for soil, plant, water, pesticides, fertilizers, food and feed samples. It is microprocessor based automatic twelve place macro block digestion system with temperature controller fitted with sensor break protection (Microprocessor based) feature and temperature range from 50-450 ^{0}C.
- **Acid Neutralizer Scrubber (Model KEL VAC):** It is used to neutralize the acid fumes, which are absorbed in 15% sodium hydroxide and dissolved in water stored in the system tank. After every 2 cycles of digestion, the 15% sodium hydroxide solution is replaced and after 3 cycles of digestion, acid fumes dissolved in water tank is drained off and refilled with fresh water in the system tank.
- **Automatic Distillation System (Model Classic DX):** It is fully automatic distillation system with programmable auto run digital features, with automatic dilution and addition of boric acid and NaOH. Both modes (auto and manual) are available for distillation reagents addition.
- **Refrigerated Water Cooling System for Condenser (Model Kel Freeze):** It is refrigerated water cooling system for distillation and condensing system with inbuilt compressor and recirculator pump.

2. Electronic balance

3. Burette
4. Pipette
5. Conical flask
6. Measuring cylinder
7. Distilled water

Reagents :

1. Concentrated sulphuric acid (H_2SO_4).
2. Catalyst mixture: Mix with 250 g potassium sulphate (K_2SO_4), 50 g cupric sulphate ($CuSO_4$. 5 H_2O) and 5 g metallic selenium powder in the ratio of 50:10:1.
3. 40 % sodium hydroxide (NaOH).
4. 4 % boric acid containing 20 - 25 ml mixed indicator /liter.
5. Mixed indicator: 0.066 g methyl red + 0.099 g bromocresol green dissolve in 100 ml of 95 % alcohol.
6. 0.02N sulphuric acid (H_2SO_4).

Procedure :

I. Digestion :

- Weigh 0.5 g of prepared plant sample or 1 g of soil sample and transfer it to the digestion tube.
- Add 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture to the sample.
- Load the digestion tubes in to the digester and heat the digestion block.
- Switch on the digestion unit and set the initial temperature 100 ^{0}C till frothing is over.
- Then block temperature is raised to 400 ^{0}C. The effective digestion starts only at 360 ^{0}C and beyond 410 ^{0}C.
- The sample turns light green colour or colorless at the end of the digestion process.

II. Distillation :

- After cooling the digestion tube, load the tube in distillation unit and other side of hose keep 20 ml of 4 % boric acid with mixed indicator in 250 ml conical flask.
- 40 ml NaOH (40 %) is automatically added by distillation unit programme.
- The digested sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 4 % boric acid containing mixed indicator solution kept in a 250 ml conical flask.
- With the absorption of ammonia, the pinkish colour turns to green.
- Nearly 150 ml of distillate is collected in about 8 minutes.
- Simultaneously, blank sample (without plant/soil) is to be run.

III. Titration :

- The green colour distillate is titrating with 0.02N sulphuric acid and the colour changes to original shade (pinkish color).
- Note the blank & sample titer reading (ml) and calculate the total nitrogen content present in plant/soil samples.

Calculations :

$$\textbf{Nitrogen content in plant (\%)} = \frac{\textbf{R}\text{ (sample titer-blank titer) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{\text{R x 0.1 x 14 x 100}}{\text{0.5 x 1000}}$$

$$\textbf{Factor} = \text{R x 0.28}$$

$$\textbf{Nitrogen content in soil (\%)} = \frac{\textbf{R}\text{ (sample titer-blank titer) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{\text{R x 0.1 x 14 x 100}}{\text{1 x 1000}}$$

$$\textbf{Factor} = \text{R x 0.14}$$

Crude protein content :

The total nitrogen is estimated by micro-Kjeldahl method as per procedure suggested by AOAC (1995) and the crude protein is calculated by the following formula:

Crude protein content (%) = micro-Kjeldahl nitrogen content (%) x 6.25 (based on the assumptions that nitrogen constitutes 16 % of protein).

References :

AOAC, (1995). *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists, Washington, DC.

Determination of Phosphorous in Soil and Plant

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The phosphorus is an essential plant nutrient and it occurs in many different forms. Therefore, a reliable procedure for measuring the amount both in soil as well as in plant is needed. There are many methods available for the determination, however, colorimetric measurement is presented here:

Principle :

Phosphorus is extracted from the soil with 0.5 m $NaHCO_3$ at a nearly constant pH of 8.5. The phosphate ion in solution treated with ascorbic acid in an acidic medium provides a blue colour complex. Measurement of the quantitative determination of phosphorous in soil (Olsen's *et al*., 1954)

Reagents :

1. **0.5 M Sodium bicarbonate ($NaHCO_3$) solution:** Dissolve 42 g of $NaHCO_3$ in distilled water to get one litre solution and adjust the pH of the solution to 8.5 by small quantity of NaOH.
2. **Activated Charcoal:** Darco G-60 (P-Free)
3. **5 N Sulphuric acid (H_2SO_4) Solution:** Add 141 ml of con. H_2SO_4 to 800 ml of distilled water. Cool the solution and dilute to one litre with distilled water.
4. **Reagent A:**
   - Dissolve 12.00 g of ammonium paramolybdate in 250 ml of distilled water.
   - Dissolve 0.2908 g of potassium antimony tartrate ($KSbO.C_4H_4O_6$) in 100 ml distilled water.
   - Above both solution mix thoroughly and made one litre in volumetric flask with the help of distilled water.
   - Add these dissolved reagents to one litre of 5N H_2SO_4.
5. **Ascorbic acid working solution (Reagent B):** Dissolve 1.056 g of ascorbic acid in 200 ml of **reagent A** and mix. This ascorbic acid (**reagent B**) should be prepared as required because it does not keep more than 24 hours.
6. **Standard phosphate solution**: Weigh 0.4393 g of potassium dihydrogen phosphate (KH_2PO_4) into one litre volumetric flask. Add 500 ml of distilled water and shake the contents until the salt dissolves. Dilute the solution to one litre with distilled water to get 100 ppm P solution. Dilute 20 ml of 100 ppm P solution to one litre to get form-working solution of 2 ppm.

Preparation of standard curve :

- Take different concentration of P (0, 1, 2, 3, 4, 5, etc ml of 2 ppm standard P Solution) in 25 ml volumetric flasks.
- Add 5 ml of the 0.5M $NaHCO_3$ extracting solution to each flask, and acidify with 5N H_2SO_4 drop by drop.
- Add about 10 ml distilled water and 4 ml of reagent 'B', then shake the solution.
- Make the volume 25 ml by distilled water.
- The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.

- Plot the curve by taking P concentration on X axis and colorimeter reading on Y axis. Repeat the process till you get straight line relationship.
- Calculate the factor i.e. 1 colorimeter reading is equal to how much ppm of phosphorus?

Procedure :

- Take 2.5 g of soil sample in 150 ml conical flask and 0.5 g Darco G-60 activated charcoal.
- Then add 50 ml of 0.5 M $NaHCO_3$ solution and shake the solution for 30 minute in a shaker. Similar processes run for a blank without soil.
- Filter the suspension through the Whatman no. 40 paper.
- Take 5 ml aliquot of the extract in a 25 ml volumetric flask, and acidify with 5N H_2SO_4.
- Add small quantity of distilled water, and then add 4 ml of reagent B.
- The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.

Observations :

1. Weight of soil sample : 2.5 g
2. Volume of extractant used : 50 ml
3. Volume of filtrate used : 5 ml
4. Absorbency : R
5. Absorbency from standard curve : A
6. Concentration of P for absorbency A : B ppm

Calculation :

$$\text{Available P (kg ha}^{-1}) = \frac{R \times F \times 50 \times 2.24}{5 \times 2.5}$$

Where, F (factor) = B / A

Limits of available P in soil :

Very low	:	Less than 5 P kg ha^{-1}
Low	:	5-10 P kg ha^{-1}
Medium	:	10-20 P kg ha^{-1}
High	:	20-40 P kg ha^{-1}
Very high	:	More than 40 P kg ha^{-1}

Determination of total phosphorus in plant :

Principle : Vanadate molybdate and orthophosphates react to give a yellow colour complex in acidic medium. The intensity of colour provide the basis of quantitative measurement of total P in plant (Koenig and Johnson, 1942).

Apparatus and reagents :

- Colourimeter/spectrophotometer
- 50 ml volumetric flask
- ammonium molybdate ammonium vanadate (in NHO_3) solution : Dissolve 2.5 g $(NH_4)_6$ $Mo_7O_2.4$ $4H_2O$ in 400 ml distilled water. Dissolve 1.25 g of ammonium vanadate in 300 ml boiling water. Add the ammonium vanadate solution to the ammonium molybdate solution and cool to room temperature. Add 250 ml conc. NHO_3 and dilute to 1 lit.
- Phosphate standard solution : Dissolve 0.2195 g KH_2PO_4 and dilute to 12%. This solution contains 50 µg phosphorus/ml.

Procedure :

Preparation of standard curve :

- Transfer 0, 1, 2, 3, 4 and 5 ml of 50 ppm P solution to 50 ml volumetric flasks in order to get 0, 50, 100, 150, 200 and 250 µg P.
- Add 10 ml vanadomolybdate reagent make up the volume and mix the content thoroughly.
- Read the transmittance/absorbance at 420 mµ (blue filter).
- Plot the reading against µg P and calculate the factor (F).

Digestion of plant material :

Take one gram of plant material in digestion flask. Add 10-15 ml of Diacid (3:1: Nitric acid : Perchloric acid) mixture and swirl the content in 150 ml volumetric flask. Place the content on hot

plate till the digestion is over. Filter the solution in 100 ml conical flask, wash the residue on filter paper several times with the hot water. Make up the volume with distilled water, store the solution in air tight container.

Estimation :

- Transfer 10 ml dilute in 50 ml volumetric flask.
- Add 10 ml ammonium molybdate vanadate solution shake the content.
- Make up the volume and record the reading as per the procedure under preparation of standard curve.

Calculation :

50 µg = R
1 R = 50/R µg (Factor)

$$\text{Total (\%) P} = \frac{\text{Factor (F) x Reading x 100x100 sample}}{10000 \times 1000 \times 10 \times 1}$$

Reference :

Koenig, R.A. and Johnson, C.R. (1942). Colorimetric determination of biological materials Ind. Eng. Chem. Analyt. Edn. 14 : 155-156.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dept. Agric. 939 : 1-19.

Determination of Potassium in Soil and Plant

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The available potassium i.e. exchangeable and water soluble potassium is determined by extracting soil with neutral normal ammonium acetate solution. The estimation of potassium is carried out by flame photometer.

1. Principle :

The principle underlying this is that a large number of elements when excited in a flame, emit radiation of characteristic wave length. The excitation cause one of the outer electron of neutral atoms to move to an outer orbit of higher energy level or the atoms may be excited sufficiently to loose an electron completely from the attractive force of the nucleus where excited atom return to lower energy level, light at characteristic wave length is emitted. Excited atoms or ions give line radiation at very definite wave length and thus K gives at 404.4 and 767 mμ. The flame photometer employees a relatively low temperature excitation and measures with a photocell the emission intensity which is proportional and to concentration in selected wave length (767 mμ) and for this red filter is used.

2. Apparatus and reagents :

a) Flame photometer with red filter,
b) Pipette, volumetric flasks and conical flask (100 ml).

3. Reagents :

(a) Neutral Normal Ammonium Acetate :

Add 58 ml of glacial acetic acid to about 600 ml H2O and then add 70 ml of concentrated ammonia (sp. gr 0.90) Dilute the solution to one litre. Then adjust pH of solution at 7.0 with the help of ammonia or acetic Acid or this can be prepared by dissolving ammo. Acetate (CH3COONH4) (77.08 eq.wt.) directly in H_2O and volume to be made one litre and then adjust the pH 7.0 .

(b) Standard Potassium Solution :

Dissolve 1.9066 g of dried KCl (AR) in distilled water and dilute to one litre. This is 1000 mg kg-1 K solution. 100 ml of this solution diluted to 1 lit. to make 100 ppm K solution.

4. Preparation of the standard curve :

Take 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of 100 mg kg-1 K solution in different 25 ml volumetric flasks. Make up the volume with 1N NH4OAc Soln. Adjust the flame photometer reading at zero with blank (zero K) solution and at 100 for 40 mg kg-1 K solution. Take the flame photometer readings for every dilution. Plot the standard curve on graph paper by taking K concentration on X axis and flame photometer reading an y axis. This will give a factor (F) of 1 flame photometer reading = 0.4 mg kg-1 K.

5. Procedure :

Take 5g soil in 100 ml conical flask and add 25 ml of 1N NH4OAc Soln. Shake the content for 5 minutes and then filter through Whatman No.1 filter paper. Potassium extract is measured by flame photometer after calibration.

6. Calculation :

$$\text{Available K (kg ha}^{-1}) = \frac{\text{RxFx25x100x20x1.121}}{\text{5 x 1000}}$$

$$= \text{R x F x 11.217.}$$

Limits of available K in soil :

Very low	:	Less than 200 K kg ha^{-1}
Low	:	200 – 250 K kg ha^{-1}
Medium	:	250 – 400 K kg ha^{-1}
High	:	400 – 600 K kg ha^{-1}
Very high	:	More than 600 k kg ha^{-1}

8. Precaution :

a) These should not be any turbidity or suspended particles in extract, it will chock the capillary feeding tube .
b) The gas and air pressure should be constant.
c) If sample reading goes beyond 100 then dilute the extract.

9. Determination of k in plant sample :

(a) Wet digestion :

Place 1-2g of ground plant sample in 100ml digestion flask. Add 20-25 ml of acid mixture Acid mixture 750 ml conc. HNO3 + 150 ml conc H2SO4 + 300 ml of HClO4 and mix the contents of the flask by swirling well. Heat the flask at a low temp and then slowly increase the flame or temp. of hot plate. Completion of digestion is confirmed when liquid is colorless. After cooling, add 20-25 ml H2O and filter through whatman No.40 into a 100 ml/250 ml volume flask and make up the volume.

(b) Determination of K :

Take the aliquot and get the reading of K through flame photometer using red filter and calculate the amount of K in the plant sample on the oven dry matter basis.

K (%) in plant sample = $X \times 4 \times 10^{-3}$

References :

Black, C.A. (1965) Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA.

Determination of Sulphur in Soil and Plant

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Principle :

Besides some amount in the soil solution, available sulphur in mineral soils occurs mainly as adsorbed $SO_4^=$ ions. Phosphate ions (as monocalcium phosphate) are generally preferred for replacement of the adsorbed $SO_4^=$ ions. The extraction is also carried out using $CaCl_2$ solution. However, the former is considered to be better for more efficient replacement of $SO_4^=$ ions. Use of Ca salts have a distinct advantage over and leads to easy filtration $SO_4^=$ in the extract can be estimated turbid metrically using a colorimeter/spectrophotometer.

A major problem arises when the amount of extracted sulphur is too low to be measured precisely.; To overcome this problem, addition of seed solution of known S concentration is added to the extract to raise concentration to easily detectable level. Sulphur in the extract can also be estimated by a colorimetric method using barium chromate (Nemeth 1964; Palaskar et al. 1981), but the turbidimetric method (Chesnin and Yien 1950) given below is mainly used in the soil testing laboratories.

Instruments :

(i) Colorimeter or spectrophotometer or autoanalyzer.
(ii) Mechanical shaker

Reagents

1. **Mono-calcium phosphate extracting solution (500 mg P L^{-1}):** Dissolve 2.035 g of $Ca(H_2PO_4)_2.H_2O$ per litre.
2. **Gum acacia-acetic acid solution:** Dissolve 5 g of chemically pure gum acacia powder in 500 mL of hot water and filtered in hot condition through Whatman No. 42 filter paper. Cool and dilute to one litre with dilute acetic acid.
3. **Barium chloride:** Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
4. **Standard stock solution (2000 mg S L^{-1}):** Dissolve 1.089 g of oven dried AR grade potassium sulphate per 100 mL.
5. **Working standard solution (10 mg S L^{-1})**: Measure exactly 2.5 mL of the stock solution and dilute to 500 mL.
6. **Barium sulphate seed suspension:** Dissolve18 g of AR grade $BaCl_2$ in 44 mL of hot water and add 0.5 mL of the standard stock solution (given above). Heat the contents to boiling and then cool quickly. Add 4 mL of gum acacia-acetic acid solution to it. Prepare a fresh seed suspension for each estimation everyday.
7. **Dilute nitric acid (approx 25%):** Dilute 250 mL of AR grade conc. HNO_3 to one litre.
8. **Acetic-phosphoric acid:** Mix 900 mL of AR grade glacial acetic acid with 300 mL of H_3PO_4 (AR grade).

Procedure :

1. Weight 20 g of soil sample in a 250 mL conical flask.
2. Add 100 mL of the monocalcium phosphate extracting solution (500 mg P L^{-1}) and shake for one hour. Filter through Whatman No. 42 filter Paper.
3. Measure 10 mL of the clear filtrate into a 25 mL volumetric flask.
4. Add 2.5 mL of 25% HNO_3 and 2 mL of acetic-phosphoric acid. Dilute to about 22 mL, stopper the flask and shake well.
5. Shake the $BaSO_4$ seed suspension and then add 0.5 mL of it and 0.2 g of $BaCl_2$ crystals. Stopper the flask and invert three times and keep.

6. After 10 minutes, invert 10 times and keep. After another 5 minutes, invert 5 times.
7. Allow to stand for 15 minutes and then add 1 mL of gum acacia-acetic acid solution.
8. Make up the volume, invert three times and keep aside for 90 minutes.
9. Invert 10 times and measure the colour intensity at 440 nm (blue filter).
10. Run a blank side by side.

Preparation of standard curve for S :

1. Place 2.5,5.0,7.5,10.0,12.5 and 15.0 mL portions of the working standard solution (10 mg S L^{-1}) into a series of 25 mL volumetric flasks to obtain 25,50,75,100,125 and 150 µg S.
2. Proceed to develop turbidity as described above for sample aliquots.
3. Read the colour intensity and prepare the curve by plotting readings against sulphur concentration (In µg in the final volume of 25 mL)

Calculation :

$$\text{Available S in soil (mg kg}^{-1}) = \frac{R \times 100}{10 \times 20}$$

Where, r stands for the quantity of S in mg as obtained on X-axis against a reading.

Determination of total sulphur in plant:

Sulphur is an essential plant nutrient and occurs in many different forms. The procedure for total sulphur estimation is as follows :

Digestion of plant material :

Take one gram of plant material in digestion flask. Add 10-15 ml of Diacid (3:1: Nitric acid : Perchloric acid) mixture and swirl the content in 150 ml volumetric flask. Place the content on hot plate till the digestion is over. Filter the solution in 100 ml conical flask, wash the residue on filter paper several times with the hot water. Make up the volume with distilled water, store the solution in air tight container.

Estimation :

Take 10 ml aligust from extract and proceed as per the method described under preparation of standard curve (Bardsley and Lancaster, 1960).

Calculation :

5 µg = R
1 R = R/5 µg (Factor)

$$\text{Total S (\%)} = \frac{\text{Factor x Sample R x1000 x 100 x 100}}{\text{1000 x 10 x 1}}$$

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Chesnin, L. and Yien, C.N. (1951). Proc. Soil Sci. Soc. Am. 15 : 149.

Determination of Zn, Cu, Fe and Mn in Soils and Plants by Atomic Absorption Spectrophotometer

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All atoms can absorb light at certain discrete wavelengths corresponding to the energy requirement of the particular atom. When at ground state the atom absorbs light it is transformed into the excited state. It is the same atom containing more energy. This energy is measured in relation to the ground state and a particular excited state say for example in case of Na may be 2.2 eV (electron volts) above the ground state.

Each transition between different electronic energy states is characterized by a different energy and by a different wavelength. These wavelengths are sharply defined and when a range of wavelengths is surveyed, each wavelength shows as a sharp energy maximum (a spectronic line). These characteristic lines distinguish atomic spectra. The lines, which originate in the ground state of atom, are most often of interest in atomic absorption spectroscopy. These are called the resonance lines. The atomic spectrum, characteristic of each element, then comprises a number of discrete lines, some of which are resonance lines. Most of the other lines arise from excited states rather than the ground state. The lines of excited states are not useful generally in atomic absorption analysis as most of the atoms in a practical atomizer are found in the ground state.

The relationship of light absorbed by the atom in ground state and their concentration in the solution is defined in the fundamental laws of light absorptions.

Lambert's Law : The portion of light absorption by a transparent medium is independent of the intensity of the incidence light and each successive unit thickness of the medium absorbs an equal fraction of the light passing through it.

Beer's Law : Light absorption is proportional to the number of absorbing atoms in the sample.

The combined Beer - Lambert law may be given as :

$$I_t = I_o - (abc)$$

$$\log \frac{I_o}{I_t} = abc = \text{absorbance}$$

Where, I_o = incident radiation power
I_t = transmitted radiation power
a = absorption coefficient
b = length of absorption path
c = concentration of absorbing atoms

i.e. the absorbance is proportional to the concentration of the elements for a given absorption path length at any given wave length.

In principle, it might be possible to calculate the concentration directly from the above equation. In practice, however, the a and b are constants hence the variation of results is directly related the concentration of atoms. For analysis, the absorbance of different concentration of standard solution is first measured with the help of atomic absorption spectrophotometer and then the results of unknown samples are compared with the standards and thus concentration of unknown sample is calculated.

Atomic absorption spectrophotometer :

Atomic absorption spectro-photo-meter is based on the principle that when atomic vapours of an element are irradiated by the radiation of a characteristic wavelength (i.e. the light from a source whose emission lines are those of the element in question), they absorb in direct proportion to the concentration of the element being determined.

Instrument features :

A wide range of atomic absorption spectrophotometer is available today, all of them have the basic features in common and consist of the following components:

(a) A Light source :

A Light source emits the spectrum of the element to be determined. The most widely used light source is hollow cathode lamp which is designed and operated in such a way that the lines to be measured are sharp, of stable intensity and free from background.

(b) Atomizer-Burner assembly :

A means of producing atomic vapours of the element to be analyzed. The solution to be analyzed is drawn by capillary and converted into stream of compressed air to a fine spray which after condensation of larger droplets is mixed with the fuel gas acetylene and burnt in a long flame (at 2100-2400^{o}C) in a stainless steel burner.

(c) A Monochromator :

It isolates the absorbing resonance lines from other non absorbing lines. When the light coming from the HCL, after traversing the flam, enters the monochromator which is already set at the wavelength of the resonance lines of the desired element, the monochromator performs its function.

(d) A Detector :

It measures the magnitude of absorption of the characteristic radiation.

(e) A Photomultiplier Tube :

It amplifies the absorption signal and converts the light radiation into electrical energy.

(f) A readout system :

It measures the absorbance in volts. It is normally a strip chart recorder, a digital display, a meter or printer. The presently available AAS have features like automatic calibration with one or more standards, automatic curve corrections, automatic and foolproof gas switching and calculation of average and standard deviations in repetitive runs.

Collection and preparation of soil and plant samples : To avoid contamination, soil samples are to be collected in plastic tub, using rust free instrument or wood and kept in polythene lined cloth bags. Samples are prepared with the help of wooden mortar and pastle and sieved through 2mm nylon screen/mosquito net cloth or stainless steel sieve.

Similarly plant samples (leaves, grains or straw) should be washed with 0.01N HCl, rinsed with glass distilled water dried in oven at 65°C and crushed with the help of stainless steel scissors.

Soil extraction : DTPA offers the most favourable combination of stability constants for the simultaneous complexing of Zn, Cu, Fe and Mn, Cd, Co, Ni and Pb (Lindsay and Norvell, 1978). Buffering of extractant in a slightly alkaline pH range (7.3) by including soluble Ca^{2+}, avoids the dissolution of $CaCO_3$ with the release of occluded micronutrients due to CO_2 partial pressure of approximately 10 times that in atmosphere, as the soil contains slightly higher CO_2 levels than found in the atmosphere.

(a) Extracting solution : (0.005 M DTPA) Dissolve 1.9679g of DTPA (Diethylene tri amine penta acetic acid) + 13.3 ml TEA (Triethanol amine) + 1.47g $CaCl_2$.$2H_2O$ in 200 ml distilled water, dilute to 900 ml, adjust pH 7.3 with 6N HCl while stirring and then make upto 1 liter and mix thoroughly.

(b) Apparatus required : Shaker (Horizontal or Rotatory), iodine value flasks (100 ml capacity) or conical flasks with glass stoppers, funnels, filter paper whatman No.1, plastic storage bottles and Atomic absorption spectrophotometer.

(c) Stock Standard Solutions : The standard solutions of different micro-nutrients should preferably be

prepared by using their wires. Dissolve 1g wire in a minimum volume of 1:1 nitric acid and dilute to 1000ml with distilled water to obtain 1000 µg/ml solution of micro-nutrient, or take salts of metals as follows:

Zn- 4.398g l^{-1} $ZnSO_4,7H_2O$
Cu- 3.929g l^{-1} $CuSO_4,5H_2O$
Fe- 4.977g l^{-1} $FeSO_4,7H_2O$
Mn- 3.598g l^{-1} $MnSO_4,H_2O$.

The prepared standards are also available in the market. Out of these standards, prepare working solution of 50 ppm. Then a series of standard solution of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm may be prepared for each metal.

(d) Background correction : The reading of a spectral line always includes any contribution from the flame and sample matrix. Failure to correct properly for the background reading can be a source of serious error. Although the need for fast background correction is most obvious with graphite furnace work, it is also a consideration with flame atomic absorption.

The most common method of background correction in atomic absorption spectrometry involves the use of a continuum source such as a deuterium lamp to measure the background. The source used is a deuterium filled discharge lamp, which emits an intense continuum spectrum from 190 nm to about 400 nm. This is the region where most atomic absorption lines occur and where the effects of background absorption are most pronounced. The poly-atomic gas D_2, is used in the lamp because a continuum is produced rather than a line spectrum.

The deuterium lamp is different from a hollow cathode lamp in construction and operation. The lamp incorporates a heated, electron-emitting cathode, a metal anode and a restrictive aperture between the two. A discharge current of several hundred milli amperes excites the deuterium gas. The discharge is forced to pass through the small aperture, forming a defined area of high excitation and hence high light emission. A suitable window transmits the light to the spectrometer's optical system.

To obtain successful background correction the deuterium lamp must be correctly aligned, and its intensity must be matched to that of the hollow cathode lamp.

It is important that both the deuterium source and the hollow cathode source are aligned to follow the same optical path. If they are not, then the two measurements may not be made on the same atom population and significant errors may occur.

In order to balance the intensity of the deuterium lamp with the hollow cathode lamp, it may be necessary to change the hollow cathode lamp current to a higher or lower value depending on the relative intensities of the lamps.

Although most modern AA spectrophotometers incorporate so called "simultaneous" background correction, they rely on two measurements separated slight in time. One measurement is of the total absorbance (atomic plus background) and the other is of the background only. The background is electronically subtracted from the absorbance to give the background corrected atomic absorbance with the continuum source method of background correction, the total absorbance is measured during the hollow cathode lamp pulse and the background during the deuterium lamp pulse. With the Zeeman method using a modulated magnetic field, the total absorbance is measured with the magnetic field off and the background with the field on.

(e) Soil analysis : Weigh 12.5g soil sample in 100 ml iodine value flasks. Add 25 ml DTPA solution. Shake this mixture for 2 hours on shaker at 70 to 80 oscillation per minute, filter through acid washed distilled water rinsed, whatman No.1 filter paper and collect the filtrate in plastic bottles. Determine the content of micronutrients on atomic absorption spectrophotometer.

(i) Plant analysis : Weigh 0.5g plant sample in a conical flask (corning, 100 ml capacity). Add 10 to 12 ml of di acid mixture (1 perchloric + 4 nitric acid) and digest the mixture on hot plate till the residue is colour less. Now take off, cool dilute with distilled water and filter through whatman No.1 filter paper. Make up the volume of digestate to 50 ml. Read for micronutrient content on atomic absorption spectro-photometer.

Factors : For soil multiply the concentration read on AAS computer sheet by "2". Similarly for plants the multiplying factor will be 100 to get concentration in mg kg^{-1}.

Reference :

Lindsay, W.L. and Norvell, W.A. (1978). Proc. Soil Sci. Soc. Am. 42 : 421-428.

Estimation of Boron in Soils, Plants and Water

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Boron occurs as anion in soils and is required by plants in very small quantity. Water soluble B makes the estimate of its availability to plants. Total boron in soils varies from 20 to 200 mg kg^{-1} and available (water soluble) boron in soils ranges from 0.03 to 12 mg kg^{-1} respectively. The threshhold value ranging from 0.1 to 0.5 mg kg^{-1} (water soluble B) depends upon the soil type, crops, and other factors, below which the response to applied boron may be expected. Some sensitive crops to boron deficiency are listed in table 1. Its availability is affected by soil pH as under:

- Deficiency of B is generally observed in old acid leached soils.
- Availability increased with the rise in soil pH having significant positive correlation with pH rising from 4.7 to 6.7.
- In neutral, saline and calcareous soils the B availability again decreases with the rise in soil pH having significant negative correlation with the rise in pH from 7.1 to 8.1. In calcareous soils B fixation occurs with the condensation of borate radical into long chains in the presence of Ca.

Table 1 : Sensitivity of crop to Boron deficiency

Sensitive	Medium	Low
Alfalfa	Apple	Barley
Cauliflower	Cabbage	Beans
Rape seed	Carrot	Corn
Conifers	Clover	Grasses
Peanuts	Cotton	Oat
Sugarbeet		Onion
Turnip		Pea
		Potato
		Soybean
		Wheat
		Rice

- In alkaline soils the availability of B is high and may be even toxic for plant growth.

Besides this the low moisture availability also causes B deficiency.

Irrigation water containing Boron between 0.3 to 0.6 mg kg^{-1} can be used safely, whereas, irrigating soils with water containing 1 to 3 mg kg^{-1} B causes toxicity of B in plants.

Boron determination (Azomethine H Method) :

Azomethine H forms coloured complex with H_3BO_3 in aqueous media. Over a concentration range of 0.5 to 10 µg B/ml the complex is stable at pH 5.1. Maximum absorbance occur at 420 nm with little or no interference from a wide variety of salts. This technique is rapid, reliable and more convenient to use than traditional procedures employing carmin, curcumin or quinalizarin (John *et al.*, 1975).

Apparatus :

(1) Spectrophotometer
(2) Poly-propylene tubes 10 ml capacity.

Reagents :

1. Distilled water
2. Buffer solution : Dissolve 250 g of ammonium acetate (NH_4OAc) and 15 g of ethylenediaminetetracetic acid (EDTA disodium salt) in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix.
3. Azomethine H reagent : Dissolve 0.45 g of azomethine H in 100 ml of 1% L ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.
4. Calcium hydroxide suspension : Add 0.4g $Ca(OH)_2$ to 100 ml distilled water.

5. 0.1 N HCl : Add 8.3 ml conc. HCl to 900 ml distilled water, mix, cool to room temperature and make up the volume to 1000 ml.
6. Calcium chloride 0.01 M Dissolve 1.11 g of anhydrous $CaCl_2$ in 900 ml distilled water and make up the volume to 1000 ml.
7. Boron standard solution : Dissolve 0.114g of Boric acid (H_3BO_3) in distilled water and adjust the volume to 1000 ml. Each ml contains 20 µg B. Dilute 10, 20, 30, 40 and 50ml of the stock solution to 100 ml with distilled water to have solution with B concentration of 2,4,6,8 and 10 µg of B/ml respectively. Include a distilled water sample for the 0.0 µg of B/ml standard solution.

Procedure :

Take 1 ml of aliquot of blank and diluted B standards into a 10 ml polypropylene tube, add 2 ml of buffer solution and mix. Add 2 ml of azomethine H reagent, mix and after 30 minutes read the absorbance at 420 nm on spetrophotometer. With the help of absorbance readings of standard solutions of different concentration of B the standard curve is drawn and a factor for concentration of B for 1 absorbance is calculated which is utilized to calculate B in the soils, plant or water sample.

Preparation of Extracts :

1. Soil extracts : The hot water soluble extraction procedure of Berger and Truog (1939) is being used widely with slight modification of adding dilute electrolyte (0.01 M $CaCl_2$) instead of water only. This provides clear, colourless extract which eliminates the need of charcoal for decolourzation. Beside this a negative error, associated with B adsorption by charcoal, is also removed.

Place 20 g air dry soil in 250 ml low B flat bottom flasks and add 40 ml of 0.01 M $CaCl_2$ solution. Attach water cooled reflux condenser to the flask. Heat the flasks for 5 minutes and then cool and filter the suspension in plastic bottles.

Transfer 20 ml aliquot to evaporating dish, add 2 ml $Ca(OH)_2$) suspension and evaporate the solution to dryness. Heat the evaporating dishes gently to destroy organic matter, cool to room temperature, add 5 ml 0.1N HCl. Triturate the residue with rubber policeman to ensure the complete dissolution of the residue (Bingham, 1982).

For analysis of B pipette 1 ml of the aliquot and proceed as for the standard curve.

2. Plant digest : Take 0.5 g plant sample in porcelain/platinum dishes Add 0.5 g $Ca(OH)_2$. Ignite the sample in the muffle furnace at 550°C for 4 hours to obtain white grey ash. Cool the dishes and moist the ash carefully with little distilled water and then add 5 ml 0.1N HCl. Transfer the content in to 25 ml volumetric flask mix and make up the volume to 25 ml with distilled water. For analysis of B take 1 ml of the aliquot and proceed as for the standard curve.

3. Water analysis : Take suitable quantity of water sample (containing 0.2 to 5.0 µg B) in porcelain dishes add 2 ml $Ca(OH)_2$ and proceed as described for soil extract. It is important to keep a definite volume of aliquot i.e. 1 ml of either soil, plant or water in final step of B determination.

References :

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Profile Studies of Deep Black Soils

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Deep black soils or Vertisoils occur globally under various parent materials and environmental conditions (Table 1).

Table 1. Distribution of Vertisols and associated soils

Juris-Diction	Location	Area (m ha)	% of Gross Black soils
Continent	Africa	105.0	38.7
	Asia & far East (Mainly India)	**70.3**	25.9
	Australia	48.8	17.7
	Latin America	27.0	9.9
	North America	10.0	3.7
	Near & Middle East	5.7	2.1
	Europe	5.4	2.0
	TOTAL	**271.4**	**100**
Country	India	**70.3**	25.9
	Australia	48.8	17.7
	Sudan	43.4	16.6
	USA	18.1	6.7
	CHAD	15.5	5.7
	China	11.6	4.3
	Others (in parts)	64.5	23.7
	TOTAL	**271.4**	**100**
India	MS	24.2	34.4
	MP	**21.2**	30.1
	GUJ.	4.9	7.0
	AP	9.4	13.4
	KTK.	5.8	8.2
	TN	2.6	3.7
	RAJ.	1.1	1.6
	UP	1.1	1.6
	TOTAL	**70.3**	**100**
MP	Vertisols	8.0	37.7
	Inceptisols	8.6	40.6
	Entisols	4.2	19.8
	Alfisols	0.4	1.9
	TOTAL	**21.2**	**100**

They clayey soils that shrink and swell extensively upon changing soil moisture conditions. Vertisols exhibit unique morphological properties such as the presence of slickensides, wedge-shaped aggregates, diapir (mukara), and gilgai. Shrink-swell phenomena are the dominant pedogenic processes in Vertisols and are attributed to changes in interparticle and intraparticle porosity with changes in moisture content.

Definition of Vertisols :

Taxonomically for defining Vertisols, there must be

1. A layer 25 cm or more thick with an upper boundary within 100 cm of the mineral soil surface, that has either SLICKENSIDES or WEDGE SHAPED PEDS that have their long axes tilted 10 to 60^o from the horizontal; and
2. A weighted average of 30 % or more clay in fine earth fraction either between the mineral soil surface and a depth of 18 cm or in Ap horizon, whichever is thicker and
3. 30 % or more clay in fine earth fraction of all horizons between a depth of 18 cm and either a depth of 50 cm or a densic, lithic or paralithic contact, a duripan, or a petrocalcic horizon if shallower and
4. Cracks that open and close periodically.

Vertisols are significant global resources that serve as the lifeline in subsistence agriculture due to their high productivity.

Efforts towards comprehension and successful utilization are imperative for continued productivity and long term sustainability of these resources for current and future civilizations.

Morphology of a soil is best evaluated from the *in situ* examination of the soil profile. A recently dug pit large enough for observation of a pedon is desirable. Old exposures such as road banks and

ditches are acceptable only for preliminary studies because morphological features often become altered after prolong exposure.

Normally the size of profile pit is kept 1.8 m long, 1.2 m wide and 1.8 m deep but for the study of black soils, the width of pit varies from place to place depending on its cyclic wave length of puffs and shelves. It should be kept in mind that atleast half wave length covering both, puff and shelve is considered while exposing profile pit in order to study the pattern of cracks and slickensides perfectly.

The profile examination begins with a first approximation and marking of soil horizon boundaries on the profile. Each horizon is then carefully observed and described. Horizon boundaries are relocated as required by the detailed study (Buol *et al*. 1998). Th description sheet containing the columns of site and soil characteristics is filled up by the profile study group during pedon studies.

Vertisols are relatively homogneous in their morphology. Although horizonation is not distinct yet a few horizons above the parent material may be identified as self mulching surface (Ap), blocky subsurface (A12), slickensided horizon and wedge shaped subsoil (Bss).

The depth of these soils may vary from shallow to very deep. Previously the black soils were grouped as shallow (<30 cm) medium (30-100 cm) and deep (>100 cm) but lateron Sehgal (2008) modified the dept of shallow soil as less than 50 cm.

Requirement of Vertisols :

Main requirements of Vertisols are the presence of high content of clay (>30 %) and predominance of montmorillonite (2:1 expanding clay). Other important parameters for the development of Vertisols are:

(i) **Parent material** having basalt, argillaceous limestone, marine clays and shales

(ii) **Weathering period** must be extensive for the development of solum with 2:1 expanding clays

(iii) **Weathering environment** should be such that no further weathering of 2:1 expanding clays takes place. Even no inter-layering exists to the extent the properties are destroyed

(iv) **Sequence of events** should continue like churning/mixing, development of argilli-pedoturbation, development of slickensides and formation of wedge shaped structures

Pedogenesis of Vertisols :

1. Separation of blocks : Deep wide cracks separate the soil into strong and massive prism like blocks in the upper part of the pedon that break into angular blocky peds of hard and firm consistence.

(a) ***Cracking of soil*** : During dry season, the soil cracks to the surface due to shrinkage of 2:1 expanding clays that may extend to a depth of 1 metre or more.

(b) ***Falling of surface soil material*** : While cracks are open, surface soil material falls into them by several mechanisms such as animal activity, wind or at the onset of rainy season by water.

2. Hydration of clays : The clay hydrate and due to their high coefficient of expansion and contraction, expand 3 dimensionally on wetting.

(a) ***Expansion of clays*** : Cracks close on the surface but because of the extra material now present in the lower part of the profile, a greater volume is attained and the expanding material presses and slides the aggregates against each other, developing a "lentil" angular blocky structure with sliekenside features on the ped surfaces.

(b) ***Shear stress development*** : The slipping occurs where shear strength is surpassed by shear stress acting

upon a soil mass. The shear stress is a major force caused by swelling and develops when volume expansion results during the wet cycle.

(c) ***Formation of slickensides*** *:* The slickensides, intersecting or close enough to intersect, also result in wedge shaped structural aggregates, the most characteristics feature of Vertisols which develop with their longitudinal axes inclined at 30 to 60° from horizontal (Sehgal and Bhattacharjee, 1988).

(d) ***Buckeling of land space*** : This expansion buckles the land scape, forming the micro relief called gilgai. The micro basins contain more organic matter than the micro ridges and probably it results from admixtures of subsurface material into micro ridge area and slight erosion of organic rich fines from the ridges to the basins.

3. Incomplete leaching : In most shrink swell soils, the temperature being high, the potential evapotranspiration suggesting incomplete leaching and inducing the process of calcification in these soils.

Cyclic movement of soil material :

Amongst several processes acting in the formation of Vertisols, the predominant process seems to be haploidization i.e. mixing by argilli pedoturbation. The specific features of such soils are :

1. Gilgai micro relief : The term gilgai is an Australian aboriginal term meaning small water hole.

Pedogenic micro topographical features like puffs (microknolls) and shelves (microbasins) develop that remain intimately associated with one another (Bhattacharjee *et al*. 1977), Columbe *et al*. (1996) introduced a term "diapir" meaning a protusion of subjacent soil material which penetrates to the overlying horizons and approaches or reaches the surface. If diapir and gilgai occur, the mound in gilgai is always developed over the diapir.

Hallsworth and Beckman (1969) classified gilgai into 6 types i.e. normal or round, melon hole, Lattice, Linear or wavy, tank or stony but lateron Paton (1974) suggested only two types of gilgai i.e. linear and circular (Nuram or Pockmarked) each of which were grouped into 4 types.

α type - Mound and depression equally developed (No shelf present)

β type - Mound of much greater extent than depression (No shelf present)

γ type - Depression of much greater extent than mound (No shelf present)

δ type - Mound, shelf and depression all present

2. Size of cyclic pedons : Half cycle linear distance (HCLD) measures the lateral dimension of a cyclic pedon. It may be small, medium or large i.e. below 1, 1 to 2 or above 2 to 3.5 metre, respectively.

3. Horizon sequence : In Vertisols, the horizon sequence has been suggested to be A1-Bss-BC-C where "ss" indicates about the presence of slickensides.

4. Thickness of horizon : Thickness of A1 in Vertisols varies with the linear frequencies of puffs and shelves of gilgai micro relief.

5. Horizon boundary (Amplitude): It is the difference between vertical distance from the surface of pedon to the lower boundary of crest of cycle and the lowest point of trough of cycle in same pedon. The amplitudes are grouped as low, medium of high according to the vertical distance as below 25, 25 to 75 or above 75 cm, respectively. Shape of apparent topography of the intermittent horizon is also graded as tongued (vertical extent > horizontal distance), wavy (vertical extent approximating the horizontal distance) and smooth (vertical extent < horizontal distances) as suggested by Bartelli (1971).

Age of Vertisols :

It is difficult to assign the Vertisols a place in the genetic scheme of soil classification as there are greater differences of opinion whether they are old, young or remain in equilibrium with the environment.

1. Views as Vertisols are old : The end product of a development sequence involves the soils whose B horizon has become so clayey that shrink-swell cycles developed and eventually "swallowed" the A horizon. It is possible because high content of fine clay and high fc/cc ratio may be produced by lessivage on a large scale.

2. Views as Vertisols are young : The fate of Vertisol may be to undergo alteration of 2:1 clays to non expanding type of clay. The profile would then cease to churn and eluviation process would dominate. This interpretation suggests that Vertisols are relatively young soils.

3. View as Vertisols are in equilibrium : Vertisols remain in equilibrium with their environment and that the 2:1 expanding lattice clays are stable and will persist, barring a climate change. Vertisols then can be considered diagnostic of environments in which the parent material is basic and gives rise to the formation of 2:1 expanding lattice silicates under the influence of wet dry climate.

Table 2 : Range in characteristics of Vertisols and Vertic Inceptisols

Horizon	Soil colour (10 YR)	Texture	Structure	Special features	Width of cracks (cm)
A. Typic Haplustert (10 YR - 2.5 YR)					
Ap/A11	4/2, 3/3, 3/2, 3/1	C	1f/1m sbk	1c/2c pr-3c pr	2-5
A12	3/3, 3/2, 3/1	C	2m/2c abk	2c pr - 3c pr	2-5
Bss	3/3, 3,2, 3/1, 2/1	C	2m/3c abk	Intersecting lickensides*	1-2
BC	4/4, 3/4	C	2m/2c abk	----do----	0.5-1
C	5/4, 4/4, 4/3	c-gc	2msbk/ massive	-	-
B. Vertic Haplustept (10 YR - 7.5 YR)					
Ap/A	5/2, 4/3, 4/2, 3/2	Cl	gr/1m sbk	1c pr-2c pr	2-2.5
AB	4/3, 3/3	cl-c	1m/2m sbk	----do----	2-2.5
B21	4/3, 3/3, 3/2	cl-c	2m sbk-3m/3c	2c pr - 3c pr or pressure faces/abk slickensides	1.5
B22	6/3, 5/3, 4/4, 4/3	gscl-cl	----do----	-	-
C	7/6, 6/3, 5/3, 4/4	gsl-gscl	1f sbk/ massive	-	-

*or parallelepipeds with long axes tilted from 35° to 55° from horizontal

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Irradiation by Gamma Chamber – 5000

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Gamma irradiation has been extensively used for food irradiation and sterilisation, killing of fungus and micro organisms, sterilisation of medical accessories and surgical equipments, high energy radiation chemistry, seed irradiation and semiconductor irradiation. Gamma Chamber can also be used in many other research applications which require irradiation of materials with ionizing radiations to varying doses.

The radiation processing of food involve the controlled application of energy from ionizing radiation such as gamma rays, electrons and X rays for food preservation. The gamma rays and X-rays are short wavelength radiation of electromagnetic spectrum, which includes radiowaves, microwaves, infrared, visible, and a violet light. Radioisotopes such as cobalt 60 and caesium-137 emit the gamma rays, while machines using electricity generate electrons and X-rays. The gamma rays and electrons are distinguished from other form of radiation by their ionizing ability. (That they are able to break chemical bond when absorbed by material). The product of ionizing radiation may be electrically charged ions) or neutral (free radicals). These there further react to cause change in an irradiated material known as the process of radiolysis. It is this reaction that causes the death of micro- organism, insect and parasites during food irradiation.

The conservation and preservation of food is a prerequisite for food security. It provides self-reliance to nation. The Indian Food Industries contributes about 25-28% towards GDP. The food processing sector provides 60-65% employment with a turnover of in US$ 36.1 billion of which US$ 27.8 billion in organized sector, any change or any stagnation in technology will inevitably have very large impact through out the economy. India is a potential producer of fruits and vegetables live stock and marine products. India has a tremendous potential as the world largest food factory.

It has been estimated that about 30-35% of fruit and vegetables of worth Rs 3000/- corers are perished every year. The reasons for such losses are seasonal nature of fruits and vegetables production. The long distance between production and consumption centers and also rising gap between demand and supply. The hot and humid climate in the country is also quite favorable for the growth of numerous insects and micro organisms that destroy stored crops and cause spoilage of food every year. The spoilage also occurs due to chemical and physiological changes in stored foods. To preserve the food and food products, technologies such as freezing caning sun drying pickling fermentation have been recommended by researchers but, each of these methods have its own merits and limitation. The search for an alternative newer economical methods to preserve food and causes least changes in sensory quality have been under taken since long back, and has been observed that radiation processing of food is one of the latest method developed for food preservation.

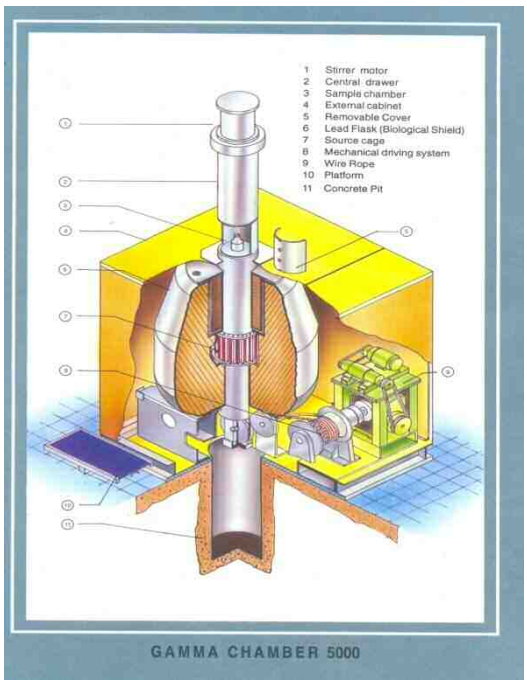
Irradiation by Gamma Chamber 5000 :

Gamma Chamber 5000 is a compact self shielded cobalt-60 gamma irradiator providing an irradiation volume of approximately 5000cc. The material for irradiation is placed in an irradiation chamber located in the vertical drawer inside the lead flask. This drawer can be moved up and down with the help of a system of motorised drive which enables precise positioning of the irradiation chamber at the centre of the radiation field.

Radiation field is provided by a set of stationary cobalt-60 sources placed in a cylindrical cage. The sources are doubly encapsulated in corrosion resistant stainless steel pencils and are tested in

accordance with international standards. Two access holes of 8 mm diameter are provided in the vertical drawer for introduction of service sleeves for gases, thermocouple, etc. A mechanism for rotating/stirring samples during irradiation is also incorporated. The lead shield provided around the source is adequate to keep the external radiation field well within permissible limits. The Gamma Chamber 5000 unit can be installed in a room measuring 4 metres x 4 metres.

GAMMA CHAMBER 5000



Features :

- **Safe and self-shielded:** The shielding provided is adequate to limit the radiation field on the external surface of the unit, well within the permissible levels. No additional shielding is required for its installation and use.
- **Automatic control of irradiation time:** Built-in timer provides accurate control of irradiation time from 6 seconds onwards. The unit can also be operated manually. Solid state programmable controls have been provided. In the event of power failure battery backup displays the programmes.
- **Manual control of irradiation temperature:** It is possible to irradiate samples at low or high temperature by circulating liquid nitrogen or hot air. These can be introduced through the service sleeves provided in the vertical drawer. The irradiation temperature is sensed by a thermocouple and displayed on the panel.
- **Remote operation:** An additional table top control panel is provided for remote operation in addition to the normal one provided on the unit.
- **Dose uniformity:** Stationary source pencils, symmetrically placed in a cylindrical cage ensure good uniformity of radiation field in the sample chamber. In addition a mechanism is also provided for rotating/stirring samples during irradiation.
- **Easy loading and unloading of samples:** Sample chamber extends to a convenient height for easy loading and unloading of samples.
- **Safety assurance:** The design of Gamm Chamber conforms to American National Standards, ANSI-N433.1-1977 for safe design and use of self-contained dry source storage gamma irradiators (Category I). It also meets the requirements of type B(U) package for safety in transport of radioactive materials as per AERB code No.SC/TR-1, 1986 of Atomic Energy Regulatory Board of INDIA.

Applications :

Gamma Chamber is a versatile equipment for research studies in many fields such as:

- Radiobiology
- Preservation of tissue grafts
- Mutation breeding
- Food preservation
- Sterile male insect technique
- Biological and genetic effects of radiations
- Radiation chemistry
- Radiation effects on materials
- Radiation sterilization
- Modification of properties of materials

Food Preservation by Gamma Radiation

The radiation processing of food is carried out inside an irradiation chamber shielded by 1.5 - 1.8 meter thick concrete walls. Food either pre-packed or in bulk placed in suitable containers is sent into the irradiation chamber with the help of an automatic conveyor. The conveyor goes through a concrete wall labyrinth, which prevents radiation from reaching the work area and operator room. When the facility is not in use the radiation source is stored under 6 meter deep water. The water shield does not allow radiation to escape in to the irradiation chamber, thus permitting free access to personnel to carry out plant maintenance. For treating food, the source is brought to the irradiation position above the water level after activation of all safety devices. The goods in aluminum carriers or tote boxes are mechanically positioned around the source rack and are turned round their own axis, so that contents are irradiated on both the sides. The absorbed dose is determined by the residence time of the carrier or tote box in irradiation position.

Measurement of radiation dose :

Placing dosimeters at various positions in a tote box or carrier we can check the absorbed dose. The dosimeters are made from a material including photographic film, Perspex and cobalt glasses. The poly vinyl chloride (PVC) dosimeters are impregnated with a dye. The Hydrogen chloride is released from the PVC by irradiation and it produces a qualitative or quantitative change in the colour of the dye to indicate the dose received.

Dose distribution :

The penetration of gamma radiation depends on the density of the food as well as the energy of the ray. At a density of 1000 kg m^{-3} half of the rays are absorbed in 11 cm. Halving the density approximately double the depth of penetration. The uniformity of dose distribution can be expressed as a ratio of D max : D min. For sensitive food such as chicken the ratio should be as low as possible1.5.

Potential Applications of Gamma Radiation :

The radiation dose administrated to a food depends on the resistance of the organisms present and the objective of the treatment. The maximum recommended dose is 15 kGy, with average dose not exceeding 100 kGy. Various application of this technology are as under:

1. Sterilisation (or radappertisation) :

It is possible to sterilize meat and other product, the dose required exceed the current limit of 10 kGy. A dose of 48 kGy is needed for 12 D reduction of *Cl. butulinum*. High dose makes the product organoleptically un acceptable.

2. Reduction of pathogens (radicidation) :

Food poisoning bacteria such as *salmonella typhimurium* are less resistant to radiation than *Cl. Botulinum*, and doses of 3-10 kGy are sufficient for destruction.

3. Prolonging shelf life (or radurisation) :

Relatively low doses are needed to destroy yeast, moulds and non-spore forming bacteria. This process is used to increase shelf life by an overall reduction of vegetative cells.

Table 1: List of radiation processing facilities available in the world :

S. No.	Country	No. of irradiators	Food Commodities
1.	Algeria	1	Potato
2.	Argentina	1	Spices, spinach, coca powder
3.	Bangladesh	1	Spices, onion, dried fish
4.	Belgium	1	Spices, dehydrasted vegetables, deep frozen foods
5.	Brazil	3	Spices, dehydrasted vegetables, fruits, vegetables, grain
6.	Canada	1	Spices
7.	Chile	1	Spices, dehydrasted vegetables, onion, potato, poultry meat
8.	China	11	Spices and vegetable seasonings, Chinese sausage, garlic, apple, potato, onion, dehydrated vegetables, sauses, rice, tomatoes
9.	Croatia	1	Spices, food ingredients, dried beef noodles
10.	Czech. Repulic	1	Spices, dry food ingredients
11.	Cuba	1	Potato, onion, beans
12.	Denmark	1	Spices
13.	Finland	1	Spices
14.	France	5	Spices, vegetable seasonings, herbs, poultry (frozen boneless chicken, dried fruit, frozen frog legs, shrimp)
15.	Hungary	1	Spices, onion, wine cork, enzyme
16.	India	2	Spices, onion, potato
17.	Indonesia	2	Spices, rice
18.	Iran	1	Spices,
19.	Israel	1	Spices, condiments, dry ingredients
20.	Japan	1	Potato
21.	Korea Republic	1	Garlic powder, spices, condiments
22.	Mexico	1	Spices, dry food ingredients
23.	Netherland	1	Spices, frozen products, poultry dehydrated vegetables, egg powder, packaging material
24.	Norway	1	Spices
25.	Poland	3	Not specified
26.	Peru	1	Spices, food additives, animal feed
27.	South Africa	4	Spices, shelf-stable food, fruits
28.	Thailand	1	Spices, fermented pork sausages, enzymes
29.	Ukraine	1	Grain
30.	UK	1	Spices
31.	USA	10	Spices, poultry, fruits, vegetables
32.	Vietnam	1	Onion
33.	Yugosla	1	Spices

(Source : ICGFI, Food & Environmental Protection Section, Update, 1997)

4. Control of ripening :

Fruits and vegetables can be irradiated to extend their shelf life about 2-3 time when stored at 10^0C The ripening and maturation of fruits are arrested by inhibition of hormone production and interupting the biochemical process of cell division.

5. Disinfestations :

Grain and tropical fruits may be infested with insect and larvae, they reduces export potential. A low dose below 1 kGy is effective for disifestation

6. Inhibition of Sprouting:

The technology is effective in inhibiting sprouting of potatoes, A dose of about 150 Gy has been recommended. Similar doses are also effective in preventing sprouting of onion and garlic.

Benefits and limitation of gamma radiation processing:

Benefits :

1. Radiation processing is a cold process and therefore, unlike heat, it can be used on agricultural commodities without changing their fresh-like character
2. Radiation processing dose not alter significantly nutritional value, flavour, texture and appearance of food
3. Radiation using Cobalt-60 cannot induce any radioactivity in food and does not leave any harmful or toxic radioactive residues on foods as is the case with chemical fumigants
4. Due to the highly penetrating nature of the radiation energy, it is a very effective method.
5. Prepackaged foods can be treated for hygienization and improving shelf-life
6. The radiation processing facilities are environment friendly and are safe to workers and public around.

Limitations:

1. Radiation processing is a need based technology and cannot be applied to all kinds of foods
2. Radiation processing cannot make a bad or spoiled food look good
3. It cannot destroy already present pesticides and toxins in foods
4. Amenability of a particular food commodity to radiation processing has to be tested in a laboratory

Plant Mutation Breeding by Gamma Radiation

Plant mutation breeding by radiation has been investigated for long time in many countries. New mutant varieties give us useful gene resources for the security of food resources, the conservation of our ecosystem, and the promotion of new industries. Using radiation technique (gamma-rays, X-rays and EB) 128 varieties were developed in Japan. Many new species were developed for disease resistant crops, i.e. 55 species of rice, 10 of barley and 2 of wheat. Other species of beans, fruits including pears resistant for black spot decease, grass, vegetables, etc, were also developed.

Recently, a lot of fascinating new mutants are generated by ion beams. Ion beams can frequently cause large DNA alterations such as inversion, translocation and large deletion rather than point mutation, which result in producing characteristic mutants otherwise attainable. Ion-beam irradiation of *Arabidopsis* seeds has produced the UV-B-resistant, the frilled-petal, and other novel mutants. The features of ion beams in the mutation induction seem 1) to induce mutants with high frequency, 2) to show a broad mutation spectrum, and therefore, 3) to produce novel mutants. New mutants of chrysanthemum and carnation with complex and striped flower-color, and new flower-shape have been produced and commercialized.

Nuclear techniques, in contrast to conventional breeding techniques, are widely applied in agriculture for improving genetical diversity. Unlike conventional breeding procedures which involve, the production of new genetic combinations from already existing parental genes, nuclear technology causes exclusively new gene combinations with high mutation frequency. Basic tool of nuclear technology for crop improvement is the use of ionizing radiation which causes induced mutations in plants. These

mutations might be beneficial and have higher economical values.

Measures of activity (A) :

The number of disintegrations, or decay events, or nuclear transformations, in a sample per unit time is its activity A. Two common informal units are disintegrations per second and disintegrations per minute.

Curie (Ci) : The US unit of activity is the curie (Ci). $3.7x10^{10}$ disintegrations per second. Common multiples are the millicurie and microcurie.

Becquerel (Bq) : The SI unit of activity is the becquerel (Bq). One becquerel is 1 disintegration per second. The common multiple is the megabecquerel (1 mCi = 37 MBq).

Half –life: The time (t) taken for the radioactivity of a sample to fall to half its initial value.

$$t_{1/2} = 0.693 / k$$

Electron volt (eV): energy of radiation (usually as mega electron volts (MeV). $leV=1.602x\ 10^{-19}$ J

Grays (Gy): Absorbed dose (where 1 Gy is the absorption of 1 J of energy per kilogram of food)

Previously rods (radiological unit) were used. 1 rad = 10^{-2} J kg^{-1}

Plant Tissue Culture Techniques for Mass Propagation of Banana

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Ex 1. Aseptic culture techniques for establishment and maintenance of cultures

Principle :

Maintenance of aseptic environment:

All culture vessels, media and instruments used in handling tissues as well as the explants must be sterilized. The importance is to keep the air surface and floor free of dust. All operations are carried out in laminar air-flow, a sterile cabinet. Infection can be classified in three ways:

1. The air contains a large quantity of suspended microorganisms in the form of fungal and bacterial spores.
2. The plant tissue is covered with pathogens on its surface.
3. The human body (a skin, breathe etc) carries several microorganisms.
4. In general, the methods of elimination of these sources of infection can be grouped under different categories of sterilization procedures:
5. Preparation of sterile media, culture vessels and instruments (sterilization is done in autoclave)
6. Preparation of sterile plant growth regulators stocks (by filter sterilization)
7. Aseptic working condition
8. Explants (isolated tissues) are sterilized using chemical sterilants, e.g. $HgCl_2$ and NaOCl.

Sterilization: It follows that all the articles used in the plant cell culture must be sterilized to kill the microorganisms that are present.

A. Steam or Wet sterilization (Autoclaving): This relies on the sterilization effect of super-heated steam under pressure as in a domestic pressure cooker. Most instruments/ nutrient media are sterilized with the use of an autoclave. The standard conditions for autoclaving have a temperature of 121ºC and a pressure of 15 psi (Pounds per square inch) for 15 minutes to achieve sterility. This figure is based on the conditions necessary to kill thermophilic microorganisms. The time taken for liquids to reach this temperature depends on their volume. It may also depend on the thickness of the vessel.

Precautions:

1. Excessive autoclaving should be avoided as it will degrade some medium components, particularly sucrose and agar breakdown under prolonged heating.
2. At the bottom of the autoclave the level of water should be verified.
3. To ensure that the lid of the autoclave is properly closed.
4. To ensure that the air- exhaust is functioning normally.
5. Not to accelerate the reduction of pressure after the required time of autoclaving. If the temperature is not reduced slowly, the media begin to boil again. Also the medium in the containers might burst out from their closures because of the fast and forced release of pressure.
6. Bottles, when being autoclaved, should not be tightly screwed and their tops should be loose. After autoclaving these bottles are kept in the laminar air-flow and the tops of these bottles are tightened on cooling.

B. Filter sterilization: Some growth regulators like amino acids and vitamins are heat labile and get destroyed on autoclaving with the rest

of the nutrient medium. Therefore, it is sterilized by filtration through a sieve or a filtration assembly using filter membranes of 0.22 µm to 0.45µm size.

C. Laminar Airflow Cabinet: This is the primary equipment used for aseptic manipulation. This cabinet is used for horizontal air-flow from the back to the front, Air is drawn in electric fans and passed through the coarse filter and then through the fine bacterial filter (HEPA). HEPA or High Efficiency Particulate Air Filter is an apparatus designed such that the air-flow through the working place flows in direct lines (i.e. laminar flow). Before commencing any experiment it is desirable to clean the working surface with 70% alcohol.

Ex 2. Preparation of stock solutions of MS (Murashige & Skoog, 1962) basal medium and plant growth regulator stocks.

Principle:

The basal medium is formulated so that it provides all of the compounds needed for plant growth and development, including certain compounds that can be made by an intact plant, but not by an isolated piece of plant tissue. The tissue culture medium consists of 95% water, macro- and micronutrients, vitamins, aminoacids, sugars. The nutrients in the media are used by the plant cells as building blocks for the synthesis of organic molecules, or as catalysts in enzymatic reactions. The macronutrients are required in millimolar (mM) quantities while micronutrients are needed in much lower (micromolar, µM) concentrations. Vitamins are organic substances that are parts of enzymes or cofactors for essential metabolic functions. Sugar is essential for *in vitro* growth and development as most plant cultures are unable to photosynthesize effectively for a variety of reasons. Murashige & Skoog (1962) medium (MS) is the most suitable and commonly used basic tissue culture medium for plant regeneration.

Plant growth regulators (PGRs) at a very low concentration (0.1 to 100 µM) regulate the initiation and development of shoots and roots on explants on semisolid or in liquid medium cultures. The auxins and cytokinins are the two most important classes of PGRs used in tissue culture. The relative effects of auxin and cytokinin ratio determine the morphogenesis of cultured tissues.

Materials: Amber bottles, Plastic beakers (100 ml, 500 ml and 1000 ml), Measuring cylinders (500 ml), Glass beakers (50 ml), Disposable syringes (5 ml), Disposable syringe filter (0.22 µm), Autoclaved eppendorf tubes (2 ml), Eppendorf stand, Benzyl-aminopurine (BAP), Naphthalene acetic acid (NAA)

MS Nutrients Stock Solutions: Nutrient salts and vitamins are prepared as stock solutions (20X or 200 X concentrations of that required in the medium) as specified. The stocks are stored at 4ºC. The desired amount of concentrated stocks is mixed to prepare 1 liter of medium.

MS major salts	mg/L medium	500 ml stock (20X)
1. NH_4NO_3	1650 mg	16.5 gm
2. KNO_3	1900 mg	19 gm
3. $Cacl_2.2H_2O$	440 mg	4.4 gm
4. $MgSO_4.7H_2O$	370 mg	3.7 gm
5. KH_2PO_4	170 mg	1.7 gm

MS minor salts	mg/L medium	500 ml stock (200X)
1. H_3BO_3	6.2 mg	620 mg
2. $MnSO_4.4H_2O$	22.3 mg	2230 mg
3. $ZnSO_4.4H_2O$	8.6 mg	860 mg
4. KI	0.83 mg	83 mg
5. $Na_2MoO_4.2H_2O$	0.25 mg	25 mg
6. $CoCl_2.6H_2O$	0.025 mg	2.5 mg
7. $CuSO_4.5H_2O$	0.025 mg	2.5 mg

MS Vitamins	mg/L medium	500 ml stock (200X)
1. Thiamine (HCl)	0.1 mg	10 mg
2. Niacine	0.5 mg	50 mg
3. Glycine	2.0 mg	200 mg
4. Pyrodoxine (HCl)	0.5 mg	50 mg

Iron, 500ml Stock (200X)

- Dissolve 3.725gm of Na_2EDTA (Ethylene diamine tetra acetic acid, disodium salt) in 250ml dH_2O. Dissolve 2.785gm of $FeSO_4.7H_2O$ in 250 ml ddH_2O
- Boil Na_2EDTA solution and add to it, $FeSO_4$ solution gently by stirring.

Plant Growth Regulator Stock: The heat-labile plant growth regulators are filtered through a bacteria-proof membrane (0.22 µm) and added to autoclaved medium after it has cooled enough (less than 60ºC). The stocks of plant growth regulators are prepared as mentioned below.

Plant Growth Regulator	Nature	Mol. Wt.	Stock (1mM)	Soluble in
Benzyl aminopurine (BAP)	Autoclavable	225.2	mg/ml	1N NaOH
Naphtalene acetic acid (NAA)	Heat labile	186.2	mg/ml	Ethanol
Indole-3-butyric acid (IBA)	Heat labile	203.2	mg/ml	EtOH/ 1N NaOH

The desired amount of plant growth regulators is dissolved as above and the volume is raised with double distilled water. The solutions are passed through disposable syringe filter (0.22 µm). The stocks are stored at –20ºC.

Ex 3. Micropropagation of Banana through shoot tip culture

Principle: Plant cells and tissues are totipotent in nature i.e., every individual plant cell or tissue has the same genetic makeup and capable of developing along a "programmed" pathway leading to the formation of an entire plant that is identical to the plant from which it was derived. The totipotency of the plant cells and tissues is the basis for *in vitro* cloning i.e., generation or multiplication of genetically identical plants in *in vitro* culture.

Micropropagation is used commercially to asexually propagate plants. Using micropropagation, millions of new plants can be derived from a single plant. This rapid multiplication allows breeders and growers to introduce new cultivars much earlier than they could by using conventional propagation techniques, such as cuttings. Micropropagation also can be used to establish and maintain virus-free plant stock. This is done by culturing the plant's apical meristem, which typically is not virus-infected, even though the remainder of the plant may be. Once new plants are developed from the apical meristem, they can be maintained and sold as virus-free plants.

Micropropagation differs from all other conventional propagation methods in that aseptic conditions are essential to achieve success. The process of micropropagation can be divided into four stages:

1. **Initiation stage:** A piece of plant tissue (called an explant) is (a) cut from the plant, (b) disinfested (removal of surface contaminants), and (c) placed on a medium. A medium typically contains mineral salts, sucrose, and a solidifying agent such as agar. The objective of this stage is to achieve an aseptic culture. An aseptic culture is one without contaminating bacteria or fungi.
2. **Multiplication stage:** A growing explant can be induced to produce vegetative shoots by including a cytokinin in the medium. A cytokinin is a plant growth regulator that promotes shoot formation from growing plant cells.

3. **Rooting or preplant stage:** Growing shoots can be induced to produce adventitious roots by including an auxin in the medium. Auxins are plant growth regulators that promote root formation. For easily rooted plants, an auxin is usually not necessary and many commercial labs will skip this step.
4. **Acclimatization:** A growing, rooted shoot can be removed from tissue culture and placed in soil. When this is done, the humidity must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting.

Materials: Beakers, Measuring cylinders, Conical flasks, Cotton plugs, Myoinositol, Sucrose, BAP (1mM stock), Agar Agar, Forceps, Blade Holder (No.3), Sterilzed blades (No.11), NAA (1 mM stock), Micropipettes, sterilized microtips, petridishes.

The shoot multiplication medium for Banana is MS basal + BAP (3 mg/l) + NAA (0.5 mg/l)

Preparation of MS medium (1000 ml)

- MS Major (20X) 50 ml
- MS Minor (200X) 5 ml
- MS Vitamin (200X) 5 ml
- Iron (200X) 5 ml
- Myoinositol 100 mg
- Sucrose 30 gm (3%)

1. Add BAP at this stage (Calculate, how much to add)
2. Make final volume to 1000 ml by double distilled water
3. Set pH at 5.8
4. Add agar agar 8 gm/L (0.8%), melt the agar agar in microwave oven
5. Sterilize the media at 15 psi/121^0 C for 15 minutes
6. After autoclaving, gently swirl the medium to mix the agar. When the agar is completely dissolved and mixed, the medium should appear clear and not turbid.
7. Add filter sterilized IBA (desired amount, calculate) once the temperature of the medium cools down to 60^0 C.
8. Dispense the medium to sterilized Petridishes (25 ml medium/plate)

Preparation and inoculation of explant: The 2-3 months old young, healthy suckers are selected for shoot tip explants. The adhering soil and dirt is removed. Remove roots and prepare rhizome of 3-5 cm with 2-4 inches of suckers, than wash thoroughly under tap water with Tween 20 for 10-15 min. There after dip the plant material in a solution of ascorbic acid 100 mg/lit and citric acid 150 mg /lit for one hour. Sterilize the explants using 0.1% $HgCl_2$ for 7 min. subsequently the explants are washed gently three times with sterile DDH_2O (double distilled water) in aseptic condition under laminar flow. Shoot tips of 1-2 cm are excised and placed the rhizome pieces on MS medium, and incubated it at 25±2°C temperature and 65-70 % RH in dark for 7-10 days, for plant regenerations.

Multiplication of shoots: Shoot multiplication is carried out on MS medium containing 3-8mg/l of BAP combined with 0.2-0.5mg/l of IBA. All cultures are maintained at temperature of 25±2°C under 16h photoperiod regime at 3000 lux and 70% RH.

Hardening of regenerated plantlets: Transfer the rooted plantlets to mixture of sand and FYM (3:1)/ Vermiculite to a poly tunnel under poly house at 25-30°C and 85-90% RH for 21-25 days. Plant the acclimatized plantlets in to polybags containing sand, soil and FYM (1:1:1) under net house conditions, after 3-4 weeks transfer polybags to open for field plantation.

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