Manual

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Soil, Water and Plant Analysis (NRM-221)

Dr. Rahul Chopra

Assistant Professor

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DEPARTMENT OF NATURAL RESOURCE MANAGEMENT

COLLEGE OF HORTICULURE AND FORESTRY

AGRICULTURE UNIVERSITY, KOTA, RAJASTHAN

Chapter 1

Methods of soil, water and plant sampling and processing for analysis

Soil sampling and processing

Introduction:- The importance of having a true representative sample can be very well realized from the fact that only a minute fraction of huge soil mass of the field is actually used for the analysis in the laboratory to find out the quantity of essential nutrients available to plants and other relevant physical and chemical characteristics. Therefore, while collecting soil samples the following aspects should be carefully considered. The soil samples collected should be representative of the area. A field can be treated as single sampling unit if it is appreciably uniform in all respects. Variation in slope, colour, texture, crop growth and management practices should be taken in to account and separate set of composite soil samples should be collected from each unit of such area.

The main purpose for which samples collected are:

a. Soil fertility evaluation and fertilizer recommendation.

b. Reclamation of problematic soils.

c. Plantation of orchards.

Tools and materials required :-

1. Soil auger, tube auger, spade, pick-axe, khurpi.
2. Bucket or tray.
3. Paper tages (Labels).
4. Information sheet
5. Cloth bags (alternatively polythene bags).
6. Ball point pen or copying pencil

Sampling for fertility evaluation and fertilizer recommendation

For soil fertility point of view, normally the samples are taken from the plough layer i.e., 0-15 cm depth. This is applicable for the fields growing cereals and other crop. In case of deep-rooted crops and under dry farming conditions, it may be necessary to obtain samples from different depths (or layers) of soil. For collecting proper soil samples following steps should be kept in mind:

1. Divide the field into small areas so that each sample represents an area of approximately 1 hectare.

2. A sample should be collected separately from areas which differ in soil colour or past management, e.g., liming, manuring, fertilization, cropping pattern etc.

3. Scrap away the surface litter and insert soil auger or sampling root to a plough depth (about 15 cm). Take at least 15 samples randomly distributed over each area and place them in a clean bucket. A spade or khurpi can be very well used if auger is not available.

4. If a spade or khurpi is used for taking samples, then dig a V-shaped hole to a plough depth and cut 1.5 cm thick slice of soil from top to bottom of the exposed face of the V-shaped hole and collect soil in a clean bucket.

5. Thoroughly mix the soil samples collected from 15 or more spots in a bucket.

6. Collect only ½ to 1 kilogram soil and discard remaining soil samples by quartering.

7. Quartering is done by dividing the thoroughly mixed soil in to four equal parts and discarding two opposite quarters. Remix the remaining two quarters and again divide it into four parts and reject two of them, repeat this procedure until about one half kilogram of soil is left.

Sampling for soil reclamation

For reclamation purpose the samples should be drawn to the plough layer but the salt crusts (visible or suspected) on the soil surface should be sampled separately. On saline and alkali soils, samples can be taken by either using a soil auger or digging a 90 cm deep pit. The samples should be collected as follow:

1. Make one side of the pit vertical (sun facing side) and put mark on it at 15, 30, 60 and 90 cm depth from the surface.

2. Hold a suitable container at 15 cm mark and scrap a uniform slice of soil from the surface down to this mark and collect about 500 gram of the soil sample. Transfer the soil sample to a cloth bag and mark it as 0-15 cm. Similarly, collect 500 gram soil sample from each layer, i.e. 15-30, 30-60 and 60-90 cm and put them separately in three cloth bags and then after dry in shade.

3. Take a separate sample of the surface crust also, if any.

4. Prepare two labels for each sample showing the depth from samples has been taken, name of farmer, name of village, exact location of the field, conditions and growth of crop if any.

5. Put up one label inside the bag and the other on the bags. Label should be

written with a copying pencil/ball pan.

6. Information sheet may also be prepared if necessary as given in soil sample information sheet.

7. Send the sample along with information sheet to be nearest soil testing laboratory.

Precautions

1. Do not draw any sample from the extreme corners of the field, area recently manured or fertilized, old bounds and marshy spots.

2. Avoid sampling from furrows, acidic or alkaline pockets.

3. Keep the sample in a bag and tag it properly.

4. Do not take less than 0.5 kg of a composite sample.

5. Sampling should be done from a uniform piece of land.

6. If there is a hard pan in the pit, it should be sampled separately and also note down its depth and thickness.

Sampling for orchard plantation

For horticultural plants, the samples may be taken from different depth or layer depending upon the root penetration of plants. The success of fruit tree plantation depends upon the physico-chemical properties and fertility status of sub-soil layers. Therefore, it is necessary to test soil before fruit tree plantation. Soil samples for plantation are to be taken as follows:

1. Dig a pit 1.80 meter deep and make its one side vertical, put marks at 15, 30, 60, 90, 120, 150 and 180 cm depths from the surface.

2. Collect samples separately from 0-15, 15-30, 30-60, 60-90, 90-120, 120-150 and 150-180 cm depths in the same way that of saline alkali soils.

3. In case there is a hard pan in the pit, sample it separately and note down its depth and thickness.

4. Pack the soil samples depth wise in separate cloth bags.

5. Put up label on each cloth bags indicating the depth, name of farmers, name of village, location of the field etc.

6. Send the samples to nearest soil testing laboratory along with detailed information.

Preparation of samples for analysis

Drying: Wet soil sample should not be stored as changes may occur in the chemical nature of certain ions and organic matter. Samples are generally air dried at temperature (25-35 0C) and

relative humidity (20-60%) then after are stored. Fresh samples from the field without any drying are required. For certain determinations such as ammonium and nitrate N, exchangeable K, acid extractable P and ferrous iron fresh sample from the field without any drying are required. Results of soil analysis are expressed on oven dry weight basis. This necessitates determination of moisture percentage by drying a small sample in an oven at 105^{0C} for 2 hours.

Sieving: Field moist samples prior to drying can be made to pass through a 6 mm sieve (about 4 mesh per inch) by rubbing with fingers. The practice seems of much advantage in case of heavy soils. Soil in the right moisture condition can be passed through a 2 mm sieve (about 10 mesh per inch). The common practice of sieving a portion of the gross sample through a 2 mm sieve and discarding the rest is undesirable as it increase the concentration of most of the elements involved in soil fertility. When the gravels in the soil exceeds 2% limit over a 2 mm sieve their exact percentage should be recorded.

Grinding: A roller, rubber pestle in an agate mortar, or a motorized grinder is commonly used. Crushing of the gravel or primary sand particles should be avoided for heavy soils, it is better to pass these through a 2 mm sieve before allowing them to get completely air dried.

Mixing: Sample should be thoroughly mixed by rolling procedure. Place the dried ground and sieved sample on a piece of cloth. Hold all the four corners of the cloth and then up the one corner and down the other corner across the sample alternatively. Now repeat the process in the reverse direction to roll the soil from one corner to another. Continue this until thorough mixing is assured.

Storage: Store the soil in paper carton (soil sample box) using a polythene bag as in inner lining. Label the carton mentioning cultivators name, plot number, date of sampling and initials.

Water sampling and processing

Methods of Water Sample Collection

A representative sample (500 ml) is collected in glass or polyethylene bottle which should be properly washed/rinsed with the same water which is being sampled. The floating debris or any other contaminant should be avoided while collecting the samples. After proper labeling, such as source of water, date of collection and the type of analysis required, the sample should be sent to the laboratory without undue delay.

Some of the anions like SO_4 and NO_3 may be quite low in irrigation waters. Hence, large volume of sample has to be first concentrated by evaporating to about 100 ml to obtain their detectable amounts.

Plant sampling and processing

Proper sampling requires that a specific plant part be taken such as a particular leaf, group of leaves or portion of the plant. Instructions also include number of individual parts, as well as the number of plants to sample. This will ensure that a sufficient quantity of plant tissue is submitted for analysis and that the collected sample is statistically representative of the area under study.

• When sampling mixed stands, particularly forages and pastures, separate plant species. Similarly, the sample should be of only leaves or petioles or whole tops and not mixtures. • When no specific sampling instructions are given for a particular crop, the general rule of thumb is to sample the uppermost recently mature leaves.

• Young emerging leaves, older mature leaves and seed are not usually suitable plant tissues for analysis since they do not ordinarily reflect the general nutrient status of the whole plant.

• The recommended time to sample usually occurs just prior to the beginning of the reproductive stage for many plants. However, sampling earlier or even later than the specified time may be recommended for specific plants or circumstances.

• Sample plants that are showing a suspected nutrient deficiency symptom at the time or shortly after the visual symptoms appear. Do not sample or include in a sample plants under a nutrient stress for an extended period of time, dead plant tissue or plants or tissue mechanically injured, diseased, or insect-damaged.

Washing to Remove Contaminants

Avoid dusty or soil-covered leaves and plants whenever possible. When leaves are dusty, brush or wipe with a damp cloth to remove the contaminants. If this is not effective or when leaves are covered with spray materials, wash in a mild detergent solution (0.30 percent) and rinse in running water to remove attached substances. Do not prolong the washing procedures or allow the plant material to stand in either the washing or rinsing baths. Wash and rinse briskly. Wash leaves which have been sprayed with nutrient solutions while they are still fresh. If iron is of primary interest, wash leaves regardless of their outward appearance. Wash whole plants sampled shortly after emergence to remove soil particles that are frequently attached to the new tissue.

Packaging Plant Tissue

• Air-dry plant tissue samples before shipment to the laboratory. Package samples in clean paper bags or envelopes for mailing to the laboratory. Never place fresh samples in a plastic bag.

What Not to Sample

Do not include diseased or dead plant material in a sample. Do not sample or include plants or leaf tissue that have been damaged by insects or mechanically injured in a sample. When whole plants are sampled, remove the roots and wash the upper portion to remove soil particles. Do not sample plants, which have been stressed extensively by cold, heat, moisture deficiency, or by excess moisture. Examine both the below ground as well as the above ground portion of the plant. The presence of nematodes or roots damaged by other insects or diseases should preclude the need to sample.

Laboratory Processing

Sample preparation is critical in obtaining accurate analytical data and reliable interpretation of plant analysis results. Proven procedures must be followed during handling in the laboratory, decontamination, drying, grinding and mixing, and storage. Such preparatory procedures enhance the accuracy and reliability of the analytical results.

Handling in the laboratory

 As soon as the plant samples are received at the plant preparation laboratory, they should be checked with the accompanying information list. Information regarding samples should be entered in a register and each sample be given a laboratory number.

 Keep plant samples refrigerated until cleaning. Take care that fermentation does not occur.

Decontamination

Decontamination procedures involving washing and rinsing should only be used for fresh, fully-turgid plant samples. After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

A. Reagents and Apparatus

 Deionized water

 0.1 to 0.3 % detergent solution (non-phosphate)

 Medium-stiff nylon bristle brush

 Plastic containers suitable for washing and rinsing tissue samples

B. Cleaning processing

 A preliminary dry-wiping can be done if the plant sample is very dirty.

 If the plant samples are too dirty and a dry-wiping is not possible, washing through the nylon bag can be done.

 The samples must be properly cleaned, but no part of it should be under water for more than a few seconds.

 Cleaning plant tissue to remove dust, pesticide and fertilizer residues, normally by washing the plants with DI water or with 0.1 – 0.3 % P-free detergent (like HCl 1%), followed by DI water.

 Rinse each portion of the plant sample into a bath of DI water, into which it is plunged, agitated, and immediately withdrawn. Change the water and repeat the rinsing. Dry by shaking vigorously by hand.

 Plant samples for soluble element determination may not be washed, particularly for long periods. However, **plant samples for total Fe analysis must be washed**.

 Excessive washing is worse than no decontamination since soluble elements, including B, K, and N, are likely to leach from the tissue.

 The wash and rinse periods should be as short as possible to avoid danger of N, B, K, and Cl leaching from the tissue.

Drying

Water is removed from plant tissue to stop enzymatic reactions and to stabilize the sample. Enzymes present in plant tissue become inactive at temperatures above 70 oC. As a result, air-drying may not stabilize samples and prevent enzymatic decomposition. Samples should, therefore, be properly dried as soon as possible after taking the sample. Some technical guidelines are as follows:

 The plant sample material should be evenly and thinly spread in a container.

 Place containers in well-ventilated drying oven.

 If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis.

 Drying time required will vary. Dry to constant weight.

 The original condition and sample size will affect drying time.

 The drying temperature should not exceed 70^{oC}, because higher temperatures may cause volatilization loss.

 Drying at temperatures less than 70^{oC} may not remove all combined water and may result in poor homogenization and incorrect analytical results.

 Drying temperatures above 70^{oC} may result in thermal decomposition and reduce dry weight.

 A drying time of 24 hours may be sufficient in normal conditions.

 Drying times longer than 24 hours may be required depending on the type and number of plant samples in the dryer.

 Quick drying of a limited number of samples can be done using a microwave oven and the drying process is closely monitored.

Grinding and Mixing

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction.

A. Apparatus

 Standard mills equipped with 20, 40, and 60-mesh screens and stainless steel contact points.

 Tecator Cyclotec sample mill (standard equipped with a 1-mm sieve) or equivalent high-speed grinder.

 Medium bristle brush.

 Vacuum system.

B. Procedure

 After drying, samples should be ground to pass a 1.0-mm screen (20 mesh) using the appropriate Wiley mill. A 20-mesh sieve is adequate if the sample aliquot to be assayed is >0.5 g. However, if the sample aliquot to be assayed is less than 0.5 g, a 40-mesh screen should be utilized.

 After grinding, the sample should be thoroughly mixed and a 5 to 8 g aliquot withdrawn for analyses and storage.

The table below shows the sampling guide for various crops

Crop	When to Sample	Where to Sample	Qunatity
Field Crops			
Alfalfa	Early bloom stage	Upper 1/3 of plant	12 - 30
Canola	Before seed set	Recently mature leaf	60 - 70
Clover	Before bloom	Upper 1/3 of plant	30 – 40
Corn / Sweet Corn	Seedling stage Before heading Tasseling to silking	All above ground portion Upper 4 leaves Opposite or below ear leaf	15 – 20 12 – 20 12 – 20
Grasses/forage mixes	Stage of best quality	Upper 4 leaves	30 – 40
Peanuts	Before / at bloom	Recently mature leaf	40 – 50
Small grains (barley, wheat, oat, rye, rice)	Seedling stage Before heading	All above ground portion Upper 4 leaves	25 – 40 25 – 40
Sorghum	Before/at heading	2nd leaf from top	23 – 30
Soybeans	Before / at bloom	recently mature leaf	20 – 30
Sugarbeets	Midseason	recently mautre leaf at center of whorl	15 - 20
Sunflower	Before heading	Recently mature leaf	20 – 30
Tobacco	Before bloom	Recently mature leaf	10 - 15
Vegetable crops			
Asparagus	Maturity	Fern from 18 – 30 inches up	10 – 30
Beans	Seedling stage Before / at bloom	All above ground portion Recently mature leaf	20 – 30 20 – 30

Source- Soil and Plant analysis. Agrimoon.com

Soil Air: Composition and Factors

Composition of Soil Air:

The gaseous constituents of soil atmosphere are as important for crop growth as nutrients and water. Aerobic respiration in roots, micro-organisms and soil fauna involve the continuous consumption of O_2 and the evolution of CO_2. The soil air contains a variety of gases like O_2, N_2, Ar, CO_2 and water vapour etc. The composition of soil air, though quite variable is similar to that of the atmospheric air, but differs in one respect.

The content of CO_2 in soil air may vary from 10-10,000 times. The composition of soil air changes with the soil conditions, locations and the season. Although the composition of atmospheric air also changes with the various locations. Soil air contains a much more CO_2 and less O_2 than the atmospheric air. At the same time, than the atmospheric air.

TABLE 5.1. Composition of Soil and atmospheric air

Types of air	*Percentage by volume*		
	Oxygen	*Carbon dioxide*	*Nitrogen*
Soil air	20.60	0.25	79.2
Atmospheric air	20.96	0.03	79.0

Factors Affecting the Composition of Soil Air:

I. Nature of the Soil – Sandy Soils have macropore, as a result of which, aeration is very good in that soil. The soils that are water-logged contain small amount of oxygen as the pore space is filled with water immediately after a heavy rains or irrigation. The surface soil contains more macropore than the sub-soil. As a result, gaseous exchange is found to be more in surface soil than the sub soil. The oxygen percentage of Soil air varies with the depth of the soil and this is true in case of carbon dioxide also.

II. Soil organic matter – Soil organic matter is decomposed by microorganism present in the soil. Microbiological decomposition leads to the production of carbon dioxide and its content increases in the soil air. Hence soil rich in organic matter contains higher percentage of carbon dioxide.

III. Season – Season and temperature also influences the carbon dioxide content of the soil. The activity of soil micro-organism increases at high temperature during summer month which results in higher production of carbon dioxide. The composition of soil air shows marked

seasonal variation, the intensity of which is affected by the texture of the soil and position of water table.

IV. Soil moisture – The oxygen content of a soil decreases when the macropores are filled with water. But when the soil is artificially drained again, the macropores are filled with air and the oxygen content of soil increases.

V. Vegetation – Soils on which crops are grown contain more carbon dioxide than fallow land as a result of respiration of plant roots. The plant takes the soil oxygen and releases carbon dioxide. As a result, the carbon dioxide content of the cropped land increases near the root zone of the plant.

VI. Soil Compaction- Compact soil suffer from poor aeration. The air of sub soil contains more carbon dioxide and less oxygen than surface soil.

VII. Soil Temperature- In general, higher the temperature increase the production of carbon dioxide in soil air.

VIII. Water logging- It affects gaseous diffusion. Since diffusion is related to the non-capillary pore space, such pore spaces are closed under water logging conditions. This increase concentration of carbon dioxide.

Gaseous Exchange between Soil and Atmosphere:

Gaseous exchange between soil and atmosphere takes place by diffusion and mass flow as follows:

I. Mass Flow:

Mass flow of air is apparently due to total pressure differences between the atmosphere and the soil air. The pressure difference arises from such meteorological factors, as changes in soil temperature, barometric pressure, wind movements and also rainfall and irrigation. The variation in soil temperature causes changes in the temperature of Soil air. When the soil air gets heated during the day, it expand and move out of the soil pore space into the atmosphere.

When the soils begins to cool, the soil air contracts and the atmospheric gases enter into the soil. When the barometric pressure increases, the volume of soil air decreases which facilitate the entry of atmospheric air to the soil and decrease in barometric pressure makes the reverse flow from soil to atmosphere. The soil pores are filled with water of rainfall or irrigation and causes poor aeration. But when drainage occurs, the pores again filled with air from the atmosphere.

II. Diffusion:

Most of gaseous interchange in soils occurs by diffusion. It is the process by which each gas tends to move in the space occupied by another as determined by the partial pressure of each gas. The partial pressure of a gas in a mixture is simply the pressure the gas would exert if it was present alone in the volume which has been occupied by the mixture of gases. Atmospheric and soil air contains a number of gases such as nitrogen, oxygen, carbon dioxide etc. each of which exerts its own partial pressure in proportion to its concentration.

The movement of each gas is regulated by the partial pressure under which it exists. The atmosphere contains more oxygen than soil pore space and soil contains more carbon dioxide than the atmosphere. So the partial pressure of oxygen is higher in the atmosphere than in the soil pore space and the partial pressure of carbon dioxide is higher in soil pore space than in atmosphere even though the total pressure in the atmosphere and soil pore spaces may be the same. Due to which oxygen moves in the soil and carbon dioxide moves out of the soil.

Fix law of diffusion-Diffusion of gases takes place under the concentration gradient.

Q= -D dc/dx

Where q is amount of gases diffusing in unit time across a plane of unit area, D, the diffusion coefficient, is equal to q when dc/dx, concentration gradient, is unity. Since the gradient is in the direction of lower concentration, dc/dx is negative.

Soil Aeration:

Soil aeration is a mechanism of rapid exchange of oxygen and Carbon dioxide between the Soil pore space and the atmosphere in order to prevent the deficiency of oxygen and toxicity of Carbon dioxide in the Soil air.

Causes of Poor Aeration:

The causes of Poor aeration are as follows:

(i) Compaction of Soil – Compact of finer texture suffers from poor aeration.

(ii) Water logging condition – Water logging condition hampers the aeration.

(iii) Addition of decomposable organic matter – Soil microorganisms decompose the organic matter. They need oxygen for their respiration and release carbon dioxide. This condition hampers the soil aeration.

Soil air is also useful in increasing the nutrient availability of the soil:

(i) By breaking down the insoluble minerals into soluble salts,

(ii) By decomposing plants and animals remains and

(iii) By bringing about nitrifying and nitrogen fixing process of bacteria.

Measuring Oxygen Diffusion Rate (ODR)

Oxygen Diffusion Rate (ODR) - **The rate at which O_2 in soil air is replenished**. ODR decreases with soil depth. Lemon and Erickson (1952) devised a method for measuring oxygen diffusion in the soil system with the help of platinum micro electrode. Critical value of ODR for plant is **$20x10^{-8}$ g cm^{-2} min^{-1}.**

Principles of Measuring Oxygen Diffusion Rate (ODR):

When a certain electric potential is applied between a platinum electrode inserted into the soil and a reference electrode, oxygen (O_2) is reduced at the platinum electrode surface. The general reaction taking place at the platinum micro electrode surface in the reduction of O_2 is in two steps involving two electrons in each step.

The reactions in two different media are:

In acid soils :

$$O_2 + 2H^+ + 2e^- = H_2O_2$$
$$H_2O_2 + 2H^+ + 2e^- = 2H_2O$$
$$O_2 + 4H + 4e^- = 2H_2O$$

In neutral or alkaline soils :

$$O_2 + 2H_2O + 2e^- = H_2O_2 + 2OH^-$$
$$H_2O_2 + 2H^+ + 2e^- = 2H_2O$$
$$O_2 + 2H_2O + 4e^- = 4OH^-$$

An electric current flows between the two electrodes and is proportional to the rate of O_2reduction. The rate of O_2 reduction is in turn related to the rate at which it diffuses to the electrode. The increase of current with potential continues until it is limited by the rate at which O_2 can diffuse from the soil environment to the electrode. So at the higher potentials, the reaction rate is limited by an extrinsic factor and the current becomes somewhat independent of the potential.

A second reaction starts at a potential of 0.7 or 0.8 volt. The second rise is caused by the reduction of ionic hydrogen to molecular hydrogen. Because the current in the plateau region is limited by the maximal rate at which O_2 can diffuse to the platinum micro-electrode surface resulting difficulty in measuring the movement of O_2.

Potential between 0.55 and 0.75 volt can be used for standard recommendation size of the electrode, however, is also an important factor and so 25 gauge electrode usually gives accurate measurement of ODR value over a wide range of soil moisture.

Theoretically, ODR increases with decreasing soil moisture. It has been also found that an increase in ODR as moisture decreased to a point beyond which ODR decreases with decreasing moisture content. This may be due to incomplete electrode wetting. Excess concentration of sulphur in the soil very often showed poisoning and that may be avoided by removing and reinstalling the electrode in the soil.

Oxygen diffusion meter

The oxygen diffusion meter measures the mobility of oxygen in the soil that is important for the availability of oxygen for plants.

The method: measuring the electric current required for the reduction of all oxygen present at the surface of a cylindrical Pt-electrode in the soil. The flow of oxygen through the air-filled pores and the water film on the electrode is measured until the steady state is reached.

The Oxygen Diffusion Rate (ODR) probe (Pt-electrode) should be placed in undisturbed soil. To this purpose a hole is predrilled to a depth of approximately 10 mm above the measuring point, after which the probe is lowered and carefully pushed into the bottom of the auger-hole. It is advised to remove the electrode from the soil after a series of measurements in order to clean it. The meter provides a stabilized voltage between the ODR-probe and the Ag-AgCl-reference electrode.

In very dry soils only part of the electrode will be covered in water. This results in rising impedance between soil and electrode. In such a situation the meter can also be used to perform a redox-potential measurement.

The measuring system consists of a read-out unit with connecting facilities for three ODR-probes, one ODR-probe, one Ag-AgCl reference electrode, KCl-solution and a brass electrode. The reference electrode is used for measuring and checking the potential between the Pt-electrode and the soil. The brass electrode is used to close the electrical circuit. The measuring range for oxygen diffusion is 0 - 999 μA and for Redox 0 - 999 Mv (resolution resp. 1 μA and 1 mV). Accuracy +/- 3 μA and +/- 3 mV. Operating temperature between 0 and 50°C and an air humidity between 30 - 80%.

Use of Radio Tracer Techniques in Soil Fertility Evaluation

India is a leading producer of radioisotopes in the world. Production of radio-isotopes in the country had started with the commissioning of first research reactor named "APSARA" (Swimming Pool type 1 MW (th) Power) in 1956. Radioisotopes are used for determining the function of fertilizer in different plants. The tracer technique is used to study the rate and direction of movement of an element in a plant. For this a radioisotope of that element is injected in the ground near the plant. After a few days the plant is laid on a photographic paper to produce an autoradiograph. The dark areas in the radiograph show the positions reached by the element. This technique gives valuable information regarding the optimum season for fertilizing crops and for poisoning weeds.

Radioisotopes:

The isotopes having unstable nuclei are known as Radioisotopes. There radioactive atoms are unstable because certain combinations of neutrons and proton produced nuclei of latent instability. Specific average life time will be characteristic of each unstable combination. The disintegration of a given radioactive atom is a matter of chance conditioned only by a set of many requirements being completely fulfilled simultaneously. They disintegrate spontaneously at a characteristic decay rate.

Type of Radioisotopes

(i). Stable Isotope: - No disintegration and no radioactivity

(ii). Unstable Isotope: - Radioactive disintegration and emitted alpha, beta and gamma radiation. These unstable nuclei have excessive energy and this is released by their disintegration into stable forms of lower energy, the excess being released in the forms of radiations, mainly alpha (α), beta (β) and gamma (¥) and they are called radioactive.

Stable Isotopes in Agriculture

Stable isotopes are used in the same way as radioactive isotopes in soil/plant studies. Whereas

radioactive isotopes emit particles which are captured in photomultiplier tubes and counted stable isotopes are separated from each other by passing a gas containing them through a strong

magnetic field, which deflects them differentially according to their mass. The most common stable isotope used is ^{15}N but a large number of other stable isotopes are produced which are increasingly being used in agricultural studies (Table1).

Table1. Some useful stable isotopes in commercial production

Atomic Number	Isotopes
6	^{13}C
7	^{15}N
8	^{18}O
12	^{25}Mg
14	^{28}Si
16	^{33}S, ^{36}S
26	^{54}Fe
30	^{68}Zn
82	^{204}Pb

Nitrogen is one of the main limiting factors for plant growth. There are twelve isotopes of nitrogen, many with extremely short half lives. Of the radioactive isotopes only ^{13}N with a half-life of 9.97 minutes has been used mainly in plant nitrogen translocation experiments (Caldwell *et al*, 1984).

Use in soil Fertility Evaluation

Tracer technique :- Tracer technique is based on the assumption that if a plant derives a particular nutrient from the soil as well a fertilizer added to it, the amount available from soil in terms of a standard fertilizer can be calculate if that derived from the fertilizers is known. The latter is possible by using with the fertilizer a radioactive or stable isotope of the nutrient in the question. Some plants are grown in soil to which is an added a phosphatic fertilizer mixed with a very small amount of phosphate having radioactive ^{32}P. The soil originally contains a known amount of non radioactive phosphorus, so also does the bulk of phosphatic fertilizers, excepting the small amount of ^{32}P added deliberately. After a suitable period of growth, the plants are harvested and the total P as well as ^{32}P contents is determined.

Estimation of available nutrients in soil: Assessment of the available soil nutrient is of considerable importance in planning optimum fertilizer use. Out of several methods available to estimate the available soil nutrient, isotopic methods that have received attention in this field.

L Value: It was first suggested by Larsen, 1952 that if labeled phosphate fertilizers was added to a soil at different rates and plants were grown, the specific activity analysis of the plant material give a constant value, if the isotope dilution of added chemical was assumed to take place in the soil system. The reason for the constant value obtained is attributed to the equilibrium which will be reached between added phosphate and the exchangeable phosphate in soil. The quantity measured in this way is known as L value and this quantity also termed as "Labile" phosphorus of the labile pool. The L- value can be consider E-value in which the plant is being used as a means of sampling the solution upon equilibrium of added P with exchangeable soil P. with the added advantage that in this procedure the conditions are identical to those prevailing in the soil – plant system.

E-value: This method is directly application of the isotope dilution principle. The amount of nutrient in the soil at equilibrium with the same nutrient in the soil solution can be measured with E- value. Reaction can be depicted as fallow:

Surface ^{31}P + Solution ^{32}P - Surface ^{32}P + Solution ^{31}P

At equilibrium,

$$\frac{\text{Surface } ^{31}P}{\text{Surface } ^{32}P} = \frac{\text{Solution } ^{31}P}{\text{Solution } ^{32}P}$$

In this method, it is possible to measure both capacity and intensity factor of the same sample. The Evalue represents labile form of nutrient and represents the total amount of nutrient undergoing isotopic dilution.

A- Value: A- Value concept was developed by Fried and Dean 1952. When the plant is confronted with two source of a given nutrient, the plant absorb from each of these in proportion

to the respective amount available. The amount of available nutrient in soil to be determined in term of fertilizer standard is known as A- value.

Mathematically A-value can be expressed as:

$$A = B \frac{1-Y}{Y}$$

Where A = Amount of available nutrient in soil

B = Amount of applied fertilizer nutrient

Y = proportion of nutrient in the plant derived from the fertilizer nutrient.

Importance:

1. Radioisotopes are use to improve the quality and productivity of agricultural products as well as optimum utilization of fertilizaers without harmful effect to plants and mankind.
2. The radio labeled fertilizer has been used to study the uptake, retention and utilization of fertilizers.

Soil Microorganisms

Soil Organisms;

A. Soil Flora

a) Microflora: 1. Bacteria 2. Fungi, Molds, Yeast, Mushroom 3. Actinomycetes, Stretomyces 4. Algae eg. BGA, Yellow Green Algae, Golden Brown Algae.

1. Bacteria is again classified in I) Heterotrophic eg. symbiotic & non - symbiotic N_2 fixers, Ammonifier, Cellulose Decomposers, Denitrifiers II) Autrotrophic eg. Nitrosomonas, Nitrobacter, Sulphur oxidizers, etc.

b) Macroflora: Roots of higher plants

B. Soil Fauna

a) Microfauna: Protozoa, Nematodes

b) Macrofauna: Earthworms. moles, ants & others.

As soil inhabit several diverse groups of microorganisms, but the most important amongst them are: bacteria, actinomycetes, fungi, algae and protozoa.

Types of Soil Microorganisms:

Micro organisms are also classified based on their ability to grow in the presence or absence of molecular oxygen as aerobes and anaerobes.

Based on Temperature

- Psychrophiles
- Mesophiles
- Thermophiles
- Micro organisms are also classified based on morphology, shape, size, biochemical transformations they carryout.
- Soil organism are classified broadly soil flora and soil fauna.
- These again may be subdivided into micro and macro.
- Micro flora again classified into bacteria, actinomycetes, fungi and algae.

Bacteria

- Single celled.
- The cells may be rod shaped or spherical.
- The rods may be about 1μm wide and up to 3μm long and about 2μm in diameter.
- Bacteria are the most abundant group of micro organism in the soil.

- Their population in the soil is not uniform.

Classification of Bacteria

1. **Based on oxygen requirement**: Aerobic and anaerobic
2. **Based on temperature**: Facultative, Psychrophiles, Mesophiles, Thermophiles
3. **Based on their food preparation**: Autotroph, heterotrophy, chemoautotroph, obligate chemotrophs (prefer specific substrates), nitrobacter (nitrite as substrate), nitrosomonas (ammonia as substrate), thiobacillus (converts sulphur compounds to SO_4), ferrobacillus (converts ferrous to ferric)
4. **Based on symbiotic relationship**:

Symbiotic N fixers – Associated with a host plant; both the host and the bacteria get the benefit; fix atmospheric N.

Non symbiotic N fixers – Bacteria present without the association of a plant; but fix atmospheric N; symbiotic, non symbiotic and cellulose decomposers come under heterotrophs; nitrifiers, denitrifiers, nitrate formers and sulphur oxidizers are autotrophs.

Role of Bacteria

- Bacteria carryout the decomposition of organic matter and synthesis of humus.
- Enzymatic transformations are carried out by bacteria.
- Bacteria oxidize or reduce many chemical reactions such as N fixation.

Conditions affecting the growth of bacteria

- Oxygen
- Moisture
- Temperature
- Organic matter
- Exchangeable Ca and pH
- High Ca concentration and pH 6 – 8 optimum. Some bacteria function at pH < 3.0.
- Exchangeable Ca is more important than pH. The bacterial population may be 10^8 – 10^9/g.
- The biomass may vary from 450 – 4500kg/ha.

Actinomycetes

- Unicellular like bacteria. Have same size as bacteria.
- Filamentous and profusely branched.

- Mycelial threads are smaller than those of fungi.
- No nuclear membrane as in bacteria.
- Also called as filamentous.
- Sensitive to acid soils.
- Potato scab a disease due to actinomycetes can be controlled by lowering the soil pH by applying sulphur.
- Heterotrophic optimum temperature 25 -30^{0}C, pH 6.5 – 8.0.
- Actinomycetes are important for organic matter decomposition.
- Chitin and phospholipids are reduced to simple compounds.
- The aroma of freshly ploughed land at certain times of the year is probably due to actinimycetes as well as certain molds.
- Actinomycete population in soil exceed all other organisms except bacteria.
- Their proportion increases with soil depth. Their population and biomass are almost equal to that of bacteria.

Fungi

- Soil fungi may be parasitic or saprophytic.
- They possess filamentous mycelium composed of individual hyphae which are 5 – 20µm in diameter and several centimeters in length.
- Most fungi are heterotrophic and hence they depend on the organic matter content of the soil.
- They are dominant in acid soils some can tolerate a pH upto 9.0.
- Fungi are strictly aerobic.
- Fungi are classified into phycomycetes, ascomycetes, basidiomycetes and fungi imperfecti.

Fungi may also be classified as molds: Molds, Yeast, Mushrooms

Molds

- Molds are filamentous microscopic molds develop vigorously in all types of soils.
- In acid forest soils – decomposing organic matter.
- The common genera – mucor, fusarium and aspergillus.
- Their average population – 10 – 200 billion / m^2.
- In humus formation and aggregate stabilization molds are more important than bacteria.

- They continue to decompose complex organic substances after bacteria and actinomycetes have stopped function.

Yeast

- Yeast is a group of fungi which exist as an unicellular organism.
- Reproduce by fission or budding.
- Used as food supplement and also for the production of alcoholic beverages.
- Yeast is not common in soils.
- They produce several plant diseases.

Mushroom

- Mushrooms are present in forests and grasslands where there are ample moisture and organic residues.
- Some mushrooms are edible.
- Mushrooms are also not common in cultivated soils.
- Their fruiting body is above the ground.

Algae

- Algae are filamentous u – 10μmin diameter. Population in soil around 1 – 10 billion / m^2.
- Their mass in soil may be 50 – 600 kg/ha of furrow slice.
- Algae are photo autotrophs.
- They are divided into 4 general groups. Blue green, green, yellow green and diatoms.
- Blue green algae are numerous in rice soils.
- Blue green algae growing within the leaves of aquatic fern.
- Azolla can also fix atmospheric N.

SOIL FAUNA – Macro fauna – Earthworm

- About 1800 species of earthworms are known.
- There are 293 species in the genus pheretima. The common earthworms found in India are pheretima, P. elongator, Lampita mautritii etc.
- There population may vary from 125000 to 1000000/ha.
- They are active in monsoon season. The worms prefer organic matter as their food.
- They also consume larvae of small animals and bacteria mixed with earth.
- They ingest soil in large quantities which pass through the alimentary canal which has many grinding gizzard.

- The ingested soil and organic matter are ejected in the form of castings.
- They make crores of burrows which make the soil porous.
- Earthworms prefer warm (21^0C) well aerated soils. The cast have low C:N ratio.
- The burrows left in the soil increase aeration and drainage. They increase the size and stability of soil aggregates.
- The factors influencing the earth worm population and activity in the soil are availability of organic matter, soil pH, temperature and soil moisture.
- The biomass or live weight of earth worm may range from 110 – 1100 kg/ha.

Soil Animalia

Ants

- They have local effects. Some have ability to break down woody materials.
- Some ant produce mounds and some have underground nests.
- There is considerable turnover of the soil due to these.
- The ants and termites can modify soil structure and till the soil.

Nematodes

- Commonly called as thread worms or eelworms. Present in almost all soils.
- They are microscopic most of the nematodes are saprophytes.
- They may feed on other nematodes, bacteria, algae and protozoa.
- Heavy infestation may cause severe damage in vegetable crops.

Protozoa

- Single celled, larger than bacteria and are more complex.
- Soil protozoa may be amoeba, celiates and flagellates.
- Soil have more than 250 species. Live weight in soil ranges from 15 – 175 kg/ha.
- They cause a number of serious disease in animals and plants.
- They thrive well in moist and well drained soils and on the surface.
- They are not abundant in soils to play a major role in the organic matter decomposition and nutrient release.

Soil Macrofauna

Rotifers

- About 100 species have been studied. They thrive under moist and swampy lands.

- Activities are confined to peat bogs and wet areas of mineral soils.

Roots of higher plants

- Since the roots grow and die in the soil they supply food and energy for the soil microflora and fauna.
- They physically modify the soil as they push through cracks and make new openings.
- By removing moisture from the soil the plant roots create physical stress that stimulates soil aggregation.
- Plant roots exudates several chemicals which stabilize the soil structure.
- The decay and supply the material for the synthesis of humus.
- Root constitute about 15 – 40 % that of above ground crop.

Mycorrhizae

- The symbiotic association between numerous fungi and the roots of higher plants is called mycorrhizae which means fungus roots.
- These association increases the availability of several essential nutrients to plants especially from low fertile soils.
- This association provides sugars and other organic exudates from higher plants as food to the fungi.
- The fungi in turn provide an enhanced availability of several essential nutrients including P, Zn, Cu, Ca, Mg, Mn and Fe.
- There are two types of mycorrhizal associations. Ecto mycorrhizae and endo mycorrhizae.
- The vasicular arbuscular mycorrhizae (VAM) is the most important endo mycorrhizae.

Average Size of Microbes:

(a) Bacteria-0.5-3.0mm

(b) Fungi-1.5-10mm

(c) Protozoa-2-200mm

(d) Viruses-100-600 Nano m(0.002mm)

(e) MLOS-0.1-0.3mm

(f) Algae – 0.1mm (BGA) to several feet (higher algae)

Importance of Soil Microorganisms:

(Involved in nutrient transformation process)

1. Decomposition of resistant components of plant and animal tissue
2. Role in microbial antagonism
3. Participate in humus formation
4. Predator of nematodes
5. Surface blooming reduces erosion losses
6. Improve soil structure
7. Involved soil structure
8. Maintenance of biological equilibrium

Major Microbiological Processes in Soil and Microorganisms Involved

Aerobic decomposition of organic matter (OM)	:	*Trichoderma, Achromobacter, Streptomyces*
Aerobic decomposition of OM	:	*Clostridium*, methane bacteria
Symbiotic N_2 fixation	:	*Rhizobium spp.*
Asymbiotic N_2 fixation	:	*Azotobactor, Azospirillum and Clostridium*
Phosphorus solubilization	:	*Acitobactor, Trycoderma*, PSB (*Bacillus, Pseudomonas, Aspergillus*), PSM
Phosphorus absorption	:	VAM (Vascular Arbuscular Mycorrhyza)
Sulfer solubilization	:	*Thiobacillus*
Iron transformations	:	*Gallionella, Ferribacterium, Leptothrix*
Copper transformations	:	*Desulfovibrio, Clostridium, Escherichia*
Manganese transformations	:	*Aerobactor, Cladosporium, Corynebacterium*

Microorganisms Responsible for Mineralization of Nitrogen

Name of Process		*Mineralization*	*Responsible Microorganisms*
1. Aminization	:	Polypeptide → Amino acid	Bacillus, Pseudomonas
2. Amonificaion	:	Amino acid → Ammonium	Nitrobactor
3. Nitrification	:	Ammonium → Nitrate (NO_3^-)	Nitrosomonas
4. Nitrification	:	Nitrate → Nitrite (NO_2^-)	Nitrobactor
5. Denitrification	:	Nitrite → Nitrogen (N_2)	Pseudomonas

Rhizobium Stains for Biological Nitrogen Fixation in Different Crops

Rhizobium japonicum	:	Soybean, Cowpea, Groundnut
Rhizobium leguminosarum	:	Gram, Pea, Lentil, Sweet pea
Rhizobium meliloti	:	Alfalfa, Medicago, Melilotus, Trigonella
Rhizobium phaseoli	:	Rajma, Beans
Rhizobium trifoli	:	Berseem (Egyptian clover)
Rhizobium lupini	:	Lupinus, Orinthopus

Important terms:

(a) Rhizosphere:

The term 'rhizosphere' was introduced in 1904 by the German scientist Hiltner to denote that region of the soil which is modified as a result of the uptake and deposition of substances by a growing root or the root surface (rhizoplane) together with that region of the surrounding soil in which the microbial population is affected, qualitatively and/or quantitatively, by the presence of a root. The rhizosphere may extend a few millimeters, or centimeters, from the rhizoplane.

(b) Rhizoplane: Root surface along with the closely adhering soil particles is termed as rhizoplane.

(C). Phyllosphere: The region/zone on leaves inhibited by microorganisms has been termed as 'phyllosphere' and the leaf surface as 'phylloplane'.

Microorganisms for Agricultural use:

I. Bacteria

A. Non symbiotic nitrogen fixating bacteria:

Azotobacter chroocochum: Azotobacter species are free-living, nitrogenfixing bacteria; in contrast to Rhizobium species, they normally fix molecular nitrogen from the atmosphere without symbiotic relations with plants, although some Azotobacter species are associated with plants, (Kasset al. 1971). Nitrogen fixation is inhibited in the presence of available nitrogen sources, such as ammonium ions and nitrates,

Azotobacter vinelandii: Azotobacter vinelandii is Gramnegative diazotroph that can fix nitrogen while grown aerobically. It is a genetically tractable system that is used to study nitrogen fixation. These bacteria are easily cultured and grown .A. vinelandii is a free-living N2 fixer known to produce many phytohormones and vitamins in soils. It produces fluorescent pyoverdine pigments.

Glucanobacter diazotrophicus: Glucanobacter is nitrogen fixing bio inoculants exclusively meant for sugarcane. G. diazotrophicus was described as a species associated with sugar rich plants, it has been found naturally associated with other types of plants, and can be recovered from inoculated, non-sugar rich plants .

Acetobacter xylinum: Acetobacter is a genus of acetic acid bacteria. Acetic acid bacteria are characterized by the ability to convert ethanol to acetic acid in the presence of oxygen. Of these, the genus Acetobacter is distinguished by the ability to oxidize lactate and acetate into carbon dioxide and water, . Bacteria of the genus Acetobacter have been isolated from industrial vinegar fermentation processes and are frequently used as fermentation starter cultures.

Azospirillum lipoferum: Azospirillum lipoferum, is a free living, gram positive, plant-growth-promoting a-proteobacteria, capable of affecting the growth and yield of numerous plant species, many of agronomic and ecological significance. The leading theory concerning its growth promotion capacity lies in its ability to produce various phyto-hormones that improve root growth, adsorption of water and minerals that eventually yield larger, and in many cases more productive plants.

B. Symbiotic nitrogen fixating bacteria:

a. Rhizobium leguminosarum: Rhizobium leguminosarum is a bacterium which lives in a mutualistic symbiotic relationship with legumes, and has the ability to fix free nitrogen from the air,. This is used in Peas, Lathyrus, Vicia, Lentil.

b. Rhizobium Tripoli: Used in berseem.

c. Rhizobium phaseoli: Used in kidney beans.

d. Rhizobium lupine: Used in lupinus, ornithopus.

e. Rhizobium japonicum: Used in soybean.

f. Rhizobium meliloti: Used in melilotus, lucerne, fenugreek.

C. Phosphorus solubilising bacteria:

a. Bacillus megaterium: Bacillus megaterium is a cytokinin promoting bacterium used to promote plant root over growth. It is a gram-positive, rod shaped, spore forming bacteria. It is used in the biocontrol of plant diseases and nitrogen fixation has been demonstrated in some strains.

b. Pseudomonas putida: Pseudomonas putida is also important in maintaining plant health. It lives in most soils and associated with plant roots, where it frequently improves plant health. The organism also produces molecules that sequester iron from the area around the plant. This deprives fungi and other bacteria of a necessary nutrient, limiting their growth. By doing so, it can affect the biological control of some plant pathogens.

D. Potash mobilize bacteria:

Frateuria aurentia: Frateuria aurantia is a species of Proteobacteria,. The microbe, Frateuria aurentia is a beneficial bacterium capable of mobilizing available Potash into near the roots of the plants. It works well in all types of soil especially, low K content soil. Use of such bacteria in powder form can increase the availability of more potash in usable form to the plants.

E. Plant growth promoting rhizobacteria (PGPR):-

a. Bacillus subtilis: Bacillus subtilis is spore forming bacteria which, when applied to the seeds or plants, it colonize the developing root system of the plants. The bacteria compete with and thereby suppress plant disease fungal organisms such as Rhizoctonia, Fusarium, Aspergillus, and others. Bacillus subtilis continue to live on the root system and provide protection throughout the growing season. Therefore, even if treated seeds are stored for prolonged periods, the bacteria stay alive, and then grow and multiply after the seeds are planted.

Bacillus polymyxa: Bacillus polymyxa is used as inoculants in agriculture and horticulture.Biofilms of B. polymyxa growing on plant roots have been shown to produce exopolysaccharides which protect the plants from pathogens. The interactions between this bacterial species and plant roots also cause the root hairs to undergo physical changes.

Pseudomonas fluorescens: This is non-pathogenic saprophytes that colonize soil, water and plant surface environments. Pseudomonas fluorescens suppress plant diseases by production of number ofsecondary metabolites including antibiotics, siderophores and hydrogen cyanide. This microbe has the unique ability to enter the plant vascular system, reach the various parts of the plant system and act as a systemic bio-control agent against various fungal and bacterial diseases. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by Pseudomonas fluorescens may also be an important factor in disease control.

Pseudomonas putida: Pseudomonas putida also interacts with other organisms in the soil. One such interaction with Saccharomyces cerevisiae in the rhizosphere led to beneficial effects on the state of the Pseudomonas putida. Fungi Saccharomyces cerevisiae produced the necessary glucose and also maintained the pH which was both favourable to the bacteria Pseudomonas putida. The complex interaction of Pseudomonas putida and Saccaromyces cerevisiae together regulate plant health. Moreover, the bacteria itself is a great maintainer of abundant plant life. The production of the siderophores, such as pyoverdine and pyochelin, protect the plants from fungal pathogens. The mutual relationship benefits both partners. While Pseudomonas putida is able to reside in the plant seed and rhizosphere, the plant is, in turn, protected from plant pathogens and able to obtain vital nutrients from the bacteria.

II. Fungus

A. Insecticide fungus

a. Metarhizium anisopliae: Metarhizium anisopliae is being used as a biological insecticideto control a number of pests such as Grasshoppers, Termites, Thrips, Catterpillers, Aphids etc.and its use in the control of malaria-transmitting mosquitos is under investigation. Metarhizium anisopliae is an entomopathogenic fungus that infects insects that come in contact with it. Once the fungus spores attach to the surface of the insect, germinate and begin to grow, they then penetrate the exoskeleton of the insect and grow very rapidly inside the insect causing the insect to die. Other insects that come in contact with infected insects also become infected with the fungus.

Beauveria bassiana: Beauveria bassiana can be used as a biological insecticide to control a number of pests such as termites, whiteflies, and many other insects. Its use in the control of malaria-transmitting mosquitos is under investigation. As an insecticide, the spores are sprayed on affected crops as an emulsified suspension or wettable powder or applied to mosquito nets as a mosquito control agent.

Beauveria bassiana is a naturally occurring entomopathogenic fungus in most part of the world. The spore of this fungus when comes in contact with the cuticle (skin) of the target insect pest they germinate and grow directly through the cuticle to the inner body of the host. The fungus proliferates throughout the insect's body, draining the insect of nutrients, eventually killing it in about 48-72 hours after spray.

Verticillium lecanii: Verticillium lecanii is an entomopathogenic fungus. The mycelium of this fungus produces a cyclodepsipeptide toxin called bassianolide and other insecticidal toxins such asdipicolinic acid, which infect aphids, whiteflies, rust fungi, scale insects and lead to death the host. This fungus was first described in 1861 and has a worldwide distribution. Insects are infected when they come into contact with the sticky fungal spores which then grow and invade the body, thus the internal organs are consumed, leading to their death. In horticulture and agriculture L. lecanii is sometimes used as a biological pesticide for controlling insect pests such as whitefly, thrips and aphids.

B. Nematicide fungus Paecilomyces lilacinus: Plant-parasitic nematodes cause significant economic losses to a wide variety of crops. Chemical control is a widely used option for plant-parasitic nematode management. However, chemical nematicides are now being reappraised in respect of environmental hazard, high costs, limited availability in many developing countries or their diminished effectiveness following repeated applications. Paecilomyces lilacinus is a naturally occurring fungus found in many kinds of soils throughout the world. As a pesticide active ingredient, Paecilomyces lilacinus is applied to soil to control nematodes that attack plant roots. It acts against plant root nematodes by infecting eggs, juveniles, and adult females.

Arthrobotrys spp.: The fungus is a biological indicator of nematodes. The annual global cost of plant-parasitic nematodes is approximately 100 billion USD. Nematode capturing fungi such as the A. oligospora can be used to control growth of nematodes. This means that they can be

potentially used as a bio-control agent to protect crops against nematode infestations. This may not be feasible since the nematodes occasionally eat the fungi .

C. Fungicide fungus

a. Trichoderma viride: Trichoderma viride is the potential antagonistic fungus which prevents the crops from diseases viz. Root rots, wilts, brown rot, damping off, charcoal rot and other soil borne diseases in c r o p s . Trichoderma is able to suppress more than 60 species of pathogens (Pythium, Botritis, Phoma, Sclerotinia, Fusarium, Ascochyta, Alternaria and others) on different plants (cucumbers, tomatoes, cabbages, peppers, various ornamentals, cereals and grain legume crops).

How to apply microorganisms

A) Seed inoculation: On the basis ofefficiency Azotobacter, other micro-organisms present in the soil benefits obtained from microorganisms and expenditure it has been fixed to use Azotobacter microorganism at the rate of 250 g microorganism for 10-15 kg seed. If one knows this proportion then take a definite quantity of seed to be inoculated. The required quantity of fresh microorganism is secured and slurry is made by adding adequate, quantity of water. This slurry is uniformly applied to seed; seed is then dried in shed and sown. Some stickers are used in order to adher microorganism to seed, viz. Jaggery or gum arebia.

B) Seedling inoculation: This method of inoculation is used where seedlings are used to grow the crop. In this method, seedlings required for one acre are inoculated using 4-5 packets (2-2.5 kg). For this in a bucket adequate quantity of water is taken and microorganisms from these packets is added to bucket and mixed properly. Roots seedlings are then dipped in this mixture so as to enable roots to get inoculums. These seedlings are then transplanted e.g. Tomato, Rice, Onion, Cole crops, flowers.

C) Self inoculation or tuber inoculation: in this method50 litres of water is taken in a drum and 4-5 kg of microorganism is added and mixed properly. Sets are required for one acre of land is dipped in this mixture. Potato tubers are dipped in the mixture of microorganism and planting is done.

D) Soil application: This method is mostly used for fruit crops, sugarcane and trees. At the time of planting fruit trees 20 g of microorganism mixed with compost is to be added per sapling, when trees becomes matured the same quantity of microorganism is applied.

Quality of Irrigation Water and Management

The suitability of irrigation water depends upon several factors, such as, water quality, soil type, plant characteristics, irrigation method, drainage, climate and the local conditions. The integrated effect of these factors on the suitability of irrigation water (SI) can be qualitatively expressed by the relationship:

SI = QSPCD

where

Q = quality of irrigation water, that is, total salt concentration, relative proportion of cations, etc;

S = soil type, texture, structure, permeability, fertility, calcium carbonate content, type of clay minerals and initial level of salinity and alkalinity before irrigation;

P = salt tolerance characteristics of the crop and its varieties to be grown, and growth stage;

C = climate, that is, total rainfall, its distribution and evaporation characteristics; and

D = drainage conditions, depth of water table, nature of soil profile, presence of hard pan or lime concentration and management practices.

Besides these factors, presence of some ions in water such as calcium, sulphate, potassium and nitrate is favorable for crop growth, as water of more salinity can be used in presence of these ions.

Irrigation Water Quality Criteria

Soil scientists use the following categories to describe irrigation water effects on crop production and soil quality:

• Salinity hazard - total soluble salt content

• Sodium hazard - relative proportion of sodium to calcium and magnesium ions

• pH - acid or basic

• Alkalinity - carbonate and bicarbonate

• Specific ions: chloride, sulfate, boron, and nitrate.

1 Salinity Hazard: The most influential water quality guideline on crop productivity is the water salinity hazard as measured by electrical conductivity (ECw). The primary effect of high ECw water on crop productivity is the inability of the plant to compete with ions in the soil solution for water (physiological drought). The higher the EC, the less water is available to plants, even though the soil may appear wet. Because plants can only transpire “pure” water, usable plant

water in the soil solution decreases dramatically as EC increases. The amount of water transpired through a crop is directly related to yield; therefore, irrigation water with high ECw reduces yield.

Table 1. Permissible limits for classes of irrigation water.

Classes of water	Concentration, total dissolved solids	
	Electrical conductivity μmhos*	Gravimetric ppm
Class 1, Excellent	250	175
Class 2, Good	250-750	175-525
Class 3, Permissible[1]	750-2,000	525-1,400
Class 4, Doubtful[2]	2,000-3,000	1,400-2,100
Class 5, Unsuitable[2]	3,000	2,100

*Micromhos/cm at 25 degrees C.
[1]Leaching needed if used
[2]Good drainage needed and sensitive plants will have difficulty obtaining stands

Sodium hazard and irrigation water infiltration –

Although plant growth is primarily limited by the salinity (ECw) level of the irrigation water, the application of water with a sodium imbalance can further reduce yield under certain soil texture conditions. Reductions in water infiltration can occur when irrigation water contains high sodium relative to the calcium and magnesium contents. This condition, termed “sodicity,” results from excessive soil accumulation of sodium. Sodic water is not the same as saline water. Sodicity causes swelling and dispersion of soil clays, surface crusting and pore plugging. This degraded soil structure condition in turn obstructs infiltration and may increase runoff. Sodicity causes a decrease in the downward movement of water into and through the soil, and actively growing plants roots may not get adequate water, despite pooling of water on the soil surface after irrigation. The most common measure to assess sodicity in water and soil is called the Sodium Adsorption Ratio (SAR). The SAR defines sodicity in terms of the relative concentration of sodium (Na) compared to the sum of calcium (Ca) and magnesium (Mg) ions in a sample. The SAR assesses the potential for infiltration problems due to a sodium imbalance in irrigation water. The SAR is mathematically written below, where Na, Ca and Mg are the concentrations of these ions in milliequivalents per liter (meq/L). Concentrations of these ions in water samples are typically provided in milligrams per liter (mg/L).

$$SAR = \frac{Na\ (meq/l)}{\sqrt{\frac{Ca\ (meq/l) + Mg\ (meq/l)}{2}}}$$

Table2 . The sodium hazard of water based on SAR Values.		
SAR values (milli mole/litre)	**Sodium hazard of water**	**Comments**
1-10	Low	Use on sodium sensitive crops such as avocados must be cautioned.
10 - 18	Medium	Amendments (such as Gypsum) and leaching needed.
18 - 26	High	Generally unsuitable for continuous use.
> 26	Very High	Generally unsuitable for use.

pH and Alkalinity: The acidity or basicity of irrigation water is expressed as pH (< 7.0 acidic; > 7.0 basic). **The normal pH range for irrigation water is from 6.5 to 8.4**. Abnormally low pH's may cause accelerated irrigation system corrosion where they occur. High pH's above 8.5 are often caused by high bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) concentrations, known as alkalinity. High carbonates cause calcium and magnesium ions to form insoluble minerals leaving sodium as the dominant ion in solution. Alkaline water could intensify the impact of high SAR water on sodic soil conditions. Excessive bicarbonate concentrates can also be problematic for drip or micro-spray irrigation systems when calcite or scale build up causes reduced flow rates through orifices or emitters.

Eaton (1950) suggested that Residual Sodium Carbonate (RSC) defined by the formula:

$RSC = (CO_3^- + HCO_3^-) - (Ca^{++} + Mg^{++})$

RSC a good index of the alkalinity hazard of an irrigation water. The anions HCO_3^- and CO_3^- in the irrigation water tend to precipitate calcium and magnesium ions in the soil resulting in an increase in the proportion of the sodium ions. For this reason, RSC was considered to be indicative of the alkalinity hazard of water.

Table 3: Classification of water based in RSC

Class	RSC (me/L)
Excellent	Below 1.25
Good	1.25 – 2.50
Unsuitable	Above 2.50

Specific ions: Chloride, Boron, and Nitrate.

Chloride: chloride is essential to plants in very low amounts, it can cause toxicity to sensitive crops at high concentrations. Like sodium, high chloride concentrations cause more problems when applied with sprinkler irrigation. Leaf burn under sprinkler from both sodium and chloride can be reduced by night time irrigation or application on cool, cloudy days.

Boron: Boron is another element that is essential in low amounts, but toxic at higher concentrations . In fact, toxicity can occur on sensitive crops at concentrations less than 1.0 ppm. Because B toxicity can occur at such low concentrations, an irrigation water analysis is advised for groundwater before applying additional B to irrigated crops**.**

Table:4 Water quality rating based in boron content

S. No	Class	Boron (ppm)
1	**Low**	**<1.0**
2	**Medium**	**1.0-2.0**
3	**High**	**2.0-4.0**
4	**Very High**	**>4.0**

Nitrogen: Nitrogen in irrigation water (N) is largely a fertility issue, and nitrate-nitrogen (NO_3^- N) can be a significant N source. High N can cause quality problems in crops such as barley and sugar beets and excessive vegetative growth in some vegetables. However, these problems can usually be overcome by good fertilizer and irrigation management. Regardless of the crop, nitrate should be credited toward the fertilizer rate especially when the concentration exceeds 10 ppm NO_3^-N (45 ppm NO_3^-).

Management of Poor Quality Water:

1. Application of greater amounts of organic matter such as FYM, compost etc., to the soil to improve permeability and structure.

2. Increasing the proportion of calcium, through addition of gypsum ($CaSO_4$) to the irrigation water in the channel, by keeping pebbles mixed pure gypsum bundles in the irrigation tank.

3. Mixing of good quality water with poor quality water in proper proportions so that both the sources of water are effectively used to maximum advantage.

4. Periodical application of organic matter and raising as well as incorporation of green manure crops in the soil.

5. Irrigating the land with small quantities of water at frequent intervals instead of large quantity at a time.

6. Application of fertilizer may be increased slightly more than the normally required and preferably ammonium sulphate for nitrogen, super phosphate and Di Ammonium Phosphate (DAP) for phosphorus application.

7. Drainage facilities must be improved.

8. Raising of salt tolerant crops such as cotton, ragi, sugar beet, paddy, groundnut, sorghum, corn, sunflower, chillies, tobacco, onion, tomato, garden beans, amaranthus and lucerne.

9. Leaching requirement (LR)

The leaching requirement may be defined as the fraction of the irrigation water that must be leached through the root zone to control soil salinity at any specific level.

The leaching requirement (LR) is simply the ration of the equivalent depth of the drainage water to the depth of irrigation water and may be expressed as a fraction or as per cent. Under the assumed conditions (uniform aerial application of irrigation water, no rainfall, no removal of salt in the harvested crop and no precipitation of soluble constituents in the soil), this ration is equal to the inverse ratio of the corresponding electrical conductivities as follows :

$$LR : \frac{D_{dw}}{D_{iw}} \times 100 = \frac{EC_{iw}}{EC_{dw}} \times 100$$

where;

LR	:	Leaching requirement expressed in percentage
D_{dw}	:	Depth of drainage water in cm
D_{iw}	:	Depth of irrigation water in cm
EC_{iw}	:	Electrical conductivity of the irrigation water in dS/m
EC_{dw}	:	Electrical conductivity of the drainage water in dS/m

Table: Guidelines for interpretations of water quality for irrigation

Parameters	**Suitable**	**Moderately Suitable**	**Not Suitable**
EC (dS/m)	<0.25	0.25-0.75	>0.75
TDS	<160	160-480	>480
pH	6.5-8.4	8.5	>8.5
Sodium Absorption Ratio	<10	10-18	>18
Sodium as Adjusted SAR	<3.0	3.0-9.0	>9.0
RSC (me/L)	<1.25	1.25-2.5	>2.5
Bicarbonate (HCO_3) (me/L)	<1.5	1.5-8.5	>8.5
NO^3-N (me/L)	<5	5-30	>30
B (mg/L)	<0.75	0.75-2.0	>2.0
Cl (me/L)	<4.0	4.0-10	>10
F (me/L)	<1.0	1.0-15	>15

Source- FAO

Table. Terms, units, and useful conversions for understanding water quality analysis reports.

Symbol	Meaning	Units
Total Salinity		
a. EC	electric conductivity	mmhos/cm μmhos/cm dS/m
b. TDS	total dissolved solids	mg/L ppm
Sodium Hazard		
a. SAR	sodium adsorption ratio	—
b. ESP	exchangeable sodium percentage	—

Determination	Symbol	Unit of measure	Atomic weight
Constituents			
(1) cations			
Calcium	Ca	mol/m^3	40.1
Magnesium	M	mol/m^3	24.3
Sodium	g	mol/m^3	23.0
Potassium	Na	mol/m^3	39.1
(2) anions	K		
Bicarbonate		mol/m^3	61.0
Sulphate	HCO_3	mol/m^3	96.1
Chloride	SO_4	mol/m^3	35.5
Carbonate	Cl	mol/m^3	60.0
Nitrate	CO_3	mol/m^3	62.0
Trace Elements		mg/L	
Boron	NO_3	mg/L	10.8
	B		

Conversions

1 dS/m = 1 mmhos/cm = 1000 μmhos/cm

1 mg/L = 1 ppm

TDS (mg/L) ≈ EC (dS/m) x 640 for EC < 5 dS/m

TDS (mg/L ≈ EC (dS/m) x 800 for EC > 5 dS/m

TDS (lbs/ac-ft) ≈ TDS (mg/L) x 2.72

Concentration (ppm) = Concentration (mol/m3) times the atomic weight

Sum of cations/anions

(meq/L) ≈ EC (dS/m) x 10

mg/L = milligrams per liter

ppm = parts per million

dS/m = deci Siemens per meter at 25° C

Leaf analysis, standards and index tissues of different crops and interpretation of leaf analytical values

Leaf analysis

Leaf analysis (also called stem leaf analysis, tissue analysis or foliar analysis) is the most precise method of monitoring plant nutrient levels.

While soil analysis reveals the levels of essential soil nutrients, leaf analysis shows the grower exactly what the plant has successfully absorbed.

Leaf analysis is especially helpful in detecting nutrient deficiencies before they affect plant health and yield.

Importance

Chemical analysis of plant foliage is an important tool for establishing and maintaining a proper fertilizer programme in soil fertility management especially for fruit plantings.

Leaf analysis can be used to confirm or diagnose a problem associated with a nutrient shortage or excess, and more importantly to prevent the development of a nutrient disorder in crops.

It would also reveal that certain fertilizers being used are not necessary and results in the most economical fertilizer programme.

Analysis must be properly taken.

In other instances, a series of analyses may be necessary to arrive at a proper explanation.

Paired comparisons, one from normal and one from the abnormal condition, are frequently helpful.

Foliar analyses made over a period of years can indicate an approaching deficiency of a nutrient element before the plant shows any visible symptoms.
It is possible then, through proper corrective fertilizer applications, to prevent the deficiency from ever occurring in the crop. B

y the same token, it is possible to learn when an element may be increasing in a crop toward a level that will reduce crop quality or bring about some other undesirable effect. When this condition is known, steps can be taken to alter the fertilizer programme and cultural practices that influence the uptake of the element from the soil.

What plant tissue analysis shows?

Plant tissue analysis shows the nutrient status of plants at the time of sampling.

1. This, in turn, shows whether soil nutrient supplies are adequate. In addition, plant tissue analysis will detect unseen deficiencies and may confirm visual symptoms of the deficiencies. Toxic levels also may be detected. Though usually used as a diagnostic tool for future correction of nutrient problems, plant tissue analysis from young plants will allow a corrective fertilizer application during the same season. Not all abnormal appearances are due to a deficiency.

2. Some may be due to too much of certain elements. Also, symptoms of one deficiency may look like those of another.

3. A plant tissue analysis can pinpoint the cause, if it is nutritional.

A plant analysis is of little value

1. if the plants come from fields that are infested with weeds, insects, and disease organisms;

2. if the plants are stressed for moisture; or

3. if the plants have some mechanical injury.

The most important use of plant analysis is as a monitoring tool for determining the adequacy of current fertilization practices. Sampling a crop periodically during the season or once each year provides a record of its nutrient content that can be used through the growing season or from year to year. With soil test information and a plant analysis report, a producer can closely tailor fertilization practices to specific soil-plant needs. It also may be possible to prevent nutrient stress in a crop if the plant analysis indicates a potential problem developing early in the season. Corrective measures can be applied during the season or, if the crop is perennial, during the next year. Combined with data from a soil analysis, a tissue analysis is an important tool in determining nutrient requirements of a crop.

INTERPRETATION

1. The sufficiency ranges are given for plant tissues of the crops at ages (or stages in the crop cycle) that research has found appropriate for sampling.

2. The analysis data can be used as a guide for attaining improved crop quality and yield.

3. For long-term orchard crops, plant tissue nutrient levels can be logged over time and used as a diagnostic tool to assist in developing a fertilizer programme.

4. If a tissue level of a nutrient is below the lower end of the sufficiency range, the nutrient should be considered deficient, whereas if the level is above the upper end of the range, the nutrient can be considered as approaching a toxic level.

5. The midpoint of the sufficiency range is the target to aim at As the level approaches the lower limit, the nutrient should be added.

6. As the level approaches the upper limit, additions of the nutrient should be withheld. It is important to be near the midpoint for most nutrients, because imbalances in the ratios of nutrients can affect crop growth.

7. Because environment plays a major role in nutrient uptake and crop development, the sufficiency ranges given here should be considered as general guides.

8. In addition to variation due to environment effects, different crop cultivars may have different critical levels.

Chemical and mineralogical composition of important horticultural crops

Introduction

The living plant is constituted of various organic compounds, which in turn are made up of several inorganic elements. The organic compounds to which the plant can be resolved at the first disintegration are called proximate constituents. The inorganic elements to which the plant can be finally decomposed are called ultimate components.

Proximate constituents are as follows:

1.	Water	:	80-95 %
2.	Carbohydrates: a. Sugars and starches b. Hemicelluloses c. Celluloses	:	1-5 % 10-30 % 20-30%
3.	Proteins	:	1-15 %
4.	Lipids	:	1-8 %
5.	Plant pigments, alkaloids, tannins and essential Oils	:	1-8 %
6.	Plant growth substances (enzymes, vitamins and hormones)	:	Small amounts
7.	Mineral elements	:	2 – 5 %

17 essential inorganic elements which make up the plant body Viz. C, H, O, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo,Cl and Ni.

Mineral composition of vegetables

TOMATO (*Solanum Lycopersicum*)

It is popularly praised vegetable commonly known as 'Love apple'. The nutrient content/ 100 g of tomato are as follows:

Water	94.1%
Protein	1.0%
Fibre	0.6%
Fat	0.3%
Carbohydrate	4.0%

Minerals

Na	3 mg	Fe	0.6 mg
K	268 mg	Cu	0.1 mg
Ca	11 mg	Mn	0.19 mg
Mg	11 mg	P	27 mg
S	11 mg	Cl	51 mg

Vitamins

Vit. A	1100 IU
Vit. C	23 mg
Vit. E	0.27 mg
Vit. B	0.2 mg
Nicotinic acid	0.6 mg

Sugar content is 1.85 – 4.27%.

Acidity	4.2 - 10.2 mg of acid / 100 ml
Ascorbic acid	20.9 - 22.5 mg / 100 g

It is an excellent source of Vitamin C, so it is commonly known as poor man's orange. It is popular salad vegetable. It is used for the preparation of soups, pickles, ketchups etc. Tomato juice is a popular appetizer and beverage.

CARROT (*Daucus carota*)

The nutrient content of the carrot per 100 g edible portion is as follows:

Water	82.2%
Energy	45 calories
Protein	1.2%
Vit. A	12,000 I.U
Thiamine	0.042 mg
Riboflavin	0.043 mg
Niacin	0.21 mg
Ca	42 mg
Vit. C	4 mg

COWPEA (*Vigna sinensis* L.)

The nutrients contents of cowpea are given per 100 g edible portion.

Moisture	84.6%
Fat	0.2 g
Fibre	2.0 g
Calories	51
P	74 mg
Vit. A	941 IU
Riboflavin	0.09 mg
Vit. C	13 mg
Protein	4.3 g
Minerals	0.9 g
CHO	8.0 mg
Ca	80 mg
Fe	2.5 mg
Thiamine	0.07 mg
Nicotinic acid	0.9 mg

ONION (*Allium cepa*)

The chemical composition per 100g of onion is as follows:

Moisture	86.8%
Protein	1.2%
Fat	0.1%
CHO	11.5%
Ca	0.18%
P	0.05%
Fe	0.7 mg
Vit. B	80 mg
Riboflavin	10 mg
Nicotinic acid	0.4 mg
Ascorbic acid	11.0 mg

Immature and mature bulbs are used as vegetable. The pungency, which is due to a volatile oil known as allylpropyl disulphide.

CABBAGE (*Brassica oleracea* var. capitata)

The chemical composition per 100 g of fresh cabbage is detailed below:

Water	92.1 g	Vit. B2	0.04 mg
Protein	1.4 g	Vit. B6	0.11 mg
Total fats	2 g	Vit. C	46 mg
Total CHO	5.7 g	P	28 mg
Fibre	1.5 g	Ca	46 mg
Vit. A	70 I. U	K	227 mg
Vit. B1	0.04 g	Na	13 mg

CAULIFLOWER (*Brassica oleracea* var.botrytis)

The composition per 100 g of cauliflower is as follows:

Water	91.7%	Vit. A	40 I. U
Energy	31 calories	Ascorbic acid	70 mg
Protein	2.4 g	Thiamine	0.2 mg
Ca	22 mg	Riboflavin	0.1 mg
Niacin	0.57 mg		

Cauliflower seedlings are used for salad and green. The curd is used in curries, soups and pickles. In abundant areas of production cauliflower curd is cut into pieces, dried and procured for off-season use.

Chemical composition of vegetables

S. No	Crop	Moisture (%)	Proteins (%)	CHO's	Fats (%)	Ca	P (mg/100g)	Fe
1.	Chillies	85.7	2.9	3.0	0.6	30	24	1.2
2.	Brinjal	92.7	1.1	5.5	0.2	0.15	0.48	0.01
3.	Bottle guard	96.4	0.2	2.5	0.1	20	5	0.7
4.	Pumpkin	92.6	1.4	4.6	0.1	10	14	0.7
5.	Bhendi	89.6	1.9	3.4	0.2	60	43	1.5
6.	Turnip	91.6	0.5	6.2	0.2	30	-	0.4
7.	Garlic	62.0	6.3	29.0	0.1	30	-	1.8
8.	Knoolkhol	92.7	1.1	3.8	0.2	20	18	0.4
9.	Bitter gourd	83.2	2.1	9.8	1.0	50	21	9.4
10.	Amaranthus	85.0	4.0	6.3	0.5	397	247	25.5

CHEMISTRY OF FRUITS

Fruits are valued for their attractive appearance, flavour and texture for a long time. In recent years their vitamin content has been recognized as an important feature. In all these characteristics, sugars either in the free stage or as derivatives play an important role. Flavour is fundamentally the result of the balance between sugars and acids. Certain flavouring constituents which are mostly glucosides also add to the flavour of the fruits.

I.Sugars

The sugar content varies from traces to 61% in fruits. Traces of sugars are present in lime and 61% in date. Sugars other than glucose, fructose and sucrose are rarely present. The average sugar content varies from 5-10%.

Apple	6-16%
Pineapple	8-18%
Grapes	10-19%
Mango	14%
Date	61%
Tomato	2-4%
Lemon	0.9-3.6

II.Proteins

The protein content of fruits is comparatively very low. The protein content varies with species, locality, season, cultural practices and other environmental factors.

Apple	0.2%	Grapes	1.3%
Banana	1.1%	Guava	0.8%
Avocado	2.1%	Lime, mango and pineapple	<1%
Tomato	1.2%	Dates	2.2%

III. Volatile compounds

The characteristic odour of many fruits is due to the presence of certain volatile compounds. These volatile compounds of the fruits are usually less than 100 ppm in concentration.

Apple	Ethyl 2 methyl butyrate
Grapes	Methyl anthranilate
Banana	Amyl acetate and isopentyl acetate
Grape fruit	Terpenes

Lemon: Hydrocarbon containing isoprene units and their oxygenated derivatives. Orange: Limonene.

IV. Fruit phenolic compounds

Phenolic compounds are responsible for colour flavour and taste. These compounds give both desirable and undesirable qualities in fruits. The fruit phenolic compounds include both “Flavonoids” and “Cinnamic acid”. The major flavonoids are anthocyanin, leucoanthocyanin, flavones and flavonols. Cinnamic acid and its derivatives are not flavonoids but related to them. The presence of these phenolic substances in fruits gives an astringemt taste.

Source- This chapter adopted from Soil and Plant analysis. AgriMoon.com

Soil and Water Pollution

Introduction: Environmental pollution is one of the most serious problems facing humanity and other life forms on our planet today. Environmental pollution is defined as "the contamination of the physical and biological components of the earth/atmosphere system to such an extent that normal environmental processes are adversely affected." Pollutants can be naturally occurring substances or energies, but they are considered contaminants when in excess of natural levels. Any use of natural resources at a rate higher than nature's capacity to restore itself can result in pollution of air, water, and land.

Pollution: The term pollution is derived from Latin word "polluere" meaning defiling. It can be defined as the undesirable change in the physical, chemical or biological characteristics of soil, water and air than can harmfully affect life and human beings and other living system.

Pollutants: The substances that cause pollution are known as pollutants. A pollutant can be any chemical (toxic metal, radionuclides, organophosphorus compounds, gases) or geochemical substance (dust, sediment), biological organism or product, or physical substance (heat, radiation, sound wave) that is released intentionally or inadvertently by man into the environment with actual or potential adverse, harmful, unpleasant, or inconvenient effects. Depending on the nature of pollutants and also subsequent pollution of environmental components, the pollution may be categorized as follows:

1. Air Pollution
2. Water Pollution
3. Soil/Land Pollution
4. Noise Pollution
5. Radioactive Pollution
6. Thermal Pollution

Among these types of pollution, air pollution is the main type threatening the environment, humans, plants, animals, and all living organisms.

Soil Pollution: Soil pollution is the presence of toxic chemicals in soil in high enough concentrations to be of risk to human health and ecosystem.

The major sources responsible for soil pollution are disposal of untreated industrial wastes, use of fertilizers and pesticides in intensive agriculture, spilling or leakage of oil or other chemicals during transportation, storage or other uses, dumping of waste and chemicals, nuclear

and radioactive waste disposal, drainage of polluted water in soils and degradation of soil due to deforestation and construction activities.

Impacts of Soil Pollution:

1. Soil pollution leads to accumulation of toxic elements in soil which adversely affect the germination of seed and plant growth.

2. Application of fertilizers and pesticide may get accumulated in the soil over time and deteriorate soil fertility and productivity

3. Excessive use of fertilizers and pesticides in soil may lead to the leaching of salts to the lower soil depths as well as water bodies through surface runoff and make water unsafe for domestic and drinking purpose.

4. Excess use of nitrogenous fertilizers results in higher concentration of NO_3^- in drinking water and make unfit for consumption. NO_3^- is converted to NO_2^-, which is absorbed in blood, causing methemoglobinemia, known as **Blue Baby Syndrome.**

5 Accumulation of PO_4^{3-} in soil along with leaching and runoff in water bodies causes eutrophication in water and adversely affects the aquatic life.

6 Heavy metals released from fertilizers and pesticides hamper the quality of water.

7 Use of pesticides not only kills pathogens but also can have negative impact on useful microorganisum in the soil.

8 Abundance of toxic and heavy metals in soils results in impaired availability of the essential soil nutrients for optimal plant growth.

Major Soil Pollution Problems in India: The major threats of soil pollution to Indian soil emerge from non judicious use of fertilizers and pesticides, nutrient imbalance, erosion, ecological imbalance and decline in biodiversity, waterlogging, salinity and alkalinity and pesticide and heavy metal pollution.

1 pollution problems with fertilizer use

Environmental problems associated with fertilizer use and the mitigation strategies

Environmental problem	Causative mechanism	Mitigation strategies
Ground water contamination	Nitrate leaching	Judicious use of fertilizers, increasing use efficiencies, using nitrification inhibitors, coated fertilizers use,
Eutrophication	Erosion and surface runoff	Reduce runoff, water harvesting, controlled

		irrigation, control erosion
Methemoglobinemia	Consumption of excess nitrate through drinking water and food	reduce leaching losses of N
Acid rain and ammonia redeposition	Nitric acid originating from reaction of N oxides with moisture in atmosphere, ammonia volatilization	Reduce ammonia volatilization losses, decrease the pH of soil, increase CEC, use fertilizer formulations and inhibitors
Stratospheric ozone depletion and global warming	Nitrous oxide emission from soil	Use of nitrification inhibitors and urease inhibitors, increase N-use efficiency

2. Nutrient Imbalance

3. Soil Erosion

4. Decline in soil biodiversity

5 Water logging

6Salinity and Alkalinity

7 Heavy metal pollution

8 Persistent organic pollutants

Remediation of Soil Pollution:

1. Oil and other related materials should be handled with care during storage and transportation. Addition of bacteria and other microorganisms in polluted soils can decompose oil and related materials.

2. In agriculture, use of chemical fertilizers and pesticides can be reduced with the adoption of integrated nutrient management and integrated pest management practices.

3. Recycling and reuse of biodegradable waste like paper , glass and woody materials and ban on non degradable waste like plastic is an alternate to disposal of waste.

4. Safe disposal of nuclear and radioactive wastes.

5. landfill locations for waste disposal should not be near to residential areas and at higher surface than highest underground water level.

6. To improve soil quality and reduce soil erosion, afforestation should be done on land.

7. Soil polluted with heavy metals, organic and inorganic toxic substances and pesticides can be ameliorated through phyto-remediation (using higher terrestrial plants), bioremediation

(enhancement of indigenous microbial activity, using enzymes isolated from bacteria or fungi), manipulation of microbial catabolic genes, and growing resistant crops.

Water Pollution: Water pollution is contamination of natural water bodies like rivers, lakes, oceans and groundwater due to deposition and inflow of pollutants by natural and anthropogenic processes.

Sources of water pollution:

1 Point sources: When water is polluted from a single or discrete like sewerage pipe, runoff from a field and from a factory outlet it is known as point source of pollution. Point source are easy to identify and easy to manage.

2 Non-point sources: When water is polluted from various sources from a large area like agricultural runoff, construction sites, and city street flows, it is known as non-point source of pollution. Non-point sources are harder to identify and also to control.

Depending upon the origin, water pollution could be of following types:

1. Biological Pollution: Growth of some microorganisms in water bodies is causing serious health issues in human beings. It also includes pollution caused by decay of organic materials. Both aerobic and anaerobic microorganism living in water bodies feed on biodegradable materials. With more use of biodegradable material, growth of microbes increases with more oxygen demand. This oxygen depletion will kill aerobic microorganisms and produce toxins like ammonia and sulphide.

2. Chemical Pollution: Chemicals released from fertilizers and pesticides in agriculture and industries are polluting water bodies and making water harmful for aquatic life. It also includes accumulation of nutrients leached in water bodies causing algal and weed growth, which make water unsafe for drinking and other domestic purpose.

Impacts of water pollution:

1. The foremost implication of water pollution is reduced water availability for both domestic and agriculture.

2. Crop production suffers from use of contaminated irrigation water.

3. High concentration of metals also has negative effects on crop production

4. Water pollution has adverse effect on human beings and on environment.

5. Drinking of contaminated water causes serious ill-effect on human health like cholera, diarrhea and malaria.

6. Eutrophication has also adverse effects on physical, chemical and biological prpperties of water. **Eutrophication:** Eutrophication (from Greek eutrophos, "well-nourished") or hypertrophication, is when a body of water becomes overly enriched with minerals and nutrients which induce excessive growth of plants and algae.This process may result in oxygen depletion of the water body. Eutrophication is often induced by the discharge of nitrate or phosphate-containing detergents, fertilizers, or sewage into an aquatic system.

7. Water pollution from oil spillage can cause changes in physiology of marine life and natural habitats of aquatic lives.

8. Irrigation with higher concentration of total dissolve solids in water can lead to soil salinity problem and reduces the crop production.

Major water pollution problems in India-

1. Surface water pollution

2. Ground water pollution

3. Water pollution from Agriculture

Remediation of water pollution:

1. To control water pollution from point sources, it is essential to treat wastewater from sources before its discharge into any water body.

2. Sewage water should be treated properly to bring the concentration of toxic and harmful chemicals to the permissible level.

3. Optimum use of fertilizers and pesticides to reduce their leaching and runoff into water bodies.

4. Storage of runoff from manures in the basin to prevent to the leaching of nutrient rich water and the same can be used in agriculture.

5. Separate drainage systems for sewage and rain water to prevent overflow of sewage.

6. Reducing erosion and leaching of fertilizers.

7. Safe use of polluted water.

Soil Biology

Soil biology is the study of microbial and faunal activity and ecology in soil. These organisms include earthworms, nematodes, protozoa, fungi, bacteria, Soil biology plays a vital role in determining many soil characteristics yet, being a relatively new science, much remains unknown about soil biology and its effect on soil ecosystems.

Distribution of various microorganisms in soil ecosystem

Organisms in Soil- Organisms presents in soil are classified into two main groups-

Soil Flora- Belonging to plant kingdom.

Soil Fauna- Belonging to animal kingdom

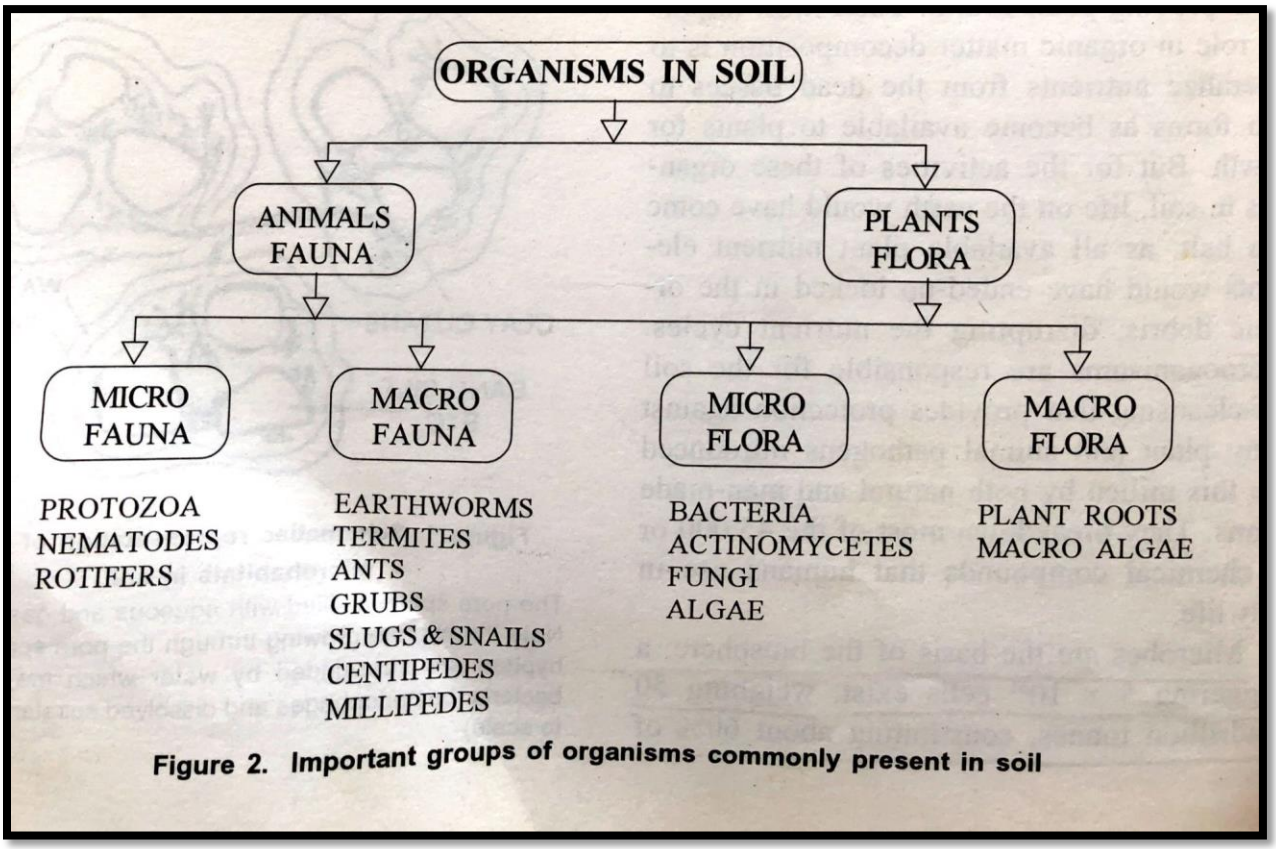


Figure 2. Important groups of organisms commonly present in soil

Classification of Microbes

A Based on the ability to grow in the presence or absence of molecular oxygen microbes are of two categories.

1 Aerobs- Azotobacter, Rhizobium

2 Anaerobs- Clostridium

Facultative aerobs- Those which generally grow and develop in the presence of oxygen but can also adopt themselves to grow under an oxygen depleted environment. Ex- Staphylococcus spp., Streptococcus spp., Escherichia coli

B Based on Temprature

Psychrophiles- Those which grow at temperature below 10^0C

Mesophiles- Those which grow between 20^0C to 40^0C. e.g., E. coli, Salmonella spp., and Lactobacillus spp.

Thermophiles- Those which grow above 45^0C.

C Based on the energy and carbon requirements for cell synthesis-

Heterotrophs- The heterotrophs derive their energy from oxidation of complex organic compounds which also serve as sources of carbon. Ex- Azotobacter, Rhizobium

Autotrophs- The autotrophs utilize carbon from CO_2. Ex Cynobacteria

Chemoautotrophs- Which drive their energy from oxidation of simple inorganic compounds.Ex - Nitrosomonas, Methanogens

Photoautotrophs- Which derive their energy from sunlight. Ex- BGA

Soil MicroFlora (Soil microorganism)- Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristics that define them and their functions in soil.

1 Bacteria- Bacteria are the smallest and most numerous of the organisms present in soil. They are single cell organisms and their size is approximately 1 micron in diameter and up to 10 micron in length. The bacteria in soil are of different shapes. Those with spherical cells are called cocci, rod- shaped cells are termed as bacilli and long spiral- shaped are termed as spirilla.

The different bacterial genera commonly occurring in diverse soils are : Pseudomonas, Arthrobacter, Clostridium, Bacillus, Achromobacter, Micrococcus and Agrobacterium. The genus Bacillus has largest representation in soils in terms of species.

2 Actinomycetes- Taxonomically actinomycetes are like bacteria which possess aerial hyphae like fungi. These organisms share characteristics of both bacteria(cell size, structure and mode of multiplication) and fungi (branching). They are next to bacteria in numbers and are fairly widely distributed in soils. They are more common in dry soils and in undisturbed pastures and grasslands. Like bacteria, they are more common in neutral to slightly alkaline soils. They are aerobic organisms. The species more commonly encountered in soils belong to the genera Streptomyces, Micro- monospora, Nocardia and Thermo-actinomyces.

3 Fungi- Fungi are filamentous organisms with much larger cell width than actinomycetes. The filaments are called hyphae and the network of hyphae collectively is termed mycelium. They are heterotrophs devoid of chlorophyll and are primarily responsible for organic matter decomposition. Soil fungi can grow in a wide range of soil pH, but their population is more under acidic condition. A majority of fungi are aerobic and prefer to grow at optimum soil moisture. Example- Pythium, Rhizopous, Mucor, Aspergillus, Penicillium , VAM Etc.

4 Alage- Soil alage are chlorophyll containing organism. They are autotrophic and therefore, their development is not restricted by organic carbon supply. Soil alage are classified on the basis of the colour (pigments) as:

1 Cyanophyta (blue green)

2 Chlorophyta (grass green)

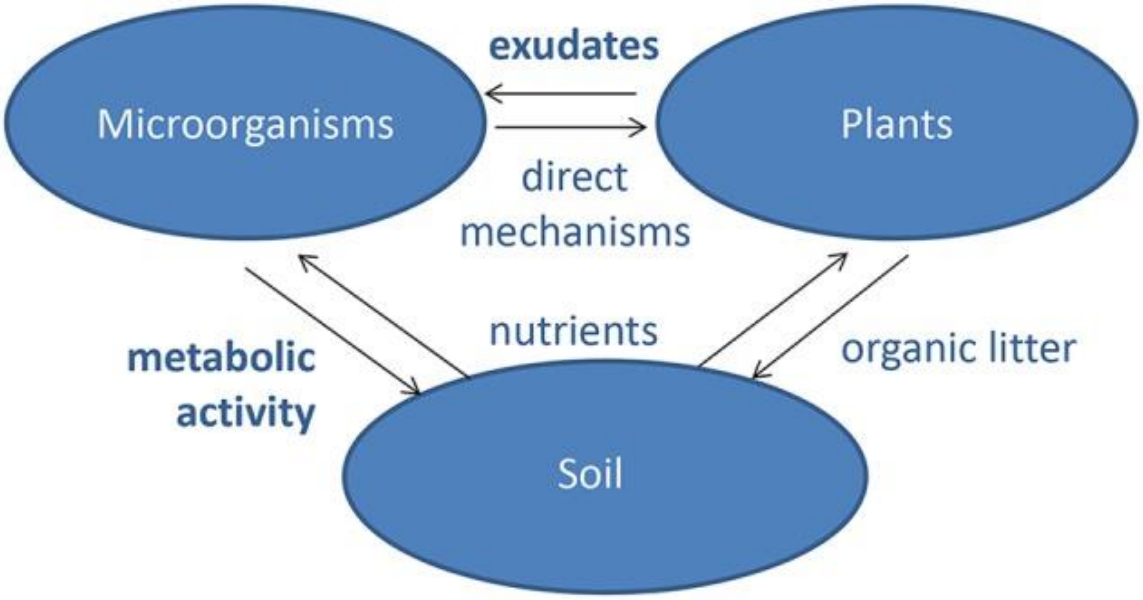
3 Xanthophyta (yellow green)

4 Bacilliriophyta (golden brown)

Blue green alage also known as Cyanobacteria are most important from the agricultural point of view because they fix atmospheric nitrogen. Some other examples- Anabaena, Nostoc etc.

5 Protozoa- Soil protozoa are single cell organisms belong to animal kingdom and larger in size than most microorganisms found in the soils.

Interactions



Interactions between plants, microbiota, and soil. Both plants and microorganisms obtain their nutrients from soil and change soil properties by organic litter deposition and metabolic activities, respectively. Microorganisms have a range of direct effects on plants through, e.g., manipulation of hormone signaling and protection against pathogens. Plants communicate with the microorganisms through metabolites exuded by the roots.

Role of microorganism in soil fertility

1 Microbes can make nutrients and minerals in the soil available to plants, produce hormones that spur growth, stimulate the plant immune system and trigger or

dampen stress responses. In general a more diverse soil microbiome results in fewer plant diseases and higher yield.

2 The group of bacteria called rhizobia live inside the roots of legumes and fix nitrogen from the air into a biologically useful form.

3 Mycorrhizae or root fungi form a dense network of thin filaments that reach far into the soil, acting as extensions of the plant roots they live on or in. These fungi facilitate the uptake of water and a wide range of nutrients.

4 Stenotrophomonas rhizophila increases drought tolerance in crops such as sugar beets and maize. The microbe excretes molecules that help plants withstand stress, including osmoprotectants, which prevent the catastrophic outflux of water from plants in salty environments.

5 Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms.

6 Soil microorganisms are responsible for the decomposition of the organic matter entering the soil (e.g. plant litter) and therefore in the recycling of nutrients in soil.

7 Soil microorganisms produce compounds that stimulate the natural defense mechanisms of the plant and improve its resistance to pathogens. Collectively, these soil microorganisms have been termed 'biopesticides' and represent an emerging and important alternative (i.e. biological control) to the use of chemical pesticides for the protection of crops against certain pathogens and pests.

Rhizosphere and Phylosphere

The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. The rhizosphere contains many bacteria and other microorganisms that feed on sloughed-off plant cells, termed rhizodeposition, and the proteins and sugars released by roots.

The active root zone of the plant contact with the soil is termed as ‘rhizosphere’ which play an important role in maintaining of plant-microbe relationship.

The microbial population of rhizosphere has an important influence on the growth of the plant. The interaction of plant root and rhizosphere microorganisms are based largely on interactive modification of the chemical soil environment by processes such as, water up take by plant system , release of organic chemicals to the soil by plant roots, microbial production of plant factors and microbial mediated availability of mineral nutrients.

Microbial population in the rhizosphere soil may benefit to the plant various ways, including removal of H2S, toxic to the roots, increased solubilization of mineral nutrients, synthesis of vitamin, amino acids, auxins and gibberellins which stimulate the plant growth and antagonism with potential plant pathogen through competition and development of a mensal relationships based on production of antibiotics.

The Phyllosphere: The stem, leaves and fruits of a plant provided suitable habitats for some microbial populations such as, heterotrophic and cyanobacteria, fungi, lichens and some algae which are occurring in the aerial plant surface. Such type of growing plants are called 'epiphytes'. The habitat in the leaf surface is known as 'phyllosphere' to denote leaf surface environment. The name was coined by Last (1955) and Ruinen (1956) independently. The term phyllosphere and phylloplane are interchangeably used in literature. Phylloplane is a natural habitat on leaf surface which support heterogeneous population both pathogen and non-pathogens. The phylloplane microbes cover a wide variety of microorganisms including yeast, filamentous fungi, bacteria, actinomycetes, blue green algae etc. The phylloplane mycofiora is of special interest from various view point because some of them have antagonistic action against fungal pathogen, degrade plant surface wax and cuticles and produce plant hormones as well as activate plants to produce phytoalexinins.

Rapid Tissue Tests for Soil and Plant

The growth and productivity of plant depends many factors of which, the nutrient Content of plant parts such as leaf, stem, etc play a vital role. Moreover the leaf and stem are considered as the indicator parts of plants for assessing the nutrients content of plant. Each crop plant requires the essential element at a specific concentration at different growth stages and it is known as 'critical level'.

When the nutrients content of plant reaches below the critical level the plants may exhibit deficiency symptoms. The availability of nutrients can be determined by-

i) Plant diagnosis

ii) Soil analysis and

iii) Plant analysis by two methods

a) Qualitative test

b) Quantitative estimation.

Based on the plant or soil tests, the required nutrients can be applied for crops to sustain the growth and rectify the deficiency disorders.

The rapid tissue test would pave way for rectifying the nutritional problems for quick recovery; however the quantitative estimation of both plant and soil for nutrients concentration will be more useful and economic for applying fertilizers either as basal or foliar and would be the long term strategy to cope up with nutritional problems.

On dry weight basis, the normal healthy cultivated crop plant will have the foliar concentration of essential elements. Nevertheless it will vary depends up on the variety, type of soil, growth stage and other environmental and cultural operations.

Nitrogen	: 1.0 to 3.0 %	Iron	: 20 to 100	ppm
Phosphorus	: 0.05 to 1.0 %	Zinc	: 15 to 50	ppm
Potassium	: 0.8 to 1.2 %	Manganese	: 2.0 to 10	ppm
Calcium	: 0.3 to 0.6 %	Copper	: 10 to 20	ppm
Magnesium	: 0.2 to 0.4 %	Boron	: 5 to 15	ppm
Sulphur	: 0.2 to 0.3 %	Molybdenum	: 0.5 to 5.0	ppm

For rapid tissue test to assess the nutrient status, different parts of plant should be taken as indicator tissue and some of the representative crops are furnished below:

Crops	Nutrients					
	N	**P**	**K**	**Ca**	**Mg**	**S**
Cereals	Stem/Midrib	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Pulses	Petiole	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Oil seeds	Petiole	Leaf blade	Leaf blade	Leaf lamina	Leaf lamina	Leaf blade
Cotton	Petiole	Petiole	Petiole	Petiole	Petiole	Petiole
Banana	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina	Leaf lamina
Papaya	Petiole	Petiole	Petiole	Petiole	Petiole	Petiole
Vegetables	Petiole Leaf blade	Petiole Leaf blade	Petiole Leaf blade	Petiole Leaf blade	Petiole Leaf blade	Petiole Leaf blade

Fruit trees- Either leaf blade/mid rib/leaf lamina can be taken.

Ornamentals, Tea, coffee, etc.,- The leaf blade should be taken.

Micronutrients: The leaf lamina/ leaf blade/ mid rib portion of leaf can be taken.

Procedure for tissue test

1. Nitrogen

Reagent: 1-% diphenylamine in conc. sulphuric acid.

Small bits of leaf or petiole are taken in a petridish and a drop of 1% diphenylamine is added. The development of blue colour indicated the presence of nitrate – nitrogen. The degree of colouration indicates the amount of nitrogen present in that leaf.

Dark blue: Sufficient Nitrogen

Light blue: Slightly deficient Nitrogen

No colour: Highly deficient Nitrogen

2. Phosphorous

Reagents: (1) Ammonium molybdate solution, (2) Stannous chloride powder.

Eight gm ammonium molybdate is dissolved in 100 ml of distilled water. To this, add 126 ml of conc. Hydrochloric acid (HCL) and volume is made up to 300 ml with distilled water. This stock solution is kept in an amber coloured bottle and at the time of use it is taken and diluted in the ratio of 1:4 using distilled water.

A tea spoonful of freshly chapped leaf bits are taken in a test tube and 10 ml of ammonium molybdate reagent is added and kept for few minutes. After shaking, a pinch of stannous chloride is added. Colour development is observed.

Dark blue: Sufficient Phosphorus

Bluish green: Slightly deficient Phosphorus

No colour: Highly deficient Phosphorus

3. Potassium

Reagent: (1) Sodium cobalt nitrate reagent, (2) Ethyl alcohol (95%).

Take 5 gm cobalt nitrate and mix with 30 gm of sodium nitrate in 80ml of distilled water. To this, 5ml of glacial acetic acid is added. The volume is made up to 100 ml distilled water. Dilute reagent prepared (5 ml) with 15 mg sodium nitrate to 100 ml using distilled water.

Finally cut leaf bits are taken in a test tube and 10 ml diluted reagent is added and shaken vigorously for few a minutes and kept for 5 minutes. Then add 5 ml of ethyl alcohol reagent, allowed to stand for 3 minutes. The solution is observed for the formation of turbidity.

No turbidity: Deficiency of Potassium

Slightly turbidity: Moderate deficiency

High turbidity : Sufficient Potassium

4. Calcium

Morgan's Reagent: 30 ml of glacial acetic acid and 100 grams of sodium acetate are dissolved in a little of distilled water

Procedure: 0.5 g of finally cut plant material is taken into a glass vial (both of healthy plant and deficient plant in different vials) and 5 ml of Morgan's reagent is added in test tube. After allowing it to stand for 15 minutes, 2 ml of glycerin and 5 ml of 10% ammonium oxalate is added and the solution is shaken for 2 minutes. The turbidity resembling after 15 minutes indicate the amounts of calcium in normal plant tissue.

5 .Magnesium Reagents

(1) 5% pure sucrose solution

(2) 2% Hydroxylamine hydrochloride

(3) Titan yellow

(4) Sodium hydroxide

150 mg of Titan yellow is dissolved in 75 ml of 95% ethyl alcohol and 25 ml distilled water. This solution is stored in darkness.

Procedure

To a tea spoonful of finely cut material, following reagents are added in sequence. One ml of 5 % sucrose solution, 1 ml of 2 % Hydroxylamine hydrochloride and 1 ml of Titan Yellow. Finally solution was made alkaline with 2 ml of 10% NaoH. Red colour indicates the presence of magnesium and yellow colour indicates absence or traces of Magnesium.

6. Iron

Finely cut leaf materials (0.5g) are taken into a glass vial and 1ml of con. Hcl is added in it. After 15 minutes, 10ml of distilled water and 2-3 drops of con HNO3 are added. 10 ml of this solution is pipetted out into a specimen tube after 2 minutes and 5ml of 20% ammonium thiocynate is added and stirred. Further, 2 ml of amyl alcohol is added, shaked well and allowed to stand for few minutes. The intensity of red colour in amyl alcohol layer indicates the quantity of iron.

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