

LECTURE NOTE

SOS 517

ANALYTICAL TECHNIQUES IN SOIL SCIENCE

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INTRODUCTION

All scientists depend on one form of analysis or the other. This is due to the need to answer questions on the content of the materials with which they work. The methods by which these analyses are carried out are referred to as analytical techniques. The types of analytical techniques may vary from field to field. This variation stems primarily from differences in the nature of materials with which people in each field works.

Soil science is branch of applied sciences that makes use of the principles of physics, chemistry, biology and mathematics in the study of soil. This is because the soil consists of matter (living and non-living) interacting together physically, chemically and biologically to provide ecosystem services at all levels of consideration. For a soil scientist to be able therefore to answer questions on the constituents of soil, he needs to carry out one form of analysis or the other. Methods of analysis in soil science consist of chemical, biological and physical techniques.

A. CHEMICAL TECHNIQUES

The soil is a mixture of elements in various states of occurrence, combination and interaction. Isolation and measurement of components of interest in soil is a major problem. Therefore, chemical analysis is aimed at determination of the component of interest in a soil sample. Chemical analysis has to be carried out in defined steps requiring possession of certain skills.

STEPS IN DETERMINATION OF CONCENTRATION

The final measurement is just only a step in the sequence of operations that are necessary for determination of component of interest (analyte) in a sample. It is therefore relevant to identify the several steps that male up the analytical process, the importance of the steps and the skill needed.

Choice of method

It is a vital step that requires both experience and intuition on the part of the analyst.

Choice of method is influenced by factors such as

- the form of component of interest
- desired level of accuracy and economy
- consideration for number of samples
- complexity of the sample to be analysed
- number of components in the sample

Collection and preparation of sample

To obtain meaningful result, analysis has to be performed on soil sample which must be representative of the bulk material (the soil of a plot, field, or an area). Obtaining representative and appropriate form of samples requires knowledge of sampling methods and statistics. Thorough mixing is needed for bulky and heterogeneous material.

Preparation of sample is to have the sample in the correct form for the analysis. The process may involve drying, homogenization, procuring the amount of the sample by

weight, volume or area, having the sample in the correct form for the analysis. Results of quantitative analysis are usually reported in relative terms (quantity of analyte per unit weight or volume of the sample) In order to express the result in meaningful manner, one should know the accurate weight or volume of the sample to be analysed.

Often, an analyte in its matrix is not in the form suitable for the chosen assay method. Bringing the analyte into the best chemical form may involve changes in the analyte or transfer from one matrix to another.

Terms for some of the methods used to optimize chemical forms in soil analysis include the following:

- *Dissolution*: Breaking down of component parts in a liquid. The transformation is driven by the binding of solutes by the solvent.
- *Digestion*: Breaking down into component parts which dissolve in a liquid. It is driven by externally applied energy.
- *Desorption*: Detachment from surface.
- *Vaporization*: Transformation into gaseous form usually by strong heating.
- *Atomization*: breaking a collection of atoms into individual atoms.\
- *Adsorption*: Binding to a surface.
- *Oxidation*: Causing the oxidation state of an element or compound to become more positive.
- *Reduction*: Causing the oxidation state of an element or compound to become more negative.
- *Extraction*: Transferring analyte from one matrix into another. The first matrix is a solid or liquid, the second a fluid.

Knowledge of descriptive chemistry and competitive equilibria are important in preparation of sample in appropriate form for analysis. Sample preparation not only helps in optimizing chemical forms of analyte but also in eliminating expected interferences (substances that prevent the direct or accurate measurement of the analyte).

Running the assay

All preliminary steps are taken in order to make the final measurement a true estimate of the analyte. The assay may involve measurement of weight or volume which can be directly or indirectly related to the amount of the analyte in the sample. It may be by measurement of some properties of the system containing the analyte, which can then be related to the concentration of the analyte in the sample. To properly relate the property measured with the concentration of analyte requires calibration of standard samples. Running an assay demands that the analyst be skillful in both molecular and elemental methods of analysis.

Calculations and interpretation

The raw data or graphical output obtained as measurement during the assay needed to be rendered in numerical answers. This may involve data reduction, statistical analysis, data transformation, and calculation.

CLASSIFICATION OF ANALYTICAL TECHNIQUES IN SOIL SCIENCE

Analytical techniques are generally divided into two groups:

- Qualitative – identifies the species composition in a sample. It answers the question of the presence or absence of the component of interest without really answering the question of how much is present.
- Quantitative – determines how much of the component of interest in the sample. It does not only indicate the presence or absence of the component of interest but actually specifies the quantity of the component of interest that is present in the sample.

Both qualitative and quantitative analyses

- Require measurement of some chemical or physical properties of the system which can be related to the desired information.
- Require preliminary treatments and procedures to ensure measurement of analyte (component of interest)

However, quantitative analysis further requires that

- the analyst works with the aim of keeping loss of components to the barest minimum
- the reaction on which the analysis is based proceeds to completion with formation of a single product
- there is reproducible relationship between the property or quantity being measured and the analyte
- systematic and orderly work habits be development of, and intellectually honest observations

QUANTITATIVE ANALYSIS

In every quantitative analysis, there is a final measurement which can be related in magnitude to the quantity of the analyte. Quantitative analysis can be generally classified on the basis of this final measurement. Majority of early analytical techniques involved weight and volume as their final measurement. These techniques are generally referred to as *classical methods* of analysis. Other methods that were later developed based on the measurement of some properties of the analyte (or the matrix containing the analyte). These methods which relate the properties of the analyte such as optical, thermal and electrical are termed instrumental methods.

Both classical and instrumental methods involve correlation of a physical measurement with the concentration of the analyte. Both methods also involve the use of instruments, and preliminary steps in the process of analysis

Classical Analytical techniques

- Gravimetric techniques** involve weight as the final measurement
 - Direct method* – involves weighing of compound that contains the analyte. For example, in determination of carbon by Dumas method, CO_2 is weighed.
 - Indirect method* – involves loss of weight due to combustion or volatilization. Example is determination of CO_2 in CaCO_3 by addition of HCl.
- Volumetric techniques** – (also titrimetric techniques) involve measurement of volume of solution that is equivalent to the analyte. Titrimetric techniques include

acid-base titration, redox titration, complexiometric titration and precipitation titration

Instrumental techniques

- a. **Spectrophotometric techniques** – involve measurements based on interaction of electromagnetic waves with analyte. This could either be absorption or emission. These techniques could be further classified based on the range of wavelength involved, whether the interaction is emission or absorption, and the instruments used.

Visible/Ultraviolet spectrometry – involves measurement in the region of visible light and the ultraviolet wavelengths. The simplest form involves colour identification. Simple colorimetry relates colour intensity to the concentration of the analyte.

Emission spectrometry – based on measurement of intensity of characteristic electromagnetic waves emitted by analyte as electrons of the excited atoms return to the ground state. Emission spectrometry is further distinguished on the basis of energy source.

Absorption spectrophotometry – involves relating characteristic electromagnetic waves absorbed by analyte to its concentration.

- b. **Electroanalytical techniques** – involve measurement of electrical properties of the chemical system containing the analyte in relation to the analyte. Electrical properties measured include potential differences, amount of charges, current, resistance and conductivity. Electroanalytical techniques include

Potentiometric technique relates the concentration of analyte to its potential difference as compared to that of reference system. We have direct potentiometric methods - e.g. use of selective ion electrodes such as the H⁺ electrode for pH determination. Potentiometric titration makes use of visual indicators that change with the potential of the solution

Conductimetric technique – involves measurement of changes in resistance of electrolyte (solution containing the analyte) with changes in concentration of the analyte. Conductance = $1/R$ where R is the resistance

Coulometric technique – relates the number of moles of analyte oxidized or reduced to total electrical charge. When electrical current is the property measured the technique is referred to as *amperometry*

Voltametric technique – combines the measurement of potential, current and time i.e. the change in current (over time) while the voltage applied to the system is changed in a precisely controlled manner.

- c. **Separation techniques** –

Solvent extraction

Chromatographic techniques – Chromatographic techniques generally include the paper, thin layer chromatography (TLC), gas liquid chromatography (GLC), column chromatography, ion exchange chromatography, and electrophoresis.

TITRIMETRIC AND GRAVIMETRIC TECHNIQUES IN SOIL ANALYSIS

Titrimetric techniques

There are four types of titrimetric techniques: Acid-base, potentiometric, precipitation and complexiometric titrations.

Acid-base titration

These are titrations that are based on the neutralization reaction. Acid-base titrations can be used to determine most strong monoprotic acids. Acid-base titration can be used to determine concentration of hydrochloric acid, sulfuric acid, acetic acid, as well as bases - like sodium hydroxide, ammonia and so on. In some particular cases, when solution contains mixture of acids or bases of different strengths, it is even possible to determine in one titration composition of a mixture - for example determination of exchangeable acidity (H^+ and Al^{3+}) in soil.

Most commonly used reagents are hydrochloric acid and sodium hydroxide. Solutions of hydrochloric acid are stable; solutions of sodium hydroxide can dissolve glass and absorb carbon dioxide from the air, so they should be not stored for long periods of time.

Type of indicator depends on several factors including the equivalence point pH.

Potentiometric titration

Potentiometric titrations are based on redox reactions.

There are many redox reagents used in redox titrations. For example, potassium permanganate is used for determination of Fe^{2+} , H_2O_2 and oxalic acid, potassium dichromate for determination of Fe^{2+} and Cu in CuCl. Determination of organic matter in soil is achieved by redox titration of Ferrous sulphate with excess potassium dichromate after oxidation of soil organic matter with dichromate -acid mixture.

Commonly used indicators are substances that can exist in two forms - oxidized and reduced - that differ in color. Potential at which the substance changes color must be such that the change occurs close to the equivalence point. Examples of such substances are ferroin, diphenylamine or Nile blue.

Complexiometric titration

Complexiometric titration can be used to determine metal concentration in soil extract with the use of Ethylenediaminetetraacetic acid (EDTA) as the reagent. The reagent acts as chelating agent as it forms complex with the metal. There are also other similar chelating agents used in complexiometric analysis, but the common one in soil analysis is the EDTA.

In the determination of metals detection of the endpoint is mainly based on substances that change color when creating complexes with determined metals. One of these indicators is eriochrome black (a substance that in pH between 7 and 11 is blue when free, and black when forms a complex with metals). This method can be employed in determination of Ca and Mg.

Precipitation Titration

This is typified by titration of chloride with silver. It is rarely applied directly to soil, but can be applied to soil extract because of interferences.

SEPARATION TECHNIQUES

QUANTITATIVE SOLID-LIQUID EXTRACTION

Generally, in quantitative solid-liquid extraction, a weighed solid is placed in a closable container, and some solvent is added. The solid and liquid are mixed well, and the liquid is separated from the solid. The procedure involves using a liquid to dissolve the analytes that are part of the solid (but not covalently bound within it).

In soil science, extraction involves the transfer of analytes from the soil matrix into solution which is then separated from the soil either through centrifugation or filtration.

The process of soil extraction is based on the ion exchange phenomenon. During extraction, ions in solution referred to as the *extractant* exchange for ions that are electrovalently bonded to the charged sites on the surfaces of soil particles.

CHROMATOGRAPHY

Chromatography can be defined as the science and art of separating the components of materials from each other. Such separations are achieved using a variety of techniques based on diverse molecular differences such as molecular charge, molecular size, molecular mass, bond polarity, redox potential, ionization constants, and arrangement of bonds such as isomer structure. However, separation methods that use electric fields to drive charged molecules so that they separate are not generally included in chromatographic techniques. Such techniques are referred to as electroseparation, electromigration and electroporesis.

ION EXCHANGE CHROMATOGRAPHY

Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on the charge properties.

Principle of Ion-exchange chromatography (IEC)

Separation in ion exchange chromatography depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge. The process of IEC is in five main stages.

1. The first stage is equilibration in which the ion exchanger is brought to a starting state, in terms of pH and ionic strength, which allows the binding of the desired solute molecules. The exchanger groups are associated at this time with exchangeable counter-ions (usually simple anions or cations, such as chloride or sodium).
2. The second stage is sample application and adsorption, in which solute molecules carrying the appropriate charge displace counter-ions and bind reversibly to the

- gel. Unbound substances can be washed out from the exchanger bed using starting buffer.
3. In the third stage, substances are removed from the column by changing to elution conditions unfavourable for ionic bonding of the solute molecules. This normally involves increasing the ionic strength of the eluting buffer or changing its pH.
 4. The fourth the removal from the column of substances not eluted under the previous experimental conditions and
 5. The fifth stage is re-equilibration at the starting conditions for the next purification.

Separation is obtained since different substances have different degrees of interaction with the ion exchanger due to differences in their charges, charge densities and distribution of charge on their surfaces. These interactions can be controlled by varying conditions such as ionic strength and pH.

There are two types: Cation chromatography, and ion chromatography

SPECTROPHOTOMETRIC TECHNIQUES

These involve measurement of some electromagnetic properties generated based on interaction with the analyte. Such interactions could be emission or absorption.

These techniques could be grouped into

- (1) Ultra violet/visible spectrometry
Involves colour identification(colorimetric method) and intensity measurement by instrumental method.
- (2) Atomic emission and absorption spectrophotometry:
Involves measurement of characteristic electromagnetic waves (visible/invisible) absorbed or emitted by the system

Atomic emission spectrophotometry is based on the principle that excited atoms emits electromagnetic energy of specific wavelengths upon returning to ground state which is proportional to the concentration of the excited atoms.

METHODS OF STUDYING SOIL MICROBIAL ECOLOGY

Much of the study of microorganisms is concerned directly or indirectly with assessment of biochemical reactions brought about by microorganisms. In order to study these reactions, methods have evolved in which organisms are grown in pure culture under conditions which encourage maximum growth. The techniques which give

information on the conditions of organisms in soil are divided into four broad groups. They include;

1. Determination of form and arrangement of microorganisms in soil
2. Isolation and characterization of soil microorganisms
3. Detection of microbial activity in soil
4. Determination of microbial biomass

Detection of form and arrangement of microorganisms in soil

Direct observation of microorganism in the soil provides a basis for ecological studies since it gives direct evidence of the occurrence of microbes in particular environments. This is because microorganisms are not uniformly distributed throughout the soil and their precise arrangement varies spatially and temporally. Methods employed for studying microbial population in soil include;

1. Microscopic examination of stained soil
2. Haemocytometer method
3. Contact slide method
4. Soil enrichment method

Microscopic examination of stained soils

This method consist of preparation of a suspension of soil in a dilute fixative solution, 1 or 2 drops of the suspension is spread upon a clean slide which is then dried and stained with acid dye and finally examined with a high magnification microscope.

The fixative solution is prepared by dissolving 0.15 g of gelatin in 1 litre of distilled water. The staining solution consist one gram of erythrosine or Rose Bengal dissolved in 100 ml of 5 % aqueous solution of phenol containing sufficient CaCl_2 (0.001-0.1 %) to give a very faint precipitate

Haemocytometer method

A known quantity of soil is suspended in molten agar gel, small drops of agar are removed and placed in the haemocytometer slide of a known depth and allowed to solidify. The films are dried and stained in a solution of acetic aniline blue followed by dehydration in alcohol. Differential counts of measured area of the film will give a quantitative estimation of the microorganism in a given amount of soil.

Contact slide method

This consists of making a slit in the soil with a sharp knife and inserting into the slit a clean slide. The soil is then pressed gently to bring it in contact with the slide, which is left in position for about 1-3 weeks. The slide is removed, clean on one side and fixed on the other side by passing it over a flame. It is washed gently in clean tap water to remove coarse soil particles, followed by distilled water and then stained with phenol erythrocin for 30 minutes at room temperature. The slide is washed, dried and examined under the microscope. The merits of this method include;

1. It is effective for determining the influence of lime, pH, nutrients, especially NPK and salts on the numbers of microorganisms.

2. It is good for the study of specific soil treatment upon the rhizosphere, or the relationship between plants and microorganisms.

Limitation

The method is inadequate for determining the function of microorganism in the soil

Direct examination of unstained soil

This method employs the use of a strong microscope to examine the soil directly. The microscope was developed by Kubiens. It has a special surface illumination; it has not been extensively used.

A frequently reported limitation of microscopic method is that the number of the microorganisms can only be estimated as small particles of soil may also be counted along with the microorganisms.

Isolation and characterization of soil microorganisms

Microorganisms more often, have to be isolated from the soil and grown in culture before they can be identified and their activities assessed. Microbial Isolation can be considered in three ways.

- i. Growth media
- ii. Transfer of organism to growth media
- iii. Isolation of organisms

Growth media

Two types of growth media are used; (a) non-selective media (b) selective media.

Non-selective media

These are also called broad spectrum media they are designed to isolate broad groups of microorganisms. In practice the media are prepared to favour the development of fungi, bacteria, actinomycetes, algae or other autotrophic forms.

Normally fungi develop best on media with a high carbon: nitrogen ratio, e.g.

Czapek Dox agar (sucrose 50 g, NaNO_3 2 g plus mineral salts).

While bacteria grow better in media with a low carbon: nitrogen ratio, e.g. nutrient agar (peptone 5 g, beef extract 3 g with no sugar).

Actinomycetes are able to attack resistant and complex nutrients and are often isolated by incorporating carbon and nitrogen sources like starch, casein, chitin, humic acid into the media.

Most algae are able to synthesize their own nutrients and therefore are grown in extremely simple inorganic salt solutions.

Selective media

These are also called selective media and are designed to encourage the growth of one or a few organisms at the expense of all the others. Media may be selective in the following ways.

1. By adding a substance used by a particular organism but not by others e.g. cellulose as a sole carbon to select cellulolytic organisms
2. By omitting substances required by most organisms but not the one being isolated, e.g. leaving out all organic matter, while supplying ammonium ions as a source of energy and nitrogen encourages the growth of nitrifying bacteria.

3. By altering the reaction (pH) of the media e.g. by acidification with acetic acid allows the slow growth of lactobacilli and inhibiting many other bacteria.
4. By adding selectively microbiocidal substance e.g. P-chloro-nitro-benzene kills most fungi but allows *Fusarium* to grow.
5. By altering the condition of incubation e.g. exposure to high temperature allows the growth of thermophilic organisms, while the exclusion of oxygen favours the development of anaerobes.

Different methods may be successfully combined to isolate very specific fraction of the microflora. For example it is possible to devise a medium for the isolation of a thermophilic anaerobic cellulose decomposing bacterium capable of utilizing inorganic nitrogen.

Selective media are used mostly for the isolation of bacteria. This is because bacteria are most conveniently studied by grouping them according to their biochemical properties. Fungi and algae are easier to identify because of their morphological diversity and biochemical uniformity. Fungi are all aerobic heterotrophic while bacteria may be aerobic or anaerobic, heterotrophic or autotrophic.

Transfer of organism to media

This can be achieved either by or indirect method.

Direct Isolation of soil microorganisms: This method involves the use of micromanipulator techniques the method is difficult because the organisms are so small. Often these methods isolate a more representative selection of the soil microflora since one is removing from morphologically visible and different organisms.

Isolates may be biased in favor of clearly visible organism e.g. dark pigmented forms while transparent hyaline forms are under-represented.

Another problem is that often the organisms failed to grow on media either because they are dead or the medium is unstable.

Indirect transfer methods: This involves the preparation of a soil suspension in water or mineral solution and the addition of this suspension or a dilution of it to the isolation medium. Often the suspension is mixed with an agar medium, poured into a petri dish and allowed to set. This method also allows the determination of microbial populations e.g. the dilution plate method.

Alternatively, a small volume of the suspension is spread over the surface of a plate of solid agar medium so that colonies develop only on the surface.

This method is useful where low numbers of organisms are present in the soil as it is often the case with fungi.

Lumps of soil or pieces of plant root may be placed on the surface of the agar or dispersed in small amounts in molten agar.

Isolation of organisms

Once the soil organisms have transfer to agar media, isolation can be achieved by sub-culturing morphologically recognized and differentiated bacteria and fungi colonies into newly prepared solid media.

Fungi develop from pieces of mycelium as well as spores, some methods have been devised to distinguish between organisms present as spores and those present as vegetative cells.

These methods include;

- i. Killing method: Drying the soil and comparing the fungal colonies that developed from isolation plates before and after drying.
- ii. Growth method: substrates are introduced into soil but are separated from the soil particles by air gap. It is assumed that the fungi that have grown across the gap are vegetative.
- iii. Washing method: if soil particles are agitated vigorously in water, saline or dilute solutions of surface active agents, spores which are readily detachable are removed while the more firmly attached fungal mycelium remains. This increases the chances of isolating fungi present as mycelium, slower growing forms and forms inside organic particles.

Detection of microbial activity in soil

The activity of microorganisms in soil can be estimated by determining rate of mycelial extension or cell division, the rate of respiration, enzyme content of the soil or by determining the rate of substrate disappearance and metabolite accumulation.

Mycelial extension:

Rate of mycelial growth in fungi can be measured by placing sterile soil in a growth tube and inoculating one end of the tube with that fungus. At a specific interval, the soil is sampled along the tube and the rate of spread is determined.

Alternatively, sterile soil can be placed in a petridish, inoculated centrally and the rate of outgrowth is determined by transferring medium into another petridish.

Rate of respiration:

The overall metabolic activity of soil microflora is determined by oxygen uptake and carbon dioxide output. When organic matter is attacked by microorganisms, the following reaction takes place



Only 60-80 % of the carbon is converted to carbon dioxide even under fully aerobic conditions because of incomplete oxidation of the substrate, giving rise to intermediates and the synthesis of cellular materials.

Fungi and actinomycetes use a greater percentage of metabolized carbon for growth than bacteria.

Theoretically, one molecule of CO_2 is liberated for every molecule of oxygen taken up i.e. the respiratory quotient (RQ) is 1. However, this is rarely observed in the soil for many reasons;

- i. Carbon : nitrogen ratio of substrate can alter the RQ,
- ii. In anaerobic respiration no oxygen is taken up.
- iii. Carbon dioxide may be liberated chemically from soil through the action of microbes producing acids on soil carbonates.
- iv. Oxygen may become bound in soil water and not liberated.

In spite of these limitations respiration still remain the most frequently used method for assessing the activities of microorganism in soil. It can be measured both in the field and laboratory

Measurement of microbial respiration in the field is done by analyzing given volumes of soil atmosphere, usually obtained by pumping air from the soil over a period of time.

It can also be done by determining the amount of carbon dioxide into an enclosed space above the soil surface.

The limitation of these methods is the inability to distinguish microbial respiration from root respiration.

Laboratory measurement of respiration is by removing known volumes of soil and placing them in containers which are incubated under controlled environmental conditions.

Limitation of this method include high rate of respiration at the beginning of the experiment due to the gross disturbance of soil samples, however after a period of equilibration, rate of respiration drops to a low but constant level.

Enzyme content of soil:

During respiration, oxidation of organic substrate is accompanied by reduction of molecular oxygen. In the process hydrogen is removed from the substrate by the action of dehydrogenase enzymes.

Therefore the activity of microorganisms can be assessed by measuring dehydrogenase activity in the soil.

Dehydrogenase activity is measured by reacting the enzymes with tetrazolium dyes which are converted to insoluble red coloured formazan compounds, the intensity of the red colour can be related to enzyme activity.

The main limitations of the technique are (i) obtaining efficient of the enzyme from the microorganisms and then from the soil, (ii) it is rare for enzyme activity to correlate with soil fertility and microbial activity because enzyme activity in the soil is a manifestation of several biological parameters.

Substrate utilization and metabolite accumulation

Rate of substrate accumulation can be measured directly through chemical analysis and indirectly by following the production of metabolites.

In a typical method described by Pochon (1957) a series of soil dilutions are inoculated into media containing the presence or absence of the substrate and its breakdown products.

The activity of the soil population in bringing about this changes is expressed in graphical form by plotting time against the first dilution in which the substrate has disappeared or in which the metabolite have appeared.

Determination of microbial mass

The activity of the soil microflora has often been linked with the number of microorganisms in a soil or occasionally with the microbial biomass.

Biomass is most conveniently determined by counting the number of microbial cells or measuring the length of mycelium present in a soil sample, calculating the volume of such cells and multiplying this figure by a notional value of specific gravity.

There are four methods of determining microbial counts.

1. **Counting by direct observation:** This includes methods discussed earlier as microscopic methods, the most accurate is the haemocytometer method.
2. **Counting cells developing in culture:** The number of living cells are usually estimated in culture techniques, the most popular being the dilution plate technique. A known weight of soil is suspended in a saline solution and shaken or stirred vigorously so that the microorganisms are detached from soil particles and the cells become dispersed. Dilutions of the suspension are made usually in tenfold and known volumes of each dilution are mixed with an agar medium in a petridish. After incubation, cells give rise to colonies and by counting these the numbers of viable cells can be calculated.
3. Bacteria in particular physiological group can be estimated using the **most probable number (extinction dilution) method**. An extended series of tenfold dilution is prepared and 1 ml of each dilution is inoculated into several tubes of medium. After incubation, the number of tubes showing growth is recorded and most probable number of organisms accounting for the result is calculated statistically. The method is less accurate than the dilution plate count.
4. **Determination of biomass without counting:** This is a more method of estimating microbial biomass. When a soil is sterilized with chloroform and then re-inoculated with non-sterile soil a small fraction of the organic matter is rapidly converted to carbon dioxide. This fraction comprising about 2.3-3.4 % of the soil carbon is considered to have been derived from the recently killed microorganism and therefore represent the percentage of carbon in the biomass.

ISOTOPE RATIO ANALYSIS AND RADIO-ISOTOPE IN SOIL PLANT SYSTEMS

The use of radio-isotope in studying processes relevant to nutrient cycling allows the easy monitoring of elements involved as they go through different transformations.

Detection of radioactivity or increased abundance in the case of stable isotopes in a given compartment of the ecosystem is a proof of its origin.

An example is the amendment of radio-isotope labeled fertilizers to a given agro-ecosystem to assess such things as fertilizer efficiency, losses to various environmental compartments and turnover in soil.

Traditionally, carbon, nitrogen and phosphorus are considered as key elements. Therefore isotope studies relating to the cycling of these three elements are usually emphasized.

Isotopic measurement of nitrogen in soil/plant system

This is the method of choice in the measurement of nitrogen fixation.

An isotope of nitrogen other than ^{14}N that makes up virtually all of nitrogen in the atmosphere is used.

In this regard two isotopes of N are useful as tracers in nitrogen fixation experiments. These are radio-active isotope ^{13}N which has a half-life of only 10.05 mins and ^{15}N

The short half-life of ^{13}N restricts its use to experiments lasting only a few hours, so that it is not generally useful for measuring nitrogen fixation but it has been used for the studies on the assimilation of the products of nitrogen fixation.

By contrast, ^{15}N has been widely adopted. It is a more stable isotope and therefore can be used without special safety precaution. The measurement of ^{15}N from $^{15}\text{N}_2$ into biological materials has become a standard technique used to prove the presence of active nitrogen fixation in the organism.

The bacteria culture or plant tissue is incubated in an enclosed atmosphere which is enriched with $^{15}\text{N}_2$. After a period of incubation the N in the biological material is purified by digestion and distillation and the proportion of ^{15}N present is determined using mass spectrometry.

The amount of nitrogen fixed can be calculated precisely from measurements of the total N and the proportion of ^{15}N in the material, if the N-enrichment of the experimental atmosphere is known.

The incubation time and ^{15}N enrichment of the atmosphere required for the experiment depend on the rate of nitrogen fixation relative to amount of N already present in the organism.

Where isotope enrichment method using ^{15}N gas is employed;

$$\text{N fixed} = \frac{E_{\text{organism}}}{E_{\text{gas}}} \times N_{\text{plant}}$$

Where E = atom % ^{15}N gas

However, where fertilizer is the sole N source;

$$\text{N from fixation} = N_{\text{fixing plant}} \times \left(1 - \frac{E_{\text{fixing plant}}}{E_{\text{fertilizer}}}\right)$$

The size and sophistication of the incubation chamber needed depends on the duration of the experiment. Care must be taken that the oxygen is not exhausted when aerobic systems are studied.

Isotopic measurement of decomposition

Decomposition experiments with uniform labeled residues have been performed with various species. The number of species involved does not adequately cover the range of relevance to tropical farming.

The general idea of decomposition experiments with labeled residues is the detection of residues derived elements in various compartments of the system and how this evolves with time. The time of measurement is important as it predetermines the level of radioactivity to add to the system.

The guiding principle in radioisotope methodology is called **ALARA principle** this recommends that radioactivity should be as low as reasonably achievable.

The length of the experimental periods also determines the kind of isotopes that should be used. Eg, the use of ^{32}P and ^{33}P is restricted to short-time experiments in view of their short half-lives, which are 14.3 and 25.3 days respectively.

Isotope labeling on plant materials

Labeling of plant materials with ^{15}N and ^{32}P can be achieved relatively easily using nutrient solution techniques.

Labeling with ^{14}C requires a relatively sophisticated growth chamber with control of the specific activity of the atmosphere.

It is mandatory to homogenous labeling i.e constant specific activity among the different fractions in the material.

The most economical way of achieving this is by supplying radioactive $\text{Na}_2^{14}\text{CO}_3$ into an acid bath at rates dictated by a preset radioactivity level in the growth chamber.

Homogenous labeling with ^{15}N or ^{32}P , ^{33}P can be achieved by (a) growing the plant in a medium contributing minimally to N and P demand of the plant and (b) amending the nutrients distributed according to the plant needs over the growing season.

For nitrogen, ^{15}N labeled $(\text{NH}_4)_2\text{SO}_4$ can be used, whereas for phosphorus ^{32}P labeled $\text{Ca}(\text{H}_2\text{PO}_4)_2$ or ^{33}P labeled $\text{Ca}(\text{H}_2\text{PO}_4)_2$ can be used.

Radioisotope material can also be used in the field if confined to small surfaces, or in small liter bags. This material can be used as a surface litter or incorporated in the topsoil depending on the experimental objectives.

The limited amount available will require that some fragmentation of the residue be done in order to achieve sufficient homogeneity.

The main advantage of using radio-isotopic techniques is that the measurements of product are provided as time average estimates which represent the integral of any changes in the system that may have occurred during the measurement period.

One of the major limitations is the requirement of sophisticated and expensive equipment such as, a mass spectrometer to accurately quantify the isotopic composition of the system.

EVALUATION OF ANALYTICAL DATA

In chemical analysis, quantitative statements of certainty are desirable. This can be done using mathematical statistical tests. Evaluation of data is most useful for an analyst to be able to say how certain a conclusion is.

Two main questions are:

How sure are you that the experimentally obtained value is close to the true value?

How are you sure that the value obtained is the same (or different) from the value obtained on the same sample at a different time of by another person?

Validation is extremely important for an individual analyst working alone, for a group within a single laboratory, and for groups of laboratory coordinating their analyses.

To validate means to ascertain that the methodology developed to determine a specific analyte in a specific type of sample produces comparative results for great majority of analysts.

Errors

Part of improving chemical analysis is to be able to identify the place where errors can occur and be able to evaluate their magnitude. There is possibility of making mistakes at any step of the analysis. These possible mistakes are called error.

One way to find out if you have made a mistake is to repeat the whole procedure for several replicate samples or replicates. The more the replicates the higher the level of certainty of the results.

Sources of errors

Errors could be classified based on the sources as:

Personal error: - These are errors that emanate from the person carrying out the analysis (the analyst). It could be due to lack of experience, dirty or unorganized work habit, lack of attention to details, or personal defect that may pose hindrance to accurate judgement (e.g. colour blindness).

Method errors: - These are errors that emanate due to use of wrong method of analysis. Some methods may not be able to eliminate certain interferences in the course of analysis, thus leading to error.

Instrumental Errors: These are errors resulting from improper functioning of the instrument used for the measurement. It could be due to improper calibration or low sensitivity of instrument.

Errors could also be classified based on whether or not the magnitude can be ascertained and correction made or not. Errors originating from fixed cause are referred to as determinate or *systematic error*; while those originating from arbitrary or indeterminate processes are *random errors*.

Measuring Errors

When several runs of replicates are made on a sample, it is not likely that the results would be exactly the same. We can make use of the Arithmetic mean (average) with deviation from mean (absolute deviation, or relative deviation).