

## **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  $^{14}\text{N}$  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  $^{13}\text{N}$  which has a half-life of only 10.05 mins and  $^{15}\text{N}$

The short half-life of  $^{13}\text{N}$  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,  $^{15}\text{N}$  has been widely adopted. It is a more stable isotope and therefore can be used without special safety precaution. The measurement of  $^{15}\text{N}$  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  $^{15}\text{N}$  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  $^{15}\text{N}$  in the material, if the N-enrichment of the experimental atmosphere is known.

The incubation time and  $^{15}\text{N}$  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  $^{15}\text{N}$  gas is employed;

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

Where E = atom %  $^{15}\text{N}$  gas

However, where fertilizer is the sole N source;

$$E_{\text{fixing plant}}$$

N from fixation = N fixing plant X (1 - \_\_\_\_\_)

$$E_{\text{fertilizer}}$$

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  $^{32}\text{P}$  and  $^{33}\text{P}$  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  $^{15}\text{N}$  and  $^{32}\text{P}$  can be achieved relatively easily using nutrient solution techniques.

Labeling with  $^{14}\text{C}$  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  $^{15}\text{N}$  or  $^{32}\text{P}$ ,  $^{33}\text{P}$  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,  $^{15}\text{N}$  labeled  $(\text{NH}_4)_2\text{SO}_4$  can be used, whereas for phosphorus  $^{32}\text{P}$  labeled  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  or  $^{33}\text{P}$  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.

## **SOIL PHYSICAL TECHNIQUES**

Soil physical properties, mainly moisture characteristics, are measured using both classical and instrumental techniques.

### **Gravimetric Techniques**

Gravimetric measurement of soil water content is based on removal of water from the sample. This can be by evaporation, leaching or chemical reaction. The amount of water removed from the sample is determined and used to calculate soil moisture content. Determination of water content removed is done by measurement of loss of weight of the sample. by collection of the water through distillation or absorption in a desiccant.

### **Instrumental Techniques**

These are indirect methods devised to ease the labour and time involved with classical techniques. Instrumental techniques in physical studies make use of interaction of electromagnetic radiation (infrared, microwave, and radio waves) with the soil, and the electrical conductivity (or dielectric property) of the soil.

Examples include the following:

### **Neutron Scattering**

The neutron scattering method is an indirect way of determining soil moisture content by relating neutron thermalization to water content. It is based on the principle that average energy loss or thermalization is much greater when neutrons collide with atoms of low atomic weight than from collisions with heavier atoms. In soils, low atomic weight atoms are primarily hydrogen. As a result, hydrogen can decelerate fast neutrons much more effectively than any other element present in the soil or vapour state, and water being the largest source of hydrogen atoms in soil. Neutron moisture probes consist of a source of fast neutrons, a thermalized neutron detector and a protective shield. Probes may also contain a scaler for registration of counts or a meter for direct display of water content. Some neutron probes come with a built-in computer for mathematic computation.

### **Microwave Methods**

Microwave methods are remote sensing method that relates the thermal and dielectric properties of the soil to its moisture content. There are two types – passive, and active microwave..

**Passive microwave (radiometric technique):** involves measurement of thermal emission from the soil surface. The intensity of observed emission is proportional to the brightness temperature

(the product of surface temperature and its emissivity). The thermal emission is related to the dielectric property of the soil.

Active microwave: Based on the principle that the scattering coefficient of microwave from soil surface is a function of soil moisture, as well as surface roughness and dielectric properties.

### **Gamma Ray Attenuation**

This is a radioactive technique that can be used to determine soil moisture content within a 1 to 2 cm soil layer. It is based on the principles of absorption by matter of gamma rays. The amount a beam of non-energetic gamma rays attenuated or reduced in intensity in soil depends upon the soil's constituent elements and the density of the soil column. Gamma ray attenuation assumes that scattering and absorption of gamma rays is related to the density of matter in their path. It also assumes that the specific gravity of a soil remains relatively constant as the wet density changes with moisture content. Changes in wet density are measured by the gamma transmission technique and the moisture content determined from this density change. If soil constituents and bulk density without water remain constant, then changes in gamma ray attenuation represent changes in water content.

### **Nuclear Magnetic Resonance**

The use of nuclear magnetic resonance (NMR) to monitor moisture is due to the ability of NMR to identify the concentration of hydrogen atoms and thus, moisture in the soil. Placement of a soil/water mixture in a fixed magnetic field and varying the magnetic field results in an increased absorption of energy at a specific frequency of the varying magnetic field. This is called nuclear magnetic resonance (NMR). Making the device specific to hydrogen allows the NMR spectrum

to be directly related to water content of the soil; its sensitivity is affected by organic matter in the soil.

### **Thermal Methods**

These methods make use of surface temperature as an indication of moisture content based on the principle that thermal conductivity and heat capacity or thermal inertia of a porous medium depends on moisture content. Both heat capacity and thermal conductivity of a soil increase with an increase of soil moisture, and so also the thermal inertia increases.

### **Time Domain Reflectometry (Tdr)**

This technique is based on the principle that the velocity of a pulse of radio frequency injected into a transmission line (soil) depends on the dielectric properties of the transmission line.

### **Tensiometric Techniques**

These are based on the principle that the energy with which water is held by the soil (suction or soil water potential) can be defined as the common log of the height of a water column in centimetres equivalent to the soil moisture tension. Tensiometers are used to measure suction and consist of a liquid-filled porous ceramic cup connected by a continuous liquid column to a manometer or vacuum gage. The ceramic cup is porous to water and solute but not to air. From changes in water flow, changes in soil water conditions or moisture content can be determined.

As the soil water increases, it is held at a lower tension. When the tensiometer reads zero, the soil is saturated, and water tension is zero. The highest tension reading that can be obtained with a tensiometer is about 1 bar. This means that the moisture content range over which the tensiometer can be used is limited.

### **Hygrometric Techniques**

These are techniques relating relative humidity of the immediate soil atmosphere to the moisture content of the soil. Such techniques make use of sensors designed for electrical resistance, capacitance, piezoelectric sorption, infrared absorption, transmission *etc*

## **Optical Methods**

Optical methods for moisture determination include polarized light methods, and near infrared methods.

**Polarized light:** Based on the principle that the presence of moisture at a surface of reflection tends to cause polarization in the reflection beam. The percentage of polarized visible light is related to moisture content. However, calibration is affected by soil type and roughness of soil surface.

**Near Infrared methods:** Based on the principle that some bands of near infrared radiation bands are absorbed by water. The amount of radiation reflected is related to the moisture content of the soil.