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Comprehensive Manual

SOIL, PLANT AND WATER ANALYSIS IN TOBACCO

भाकृअनुप – केन्द्रीय तम्बाकू अनुसंधान संस्थान
ICAR - CENTRAL TOBACCO RESEARCH INSTITUTE
(ICAR-NATIONAL INSTITUTE FOR RESEARCH ON COMMERCIAL AGRICULTURE)
(An ISO 9001 : 2015 Certified Institute)
RAJAHMUNDRY - 533 105, ANDHRA PRADESH, INDIA

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Comprehensive Manual - Soil, Plant and Water Analysis in Tobacco

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CONTENTS

PREFACE

Tobacco a commercial crop in India, known for its contribution to the economy, employment generation and farmers' prosperity. Its rich history and economic significance demands a meticulous attention in its cultivation for enhancing the productivity, quality and profitability. Understanding the soil nutrient availability, plant nutrient status, quality composition, water quality is an integral part in tobacco supply and value chain.

In today's world of sustainable agriculture, effective resource management and enhancing their efficiency is a crucial step for sustainable and profitable tobacco production. This manual is a comprehensive guide for soil, plant and water analysis, encompassing the details of scientific sampling, analytical procedures of extraction, estimation and interpretation of the results *etc.*, The soil analysis covers the updated methods on physical, physico-chemical, chemical and microbial aspects. Plant and water analysis covered estimation of different nutrients and water quality indices. The manual also provides the information on fertilizer recommendation for different tobaccos, soil health card, laboratory safety guidelines *etc.*This comprehensive manual is of immense help to all the tobacco stakeholders including researchers, Tobacco Board officials, farmers and trade.

The previous manual on soil analysis was published 23 years ago and did not include plant nutrient analysis. Since then, significant advancements and improvements have been made in the methods for analysing soil, plants, and water. This comprehensive manual now incorporates all the latest and most updated procedures for soil, plant, and water analysis in tobacco.

The authors made all possible efforts in compiling and editing all the relevant information for soil, plant and water analysis pertaining to tobacco cultivation. I firmly hope that this manual is trusted companion and guide to all tobacco stakeholders for decision making on resource characterization and improving efficiency.

Date: 24th August, 2024

(M. SHESHU MADHAV)
DIRECTOR

INTRODUCTION

Tobacco one of the commercial crops grown in India across the different agro-ecological regions, plays a significant role in Indian economy by contributing ~Rs. 45, 000 crores towards excise revenue and export earnings. Apart, it provides employment to 45.7 million people. India is a major global player in the tobacco market, ranking second only to Brazil in tobacco exports. In India, > 15 tobacco types ***viz.,*** FCV, ***Bidi***, Burley, Chewing, Cigar, Cheroot, ***Natu, Lanka, Rustica***, ***Motihari,*** HDBRG, Oriental, ***Pikka,*** DWFC and ***Jati*** are cultivated in different states. Among them, FCV and ***Bidi*** tobaccos occupy~70% area and production. FCV tobacco is cultivated mainly in Andhra Pradesh in four different zones (Northern Light Soils, Southern Light Soils, Southern Black Soils and Traditional Black Soils) and in the transitional light soil zone of Mysore, Karnataka. ***Bidi*** and Rustica tobacco types are cultivated in Gujarat. Other important states cultivating tobacco include West Bengal, Bihar, Uttar Pradesh and Tamil Nadu.

Tobacco is a quality conscious commercial crop, where in climate and soil play a pivotal role in tobacco productivity and quality. Deficiency or excess of the certain nutrients hamper the tobacco quality thereby the exports. Each soil type presents unique challenges, from managing nutrient availability to water retention, all of which influence the growth, quality, and marketability of the tobacco produced. Hence, utmost care is to be taken for soil, nutrient and water management while selecting soils for tobacco cultivation.

Ideal soil characteristics for tobacco cultivation includes (i.) sandy surface soil up to 15 to 25 cm deep, (ii.) Sandy clay sub-soil extends up to a depth of 150 cm, (iii.) Acidic soil reaction (pH 5.5 to 6.5), (iv.) Low reserve of essential plant nutrients, (v.) Low organic matter, (vi.) Very low chloride content (less than 100 mg/kg), (vii.)The soil should be free-draining and well-aerated throughout the season, (viii.)Soil fertility status should not be high. Nitrogen starvation should prevail during leaf maturation. In modern agriculture, soil testing is the most important practice to manage fertilizer application and crop production. Without soil testing, it is very difficult to ensure the right application of fertilizers for the crop and get the optimum yield. It helps in balanced fertilizer use apart from improving the resource use efficiency. For assessing the soil health, it is imperative to identify and use a right method by which the crop response to the fertilizer can be predicted.

Plant analysis is an essential tool in tobacco cultivation, playing a vital role in nutrient management, quality control, and yield optimization. Plant analysis helps in identifying nutrient deficiencies or imbalances in the tobacco plants. By analyzing the nutrient content in the plant tissues we can optimize the fertilizer use, adjust

fertilizer dose more accurately, ensuring the plants receive the right nutrients at the right time, leading to more efficient use of resources. The quality of tobacco leaves, which directly impacts market value, is influenced by the plant's nutritional status.

Water is an important resource in tobacco cultivation and the quality of the applied water directly affects the soil and plant health. Analyzing water helps in detecting chlorides and excessive salts, that could damage the tobacco plants or degrade the soil. High concentrations of certain elements like sodium, chloride, or boron in water can lead to toxicities in tobacco plants. Water analysis helps in identifying such risks and taking corrective measures.

The previous manual on soil analysis was published 23 years ago and did not include plant nutrient analysis. Since then, significant advancements and improvements have been made in the methods for analysing soil, plants, and water. This comprehensive manual now incorporates all the latest and most updated procedures for soil, plant, and water analysis in tobacco.

An attempt is made in compiling the right methods of soil, plant, and water analysis for improving the productivity and quality of tobacco. The manual describes the various methods encompassing soil physical, physico-chemical, chemical, microbiological properties, plant analysis for various plant nutrients including major, secondary & micro nutrients, chemical quality parameters and water analysis to determine its quality indices for tobacco cultivation. This manual helps the stake holders for identifying right soil type, assessing the fertility status for balanced fertilizer use and also identifying the nutrient deficiencies & hidden hunger for ensuring the appropriate corrective measures. The manual also contains the information on laboratory safety measures, fertilizer doses for different tobaccos, soil health cards *etc.* for the benefit of the stake holders. As a whole the manual is one stop information for soil, plant and water analysis for sustainable tobacco cultivation.

Chapter - 1

Soil Analysis

SOIL SAMPLING AND PROCESSING

Soil sampling is a technique by which a true representative sample of a given area is collected. Collection of representative samples is most important in an effective soil testing programme as the entire analysis and recommendation depends on the sample collected. Soils and fields are heterogeneous and utmost care is needed in sampling. "The accuracy and reliability of an analysis are only as good as the quality of the sample used."

Time of sampling: Soil sampling should ideally be done before planting, typically in the summer. Pre-season sampling helps to determine nutrient levels, pH, and organic matter content, enabling farmers to make necessary adjustments before the growing season begins. After the tobacco crop is harvested, soil sampling can be done to assess how the growing season impacted soil nutrient levels. Post-harvest sampling provides insights into nutrient depletion or buildup, in planning for the next crop. In some cases, mid-season soil sampling might be necessary, especially if there are signs of nutrient deficiencies or other soil-related issues.This can guide in-season adjustments in fertilization and irrigation practices to ensure optimal plant growth. For enumeration of microorganisms and rhizosphere microbiome studies, soil sampling should be done at active crop growth stage.

Sample Size: One composite sample is to be collected for every 2 ha. area. A total No. of 15 to 20 sub samples per sampling area is to be taken and mixed to get a representative sample or composite sample. Soil samples should be taken once in every three years.

Depth: As most of the active roots of tobacco plant are known to be concentrated in 0-45 cm depth of the soil from surface, soil samples are to be taken from surface to 22.5 cm depth and subsoil sample from 22.5 cm to 45.0 cm depth for routine soil tests. For estimating carbon stocks and carbon sequestration potential of soils, depth wise sampling is to be done upto 0-60 cm. For microbial population enumeration collect samples from 0-15 cm depth.

Soil sampling tools : For soft, moist soils (light textured soils), soil tube, spade or a khurpi (trowel) are used. For hard soils (heavy soils), screw type auger or crow bar is more convenient. Scale, labels, cloth bags or polythene bags, pencils, information sheet are the other tools required for soil sampling.

Soil sampling Procedure

- Traverse through the entire field for features like gravelliness, slope, soil color, *etc.* pay attention to salinity/alkalinity patches and water logging
- Demarcate the field/entire land approximately into several uniform subplots or portions each of which must be sampled separately
- In each subplot, collect samples from 15-20 spots randomly in zigzag path
- At each spot, remove surface litter or leaves, small stones, gravels, pebbles, roots *etc.*
- Give a V-shaped cut into a depth of 22.5 cm with spade or pick-axe. 2 cm thick, uniform slice of the soil from surface to bottom is to be taken carefully and collect the soil samples from all the spots into a bucket
- Reduce sample size by composite soil sampling till you get 0.5 kg/500 g by "quartering" method

Quartering: Collect the soil at one clean place, mix thoroughly by hand, spread and make four quarters, discard the two opposite ones. Remix the remaining two quarters. Repeat the process to reduce the quantity to about 500 g. Air-dry the sample under shade and keep it in a clean polythene bag. Prepare two labels, put one label inside the bag and the other to be tied on to the neck of the bag.

Precautions while soil sampling

- Avoid collecting soil sample near bunds, near roads, near FYM/ compost pits, below the trees, near buildings, near nalas/ streams/ ponds/ wet spots, irrigation canals and drainage lines and other unrepresentative spots
- Do not collect the soil sample immediately after application of fertilizers, manures and amendments. There should be a minimum 3 months gap after the application of fertilizers, manures and amendments
- If the soil sample has to be analyzed for micro nutrients, avoid tools made of iron, copper and brass. Use only stainless steel, wooden, aluminium and plastic tools
- Do not dry the sample near fertilizer bags or in fertilizer/pesticides/seed godowns and do not use fertilizer or seed bags for sampling or for drying

- All sampling tools and storage bags should be perfectly clean to avoid contamination
- For micro nutrient analysis like Cu, Fe ***etc.*** metal sieves should not be used. Plastic or nylon sieves are preferred
- Problematic spots like low-lying, saline and alkali areas should be sampled separately for critical evaluation of the problem soil

On receipt of the soil samples at the Soil Testing Laboratory, these samples will be powdered, sieved to pass through 2.00 mm sieve, cartoned and subjected to chemical analysis. For organic carbon estimation samples will be sieved to pass through 0.2 mm sieve.

While collecting soil samples for microbial studies, use sterile tools and containers to prevent contamination and handle soil gently to avoid disturbing microbial communities. Avoid sampling during extreme weather and label samples clearly. Store samples in a cool, shade place and process them promptly to maintain microbial activity. Prevent exposure to chemicals and ensure personal hygiene to avoid introducing external microorganisms. For microbial population enumeration, keep the samples in a cool, shaded environment to preserve microbial activity. Ideally, store samples at 4°C (39°F) and avoid freezing, which can damage microbial cells. Use clean, airtight containers to prevent contamination and moisture loss.

SOIL REACTION (pH)

Soil reaction (pH) is defined as the negative logarithm of Hydrogen ion activity of a solution. Soil reaction or soil pH is the term used to indicate acid-base reaction of a soil i.e., to characterize it into an acid, alkaline or neutral soil. Soil reaction is measured in pH scale ranging from 0-14 and is meant to express acidity or alkalinity of soil. Soil pH is one of the important electro-chemical properties having an influence on various physical, chemical and biological properties. The ideal soil reaction for tobacco plant growth falls in the range of pH 5 to 6.

Principle

The soil pH is measured using pH meter, where a glass electrode is made up of glass membrane. The electric potential developed across the glass membrane is in proportion to the difference in pH of KCl filled inside the membrane and the pH of soil-water suspension outside the membrane. The KCl that diffuses through fine holes of the reference calomel electrode into the soil-water suspension forms the ionic invisible bridge between the electrodes through which current passes. The difference in electric potential developed across the electrodes is measured through a galvanometer.

Reagents

Standard buffer solutions of pH 4, 7 and 9.2 are prepared by using commercially available tablets i.e., buffer tablets. Dissolve the respective tablets in freshly prepared distilled water and make up the volume to 100 ml.

Procedure (Jackson, 2018)

Weigh 20 g of 2 mm sieved soil into a clean 100 ml beaker

Add 50 ml of distilled water (1:2.5:: Soil: Water)

Stir the suspension intermittently for 30 mins

Record the pH using calibrated pH meter

Calibration of the pH meter

Switch on the instrument for 15-20 mins for stability

Set the temperature correction knob to the temperature of the sample solution

Dip the electrode in standard buffer solution of pH 4.0

Remove the electrode, wash with distilled water and wipe it with ordinary filter paper

Dip the electrode in the buffer solution of pH 7.0 or pH 9.2

If the instrument records the pH of the buffer exactly, then the pH meter is calibrated for the pH measurement

Interpretation

pH range	Soil reaction rating	Associated Conditions
< 4.6	Extremely acidic	Ca or Mg deficiency, poor crop growth due to low CEC and Al toxicity
4.6 – 5.5	Strongly acidic	
5.6 – 6.5	Moderately acidic	Soil is lime free, should be closely monitored. Satisfactory for most of the crops
6.6 – 6.9	Slightly acidic	Ideal range for crop production. Soil CEC is near 100 % of base saturation
7.0	Neutral	Ideal range for crop production
7.0 – 8.5	Moderately alkaline	Free lime ($CaCO_3$) exists in soil
>8.5	Strongly alkaline	Invariably indicates sodic soil

At low pH (acidic soils), essential nutrients like phosphorus, calcium, and magnesium become less available, while toxic elements like aluminum may increase. High pH (alkaline soils) can lead to deficiencies in micronutrients such as iron, manganese, and zinc. Maintaining the correct pH balance ensures that nutrients are in forms readily available to plants, promoting healthy growth and optimal yields. Regular soil testing and adjustment of pH can help maintain this balance.

TOTAL SOLUBLE SALTS (EC) IN SOIL

The determination of total water-soluble salts is of special importance for arid and semi-arid regions and for irrigated areas because soils under these conditions tend to develop salinity or alkalinity due to the accumulation of salts. The nature of ions present will determine whether the soil is saline or alkaline. A high salt content of the soil interferes with the absorption of water and nutrients by the plant. Usually, higher EC values are associated with higher chloride ion concentration in soil. It is observed that when EC value of the soil-water suspension (1:2.5) exceeds 0.50 dS/m, the chloride content of the soil will be generally more than 100 mg/kg. Such high chloride soils are not recommended for FCV tobacco cultivation as the tobacco grown on these soils invariably contains more than 2% chlorides and exhibits very poor burn and poor storage properties.

Principle

Water is a poor conductor of electricity. The presence of salts in the aqueous media enhances conductivity. Soluble salts present in soil are extracted with water and the solution conductivity is determined to estimate the salt content using conductivity meter. Thus, EC used as an index of salt content. Higher the salt content lower is the resistance to flow of current and higher is the electrical conductance. EC is a reciprocal of resistance and increases with increase in salt concentration.
Two metal plates made of platinum, graphite, stainless steel, or other metallic material is inserted into the solution. A potential, typically a sine wave voltage, is applied across the plates, and the EC meter measures the resulting electrical current that flows between the electrodes. This current is directly proportional to the number of ions in the soil solution, which in turn reflects the soil's ability to conduct electricity.The conductivity (G), which is the inverse of resistivity (R), is calculated using Ohm's law based on the voltage and current measurements.

According to ohm's law:

G = 1/R =I/E

Where, G = conductivity; R = resistivity; E = electrical potential in volts; I = current in amperes

Since the charge on ions in solution facilitates the conductance of electrical current, the conductivity of a solution is proportional to its ion concentration.

Procedure (Jackson, 2018)

Weigh 20 g of 2 mm sieved soil into clean 100 ml beaker

Add 50 ml of distilled water

(Same soil water suspension prepared for pH estimation can be used after 24 hrs.)

Stir the suspension intermittently for 30 minutes

Allow the suspension to settle for overnight

Measure the EC in the supernatant solution

Conversion formulas used in EC

- meq/l = 10 X EC
- ppm of salts = 640 X EC
- % Salts in solution = 0.064 X EC
- Osmotic pressure of solution in atmosphere OP = 0.36 X EC

INTERPRETATION (EC: dS/m)

Rating	Sandy soils	Loamy soils	Clay soils
Normal	0.01 to 0.40	0.01 to 0.70	0.01 to 0.80
Critical	0.41 to 0.80	0.71 to 1.40	0.81 to 1.60
Injurious	Above 0.80	Above 1.40	Above 1.60

SOIL CATION EXCHANGE CAPACITY

The Cation Exchange Capacity (CEC) of the soil is a measure of the quantity of readily exchangeable cations neutralizing the negative charge in the soil and usually expressed in c mol (P^+) kg^{-1} soil.

Reagents

- Ammonium acetate (1 N): Dissolve 77.08 g of ammonium acetate in 500 ml of distilled water and make the volume to 1 L . Add few drops of acetic acid or NH_4OH to adjust the reaction of the solution of pH to 7
- Potassium chloride (1 N KCl): Dissolve 74.5 g of KCl in 500 ml of distilled water and make the volume to 1 L
- Ethanol (95%)
- Boric acid (4%) with mixed indicator (Methyl red and Bromocresol green): Dissolve 40 g of boric acid in 500 ml of warm distilled water and make the volume to 1 L of distilled water and add 50 ml of mixed indicator to 1 L of 4% boric acid
- Mixed indicator: Dissolve 0.5 g bromocresol green and 0.2 g of methyl red in 100 ml. of alcohol
- Sodium hydroxide (40 % NaOH): Dissolve 200 g NaOH in 500 ml of distilled water.
- Sulphuric acid (0.01 N H_2SO_4): Dilute 3.0 ml of conc. H_2SO_4 to 1 L with distilled water to get 0.1 N and then 100 ml of 0.1 N solution is diluted to 1 L to get 0.01 N of H_2SO_4. Standardize with 0.01 N sodium carbonate
- Sodium carbonate (0.1 N Na_2CO_3): Dissolve 5.29 g of AR grade Na_2CO_3 in 1 L of distilled water

Procedure (Jackson, 2018)

Transfer 1g of soil into a 50 ml centrifuge tube, add 33 ml of neutral normal ammonium acetate solution, shake for half an hour and centrifuge for 10 minutes @ 10,000 rpm and collect the supernatant into a suitable container

Repeat this procedure twice using 100 ml of ammonium acetate
Add 33 ml of alcohol, shake for half an hour and centrifuge for 10 mins discard the supernatant liquid

Repeat the step for 2 or 3 times (till it is free of ammonia)
Then add 33 ml of 10% KCl, shake for half an hour, centrifuge @ 10,000 rpm for 10 minutes and collect the supernatant

Repeat this procedure 3 or 4 times or till all the ammonia adsorbed on the exchange complex are replaced by K^+ ions

Transfer 50 ml of aliquot KCl extract into the distillation tube and connect to the distillation unit

Then add 10 ml sodium hydroxide and distilled water to be automatically added to the aliquot by the distillation unit

Fill 25 ml boric acid into a receiving flask and place in position for distillate and start the distillation

Titrate the distillate with 0.01N H_2SO_4

Calculations

$$\text{CEC [c mol } (P^+) \text{ kg}^{-1}] = \frac{\text{TV X N of Acid X 100}}{\text{S}}$$

Where, TV = titre value or ml of standard acid used; N = Normality of acid; S = Weight of soil

SOIL ORGANIC CARBON (OXIDISABLE) BY WET DIGESTION METHOD

Soil organic matter is primarily made up of carbon, which constitutes about 48-50% of its total weight.Therefore, organic carbon determination is often used as a basis for organic matter estimates through multiplying the organic carbon value by a factor. For many years the Van Bemmelen factor of 1.724 was used based on the assumption that the organic matter contains 58% C. The Walkley and Black (1934) method, which provides organic carbon content of soil, is frequently employed in soil testing and surveying.

Principle

Hot $K_2Cr_2O_7$ and conc. H_2SO_4 mixture treatment oxidizes organic matter in the soil. The excess $Cr_2O_7^{-2}$ is titrated with ferrous ammonium sulphate, and it is assumed that the $Cr_2O_7^{-2}$ reduced during the reaction with soil is equal to the organic carbon in the sample. A redox indicator, such as diphenyl amine or ferroin, which produces a strong and noticeable colour change, must be used because the green colour of the solution caused by the Cr^{+3} ions formed by the reduction of potassium dichromate makes it difficult to determine the end point of a dichromate titration by simple visual inspection of the solution.

Reagents

- 1N Potassium dichromate ($K_2Cr_2O_7$): Dissolve 49.04 g of $K_2Cr_2O_7$ in distilled water and make up the volume to 1 litre
- Conc. Sulphuric acid (H_2SO_4)
- Conc. Ortho-phosphoric acid (85 % H_3PO_4)
- 0.5 N Ferrous Ammonium Sulphate ($FeSO_4$ $(NH_4)_2SO_4.6H_2O$): Dissolve 196.1 g of Ferrous ammonium sulphate (AR) in about 600 ml of distilled water and add 20 ml of conc. H_2SO_4 and make up the volume to 1 litre using distilled water
- Diphenylamine (DPA) indicator: Dissolve 0.5g of diphenylamine in 20 ml of distilled water and make the volume to 100 ml with conc.H_2SO_4
- Ferroin indicator: Dissolve 0.695g of $FeSO_4.7H_2O$ and 1.485 g of ortho-phenonthroline monohydrate in 100 ml distilled water in a volumetric flask

Procedure (Walkley and Black, 1934)

Finely grind a small portion of 2 mm sieved soil sample using agate pestle and mortar till the sample completely passes through 0.2 mm sieve

Weigh exactly 0.5 g of soil and transfer to 500 ml conical flask

Using pipette, add exactly 10 ml of $K_2Cr_2O_7$ solution to conical flask

Add 20 ml of conc. H_2SO_4 through sides of the flask and swirl gently for approximately one minute so that all the organic matter comes in contact with the solution

Swirl the flask periodically and after 30 minutes, add 200 ml distilled water
Add 10 ml H_3PO_4

Add 10 drops of diphenylamine or Ferroin indicator

Carryout titration using 0.5 N Ferrous Ammonium Sulphate with rapid stirring till colour changes from dark blue to green for diphenylamine indicator. (Use of ferroin indicator gives colour change from yellowish orange through green to reddish brown)

Run a blank without soil sample

Calculation

$$\%OC = \frac{\text{(BTV-STV) X N Ferrous Ammonium Sulphate X 0.003 X 100}}{\text{Wt. of Soil sample (g)}}$$

% Organic matter = % OC X 1.724
OC = Organic carbon; BTV = Blank titre value;STV = Sample titre value
0.003 = Conversion factor from me. of dichromate to grams of carbon (Van Bemmelean factor)
1.724 = Factor for converting organic carbon to organic matter

Interpretation

% Organic carbon	Rating
<0.5	Low
0.5 – 0.75	Medium
> 0.75	High

SOIL AVAILABLE NITROGEN

The total nitrogen taken up by plants is represented by the amount of nitrogen that is available in the soil and the amount added in the form of organic and inorganic fertilizer. Under aerobic conditions, nitrogen is typically absorbed as nitrate, whereas in anaerobic situations, nitrogen is absorbed as ammonium ions during plant growth. However, the overall amount of nitrogen that is made available to the plants during growth is greater than the sum of the ammonical and nitrate nitrogen. The organic forms of nitrogen (proteins, amino acids and sugars ***etc.*** gradually become available to plants for absorption, depending upon the nature of their combination and soil conditions. Nitrogen is the key nutrient for plant growth, yield and quality of plants. In tobacco, leaf being economic product, too excess or too short a supply of nitrogen fertilizer leads to low yields, poor quality and very low market price. Further, the balanced nutrition of N and K holds key for successful tobacco production and higher net returns (Krishnamurthy and Anuradha, 2011).

Principle

The readily oxidizable fractions of organic nitrogen can be extracted by the alkaline $KMnO_4$, which is a mild oxidizing agent. An excessive amount of alkaline $KMnO_4$ is applied to a known weight of the soil. Boric acid is used to absorb the ammonia that generated during the distillation process, and standard acid is used to titrate it.

Reagents

- Potassium permanganate (0.32 % $KMnO_4$): Dissolve 3.2 g of $KMnO_4$ in distilled water and make up the volume to 1 litre
- Sodium hydroxide (2.5 % NaOH): Dissolve 25g of NaOH flakes in distilled water and makeup the volume to 1 liter
- Sulphuric acid (0.01N H_2SO_4): Pipette out 0.28 ml conc. H_2SO_4 into 1000 ml vol. With distilled water, standardise with 0.01 N Na_2CO_3 solution
- Boric acid (2% H_3BO_3): Dissolve 20g H_3BO_3 in 1000 ml of warm water
- Mixed indicator: Dissolve 0.5g bromocresol green and 0.1g Methyl red in 100 ml ethanol and adjust pH to 4.5 or bluish-purple colour with NaOH or HCl

Procedure (Subbiah & Asija., 1956)

Kelplus method

Weigh 5 g of soil and transfer it into the distillation tube, add 10 ml distilled water

Add 25 ml of 0.32 % $KMnO_4$ solution and fix the tube to the distillation unit

Pipette out 25 ml of boric acid mixed indicator solution into 250 ml conical flask and dip the end of the delivery tube into it. (Note that boric acid should be kept at receiving end before starting the unit)

Distill the ammonia gas from the distillation tube in the N distillation unit, collect the distillate in boric acid mixed indicator solution

After completion of distillation, take out the conical flask from the receiving end

Titrate with standard sulphuric acid till the end point is obtained. (colour changes from green to pink)

Calculation

$$\textbf{Available N (Kg/ha)} = \frac{\textbf{(TV of std. acid) X Normality of acid X 0.014 X 2.24 X } 10^6}{\textbf{Weight of soil (g)}}$$

Where, TV = titre value; 0.014 = conversion factor from 1 me of NH_4^+ to g of N

Procedure (Subbiah and Asija, 1956)

Weigh 20 g of soil sample in an 800 ml Kjeldahl flask

⇩

Moisten the soil with about 10 ml of distilled water, wash down the soil adhering to the neck of flask, if any

⇩

Add 100 ml of 0.32% $KMnO_4$ solution

⇩

Add a few glass beads or broken pieces of glass rod

⇩

Add 2-3 ml of paraffin liquid, avoiding contact with upper part of the neck of flask

⇩

Measure 20 ml of 2% boric acid containing mixed indicator in a 250 ml conical flask and place it under the receiver tube

⇩

Dip the receiver tube end in the boric acid

⇩

Run tap water in the condenser

⇩

Add 100 ml of 2.5% NaOH and immediately attach to the rubber stopper fitted in the alkali trap

⇩

Switch the heaters on and continue distillation until about 100 ml of distillate is collected

⇩

Remove the conical flask containing distillate and then switch off the heater to avoid back suction

⇩

Titrate the distillate against 0.02N H_2SO_4 taken in burette until pink colour starts appearing

⇩

Run a blank without soil with each set of five samples

⇩

Carefully remove the Kjeldahl flask after cooling and drain the contents in the sink

Calculation

$$\textbf{Available N in soil in kg/ha} = \frac{\textbf{(s-b) X 0.00028 X 2.24}}{\textbf{20}}$$

Where, s = sample titre value; b = blank titre value

Interpretation

Available N (kg/ha)	Rating
<200	Very low
200-280	Low
280-560	Medium
>560	High

SOIL AVAILABLE PHOSPHORUS

Next to the nitrogen, phosphorus is an important essential nutrient for plant growth as it is a component of cell membrane, chloroplast and mitochondria and constituent of ADP, ATP, nucleic acids ***etc.,*** It plays an important role in energy transformation and metabolic process. Plants take up P in the form of $H_2PO_4^-$ and $HPO_4^=$. In acid soils, Fe and Al are in higher concentration and P is mainly in the form of Fe-P and Al-P. In acid soil, $H_2PO_4^-$ form is dominant. In alkaline soils, P is present mainly as Ca-P because of higher concentration of Ca in soil solution. In alkaline soil $HPO_4^=$ and PO_4^{3-} forms of P are dominant.

Phosphorus requirement of FCV tobacco crop is low and may vary between 46-60 kg P_2O_5/ha and the leaf concentration ranges from 0.2-0.4% Phosphorus. Several methods are available for the extraction of available P from soil. Number of inorganic acids have been used for the extraction of P from soil. Most widely used methods are 0.03N NH_4F + 0.025 N HCl (Bray and Kurtz No. 1 solution) for acid soils and 0.5 M $NaHCO_3$ solution adjusted to pH 8.5 (Olsen's method) for neutral to alkaline soils. Once 'P' is extracted from the soil, the further estimation is by spectrophotometer.

Spectrophotometer

The principle of a spectrophotometer is based on the absorption of light by a substance.

- Light Source: The spectrophotometer emits light, usually across a range of wavelengths (ultraviolet, visible, or infrared)
- Sample Interaction: When this light passes through the sample, certain wavelengths are absorbed by the substance depending on its properties, while others pass through
- Detection: The spectrophotometer measures the intensity of the transmitted light and compares it with the intensity of the light before passing through the sample
- Absorbance: The difference between the incident light and transmitted light is the absorbance, which follows Beer-Lambert's Law, stating that absorbance is directly proportional to the concentration of the substance and the path length

By analyzing the absorbance, the spectrophotometer determines the concentration of the substance in the sample

Extraction of P in acid soils by Bray and Kurtz No. 1 solution

Principle

The F^- ions have the special property of complexing Al^{+3} and Fe^{3+} ions in acid solution with consequent release of P held in the soil by these trivalent ions. Inclusion of acid (0.025 N HCl) in extracting solution results in the dissolution of more active Ca-P and prevents the precipitation of P released from Fe-P and Al-P. Extraction of P from acid soils with 0.03N NH_4F + 0.025 N HCl has been found to give results that are highly correlated with crop response to P fertilization.

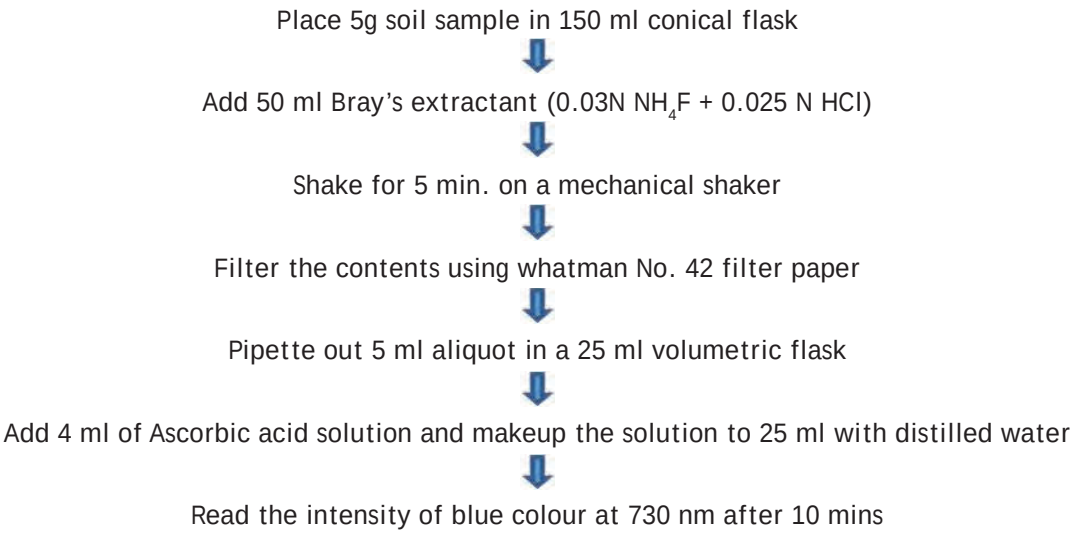
Reagents

- Brays reagent [0.03 N Ammonium fluoride (NH_4F) in 0.025 N HCl]: Dissolve 1.11 g of NH_4F in 1 Lit of 0.025N HCl
- Std. Phosphorus solution (50 ppm): Dissolve exactly 0.2196 g of potassium dihydrogen orthophosphate (KH_2PO_4) AR grade in half litre of distilled water. Add 5 ml of conc. H_2SO_4 and make up volume to 1 L with distilled water
- Std. P solution – 2 ppm : Dilute 4 ml of 50 ppm 'P' solution to 100 ml with distilled water
- Molybdate tartarate solution: Dissolve 12 g of ammonium molybdate in 250 ml distilled water to get solution A. Prepare solution B by dissolving 0.291g of antimony potassium tartarate in 100 ml distilled water. Prepare 1 lit of 5N H_2SO_4 and add solution A and B to it. Mix thoroughly and make the volume to 2 lit with distilled water
- Ascorbic acid solution: Dissolve 1.056 g of ascorbic acid in 200 ml of molybdate tartrate solution and mix well. This reagent should be prepared fresh as and when required
- P-nitrophenol indicator: Dissolve 0.5g p-nitrophenol in 100 ml of distilled water.
- 5N Sulphuric Acid (H_2SO_4): Carefully dilute 140 ml of conc. H_2SO_4 to 1L with distilled water to get approx. 5N H_2SO_4

Preparation of standard curve

- Pipette out 0, 1, 2, 3, 4 and 5 ml of 2 ppm P solution in to separate 25 ml volumetric flasks and make up with distilled water to get 0, 0.08, 0.16, 0.24, 0.32 and 0.40 ppm 'P' solutions, respectively
- Add 5 ml of the extractant (Brays or Olsens)
- Add ascorbic acid solution as for Brays or Olsens method and proceed to develop colour as done for the test sample
- Measure the intensity of blue colour at 730 nm after 10 mins
- Draw the calibration curve by plotting absorbance on Y-axis and concentration of 'P' on X-axis

Procedure (Singh *et al.*, 1999)



Extraction of 'P' in neutral to alkaline soils by Olsen's method

Principle

The 0.5 M $NaHCO_3$ solution at pH 8.5, when added to the soil, suppresses the ionic activity of Ca due to increased activity of HCO_3^-. Thus some phosphate from the surface of calcium phosphate is extracted through the solubility product of calcium phosphate. As Ca activity decreases P activity increases.

Reagents

- Olsen's reagent (0.5 M $NaHCO_3$ solution adjusted to pH 8.5): Dissolve 42g of sodium bicarbonate in about 900 ml distilled water and adjust the pH to 8.5 using NaOH or HCl and make up the volume to 1000 ml
- Ammonium molybdate (1.5 %): Dissolve 15 g of ammonium molybdate in 300 ml of hot distilled water (50-60 ºC), filter if there are any sediments. Add 400 ml of 10 N HCl and make up the volume to 1 L
- Molybdate tartarate solution: Dissolve 12 g of ammonium molybdate in 250 ml distilled water to get solution A. Prepare solution B by dissolving 0.291g of antimony potassium tartarate in 100 ml distilled water. Prepare 1 lit of 5N H_2SO_4 and add solution A and B to it. Mix thoroughly and make the volume to 2 lit with distilled water
- Ascorbic acid solution: Dissolve 1.056 g of ascorbic acid in 200 ml of molybdate tartrate solution and mix well. This reagent should be prepared fresh as and when required

Procedure (Singh *et al*. 1999)

Weigh 2.5 g of soil into a 150 ml conical flask

Add a pinch of 'P' free Darco-G 60 and 50 ml of 0.5 M $NaHCO_3$ solution

Shake for 30min. and filter the contents using whatman No. 42 filter paper

Pipette 5ml of the Olsen's reagent into 25ml flask and add 2-3 drops of p-nitrophenol indicator, develops yellow colour

Add known quantity of 5N H_2SO_4 drop by drop to acidify the Olsen's reagent to pH 5 at which yellow colour will disappear. Note the volume of 5N H_2SO_4 used

Take 5 ml of the filtrate into 25 ml volumetric flask

Add 4 ml of Ascorbic acid solution

Make up the volume to 25ml with distilled water and shake well

Wait for 10 min and then measure the intensity of blue colour at 730 nm

Run a blank with extracting solution (without soil)

Calculation

$$\textbf{Available P (kg ha}^{-1}\textbf{)} = \frac{\textbf{Graph ppm X vol. of extractant X vol. made X 2.24 X } 10^6}{10^6 \textbf{ X weight of soil X volume of aliquot}}$$

STANNOUS CHLORIDE METHOD

Bray's P-1 (Bray and Kurtz 1945)

Reagents

- Bray's P-1 extractant: Dissolve 1.110 g of AR grade ammonium fluoride in one litre of 0.025N HCl
- 1.5% Dickman and Bray's reagent: Dissolve 15 g of AR grade ammonium molybdate in 300 ml of warm water, cool and add exact 350 ml of 10N HCl Make the volume to one litre
- 40% Stannous chloride ($SnCl_2$) stock solution: Weigh 10 g of stannous chloride in a 100 ml glass beaker. Add 25 ml of conc. HCl and dissolve by heating. Cool and store in an amber coloured bottle in dark, after adding a small piece of Zn metal (AR grade) to prevent oxidation. From this prepare a dilute $SnCl_2$ solution (0.5 ml diluted to 66 ml) immediately before use
- 100 mg P L^{-1} stock solution: Weigh 0.439 g of AR grade KH_2PO_4 dried in oven at 60°C for 1 h in a one litre beaker, add about 500 ml of distilled water and dissolve. Add 25 ml of approx. 7N H_2SO_4 and make the volume to one litre
- 2 mg P L^{-1} solution: Dilute a suitable volume of 100 mg P L^{-1} solution by 50 times to get 2 mg P L^{-1} solution

Procedure

Weigh 5 g of soil sample in a 150 ml conical flask

⇩

Add 50 ml of Bray's P-1 extractant and shake for 5 min

⇩

Filter through Whatman No.1 filter paper quickly
so as to collect the filtrate within 10 minutes

⇩

Transfer 5 ml aliquot into a 25 ml volumetric flask

⇩

Add 5 ml of ammonium molybdate solution, shake a little and dilute to about 22 ml.

⇩

Add 1 ml of diluted $SnCl_2$ (0.5 ml diluted to 66 ml), mix by shaking a little,
and make up the volume

⇩

Run a blank without soil under identical conditions

⇩

Measure the intensity of the blue colour developed, using 660 nm wave length (red filter)

Olsen's method (Olsen *et al.* 1954)

Reagents

- 0.5M Sodium bicarbonate ($NaHCO_3$): Dissolve 42 g of P-free sodium bicarbonate in about 500 ml of hot water and dilute to one litre. Adjust the pH to 8.5 using dil. NaOH solution or dil. HCl
- Activated charcoal: Wash pure activated charcoal or commercially available Darco G-60 with acid to make P-free, even if having traces of P
- 1.5% Ammonium molybdate solution: Similar to that described in case of Bray's P-1 except that use 400 ml of 10N HCl instead of 350 ml per litre
- 40% $SnCl_2$ solution: Same as in case of Bray's P-1
- 100 ppm P solution: Same as in case of Bray's P-1
- 2 ppm P working solution: Same as in case of Bray's P-1

Procedure

Weigh 2.5 g of soil sample in 100 ml conical flask

⇓

Add a pinch of Darco G-60 and 50 ml of Olsen's reagent (0.5M $NaHCO_3$, PH 8.5)

⇓

Shake for 30 min. on a mechanical shaker

⇓

Filter through Whatman No.1 filter paper

⇓

Transfer 5 ml of clear and colourless filtrate into a 25 ml volumetric flask

⇓

Gradually add 5 ml of ammonium molybdate containing 400 ml of 10N HCl per litre

⇓

Shake slowly and carefully to drive out the CO_2 evolved

⇓

When frothing completely ceases, add distilled water, washing down the sides, to bring the volume to about 22 ml

⇓

Add 1 ml of freshly diluted $SnCl_2$ solution, shake and make the volume to 25 ml

⇓

Read the blue colour intensity at 660 nm (red filter)

⇓

Run a blank without soil under identical manner

Calculation

$$\text{Available P (kg h}^{-1}\text{)} = \frac{\text{Q X V X 2.24}}{\text{A X S}}$$

Where, Q = quantity of P in µg read on X-axis against a sample reading; V = volume of extracting reagent used (ml); A = volume of aliquot used for colour development (ml) and S = of soil sample taken (g).

Bray's P = Q X 4.48; Olsen's P = Q X 8.96

Interpretation

Aailable P (kg ha^{-1})	Rating
<11	Low
11-22	Medium
>22	High

SOIL AVAILABLE POTASSIUM

Potassium is a key essential plant nutrient and performs a wide range of vital roles in plant systems including, enzyme activation, photosynthesis, respiration, stomatal control ***etc.*** It plays a critical role in enhancing plant tolerance to abiotic and biotic stresses and improving the quality of produce in crops like tobacco. Concentration of K in leaf tissue is the key factor that enhances tobacco quality in terms of improved leaf size, specific leaf weight, leaf colour, pliability and combustibility. Generally, K is absorbed by the tobacco in larger quantity than any other nutrient. On an average, a tobacco crop yielding 2000 kg ha^{-1} takes up 100-120 kg K ha^{-1}. Alfisols supporting FCV tobacco under Karnataka Light Soils and Northern Light Soils of Andhra Pradesh have low native K fertility and require liberal input of K. Sufficient K not only increases the yield of tobacco leaves, but also promotes the normal maturation of the leaves, thereby improving the leaf quality (Zhang ***et al***. 2017). In addition, K also plays an important regulatory role in the response of plants to various biotic and abiotic stresses (Chen ***et al***. 2010). Plants take up potassium in its ionic form (K^+), which occurs in soil solution and on the exchange complex. This available form of K^+ is extracted with neutral normal ammonium acetate from soil.

Potassium (K) is one of the essential nutrient elements for plant growth and development. It plays a critical role in enhancing plant tolerance to abiotic and biotic stresses and improving the quality of produce in crops such as tobacco (*Nicotiana tobaccum* L.). Generally, K is absorbed by flue-cured tobacco in larger amounts than any other nutrient (Krishnamurthy ***et al.*** 2003). Concentration of K in leaf tissue is the key factor that enhances tobacco leaf quality in terms of improved leaf size, specific leaf weight, leaf color, pliability, and combustibility (Krishnamurthy ***et al.*** 2003). Consequently, plant-tissue analysis is routinely done to monitor K status of crops for developing appropriate fertilization strategy and assessing leaf quality.

Principle

When neutral normal ammonium acetate is added to soil, NH_4^+ of NH_4OAC replaces K^+ from exchange site to solution. Concentration of potassium in solution is estimated by flame photometry.

Reagents

- 1 N Ammonium Acetate (NH_4OAC): Dissolve 77.09 g NH_4OAC in distilled water and makeup vol. to 1 lit and adjust pH to 7
- 1000 ppm K: Dissolve 1.91 g of KCl in distilled water and makeup vol. to 1 lit

Preparation of standard curve

- Dissolve 1.91 g of KCl in distilled water and make up the volume to 1 L to give 1000 ppm solution of K
- Pipette out 5, 10, 20, 30 ml of 1000 ppm K-solution to separate 500 ml volumetric flasks and make up the volume to give 10, 20, 40, 60 ppm K respectively
- Record the flame photometer reading for each standard after standardising the flame photometer
- Draw calibration curve by plotting flame photometer reading (FPR) on Y-axis and conc. on X-axis

Procedure

Transfer 5 g soil sample into 150 ml conical flask

⬇

Add 25 ml of 1N NH_4OAC and shake for 10 min

⬇

Filter through Whatman No. 1 filter paper

⬇

Read in the calibrated flame photometer and find out the conc. of K

Flame photometer

When a sample is introduced into a flame, the atoms of certain elements (like sodium and potassium) get excited and emit light. The intensity of the emitted light is proportional to the concentration of the element in the sample. A detector measures this light to determine the concentration of the element.

Calculation

$$\text{Available K (kg/ha)} = \frac{\text{graph ppm X vol. of extractant X 2.24 X } 10^6}{10^6 \text{ X wt. of soil}}$$

Where,

C = Concentration of K (mg L^{-1}) in the extract; V = Total volume of extractant (25 ml)
W = Air dried weight of soil sample (5 g);
To express the value in terms of kg ha^{-1}, the mg L^{-1} value is to be multiplied by a factor of 2.24

Interpretation

Available K (kg/ha)	Rating
<120	Low
121-280	Medium
>280	High

SOIL EXCHANGEABLE CALCIUM AND MAGNESIUM

Exchangeable calcium and magnesium in soil for tobacco cultivation is very important for ensuring optimal plant growth and yield. The critical limits vary depending on factors like soil type, climate and tobacco variety. The critical limit for calcium in soil for tobacco is around 400-600 mg/kg and optimum is 600-1200 mg/kg. The critical limit for magnesium is around 50-100 mg/kg and optimum range is 100-200 mg/kg. The Ca: Mg ratio is crucial, with an optimal range of **5:1 to 7:1** for tobacco. Imbalances, particularly excessive magnesium relative to calcium, can lead to poor soil structure and nutrient availability issues. Calcium helps in strengthening of tobacco leaf structure and root development, reduces leaf tip burn and improves quality. Magnesium is crucial for chlorophyll and photosynthesis, ensures vibrant leaf color and overall plant health. Both nutrients are vital for optimal tobacco growth, enhancing yield and quality, and supporting efficient nutrient uptake.

The complexometric titration using EDTA as titrant was first proposed by Schwarzenbach,1955. This method is also called as "Versenate method", as the trade name of EDTA is "Versene".

Estimation of Calcium

Principle

For the estimation of Ca, the pH of the aliquot is maintained at 12.0 or above by adding NaOH solution which eliminates Mg as Mg $(OH)_2$ precipitate and allows only Ca to remain in the solution. After maintaining the pH of an aliquot at 12.0 and above, ammonium purpurate (murexide) or calcon indicator is added to the aliquot which immediately combines with Ca^{2+} and forms a pinkish (with murexide) or reddish (calcon) complex. When EDTA solution added to the aliquot containing Ca^{2+} -indicator complex, the EDTA being a powerful chelator, snatches Ca^{2+} from the Ca^{2+} -indicator complex till all Ca^{2+}-indicator complex molecules dissociate to loose Ca^{2+} for complexation with EDTA. At the end point, the original colour of indicator alone i.e., violet (in the case of murexide) or sea blue (in the case of calcon) becomes evident.

Reagents

- 1N Neutral Ammonium Acetate: Weigh 77.08 g of ammonium acetate (NH_4OAc), dissolve in 500 ml of distilled water. Adjust the pH to 7.0 either with glacial acetic acid or ammonia solution as per the requirement and make up the volume to 1-L mark

- Sodium hydroxide solution (10 % NaOH): Weigh 100 g of pure sodium hydroxide and dissolve it in distilled water and makeup the volume to 1 L mark in a volumetric flask
- EDTA solution (0.01 N): Weigh 1.86 g of EDTA in distilled water and make up the volume to 1L mark in a volumetric flask. Titrate this solution with standard solution of 0.01 N Ca solution for standardization
- Murexide indicator powder: Take 0.2 g of murexide and mix it with 40 g of powdered potassium sulphate. Potassium sulphate is to be added to improve their stability and performance

Procedure

Weigh 5 g of soil sample in a 150 ml, conical flask

Add 25 ml of neutral normal ammonium acetate solution

Shake the contents on a mechanical shaker for 5 min and filter the contents using Whatman No. 1 filter paper

Take 5 ml of aliquot in a 150-ml porcelain dish

Add 5 ml of 10% NaOH solution

Add 50 mg of the murexide and titrate it with 0.01 N EDTA solution until the colour changes gradually from pink to purple

Add a drop of EDTA solution at Intervals of 5-10 sec. as the colour change is not instantaneous

Record the volume of EDTA consumed for titration

Run a blank

Calculation

$$\text{Exchangeable Ca (meq } 100g^{-1} \text{ soil)} = \frac{\text{(STV-BTV) X N of EDTA X 25 X 1000}}{\text{5 X ml of aliquot taken X 10}}$$

Estimation of Ca+Mg

Principle

For estimation of Ca and Mg together, after addition of suitable masking agents to eliminate interferences due to heavy metal ions, the pH of aliquot is to be maintained to 10.0 by adding ammonium hydroxide-ammonium chloride buffer which keeps both Ca^{2+} and Mg^{2+} ions in solution. Eriochrome Black-T (EBT) indicator is then added to the aliquot which forms a complex with both Ca^{2+} and Mg^{2+} and produces a reddish-purple complex. When EDTA is added to this aliquot, it first snatches Ca^{2+} from the metal-indicator complex and later Mg^{2+} from the indicator complex. At the end point, all Ca^{2+} and Mg^{2+} ions form complexes with EDTA and the original colour of indicator (sea blue) becomes evident.

Reagents

- 1N Neutral Ammonium acetate: Weigh 77.08 g of ammonium acetate (NH_4OAc) and dissolve in 500 ml of distilled water. Adjust the pH to 7.0 either with glacial acetic acid or ammonia solution as per the requirement and make up the volume to 1-L mark
- Ammonium chloride-ammonium hydroxide (NH_4Cl-NH_4OH) buffer solution: Weigh 67.5 g of ammonium chloride and dissolve it in 250 ml. distilled water, add 570 ml. of concentrated ammonium hydroxide, and make up the volume to 1L with distilled water
- EDTA solution (0.01 N): Weigh 1.86 g of EDTA in distilled water and make up the volume to 1L mark in a volumetric flask. Titrate this solution with standard solution of 0.01 N Ca solution for standardization
- EBT indicator: Take 100 ml of ethanol and dissolve 4.5 g of hydroxylamine hydrochloride in it. Add 0.5 g of EBT indicator and prepare the solution

Procedure

Weigh 5 g of soil sample in a 150 ml flask

Add 25 ml of neutral normal ammonium acetate solution

Shake it in a mechanical shaker for 5 min, and filter using Whatman No. 1 filter paper

Pipette out 5 ml of aliquot in a 150- ml porcelain cup

Add 5ml of buffer solution

Add 3-4 drops of EBT indicator

Titrate it with 0.01 N EDTA solution until the colour changes from purple to sea blue and no tinge of wine red colour remains

Titration is repeated with blank

Calculation

$$\textbf{Exchangeable Ca + Mg (meq kg}^{-1}\textbf{ soil)} = \frac{\textbf{(STV-BTV) X N of EDTA X 25 X 1000}}{\textbf{5 X ml of aliquot taken X 10}}$$

SOIL AVAILABLE SULPHUR

Sulphur is an important secondary nutrient required for the synthesis of S-containing essential amino acids like cysteine and methionine. It is also required for the oil synthesis. Sulphur occur in soils both in organic and inorganic forms, with the organic 'S' accounting for 95% of the total "S' in most soils from humid and sub humid regions. The proportion of organic and inorganic 'S' in a sample, however varies widely according to soil type and depth of sampling.The total sulphur content of soil ranges from 20 ppm in sandy soils to 600 ppm in heavy textured soils. Plants absorb 'S' as SO_4^{-2}. Estimation of SO_4^{-2} in the soil is better index of available 'S' in soil. Sulfur plays a role in the quality of cured tobacco leaves. It influences the aroma, taste, and overall chemical composition of tobacco. Sulfur improves the efficiency of nitrogen utilization. When nitrogen and sulfur are available in balanced proportions, plants can use nitrogen more effectively, leading to better growth and yield.

Principle

Sulphate sulphur is extracted from soil by using 0.15% $CaCl_2$ solution, Sulphate in solution is converted to $BaSO_4$, by treating with $BaCl_2$ and estimated at 420 nm using spectrophotometer.

Reagents

- Calcium Chloride (0.15 % $CaCl_2.2H_2O$) - extractant: Dissolve 1.5 g of $CaCl_2.2H_2O$ in about 700 ml of distilled water and make up the volume to one litre
- Barium Chloride ($BaCl_2$) crystals-powdered to 20-60 mesh
- Conditioning agent: Dissolve 15 g sodium chloride in 300 ml distilled water, add 30 ml conc. HCl and 100 ml 95% ethyl alcohol and 500 ml glycerol and mix well
- Standard sulphur solution: Dissolve 0.5434 g K_2SO_4 in distilled water and make up the volume to one litre to get 100 ppm S-stock solution

Preparation of standard curve

- Transfer of 0, 1, 2, 3, 4, 5, 10, 15 and 20 ml of 100 ppm S-stock solution to 50 ml volumetric flasks separately, to get 0, 2, 4, 6, 8, 10, 20, 30 and 40 ppm of S-working standards respectively
- Add 1 ml of conditioning agent and mix well
- Add 0.5 g barium chloride crystals and continue to stir for one minute
- Immediately after one minute pour some solution into absorption cell of spectrophotometer and measure optical density at 420 nm
- Prepare standard graph between optical density v/s concentrations of sulphate

Procedure

Weigh 5 g of soil into a 150 ml conical flask

⇩

Add 25 ml of 0.15 % $CaCl_2$ solution

⇩

Shake for 30 mins and filter using Whatman No. 42 filter paper

⇩

Take 10 ml extract into a 50 ml vol. flask

⇩

Add 1 ml of conditioning reagent and mix well

⇩

Add 0.5 g barium chloride crystals and continue to stir for one min.

⇩

Make up the vol. with distilled water and measure turbidity at 420 nm using spectrophotometer

Calculation

$$\text{Available S (ppm)} = \frac{\text{graph ppm X vol . of extract X vol. made}}{\text{Weight of soil X aliquot taken}}$$

Interpretation

Available Sulphur (ppm)	Ratings
<10	Low
10-20	Medium
>20	High

AVAILABLE SOIL CATIONIC MICRONUTRIENTS

The micronutrients including Copper, Iron, Manganese and Zinc are required in small quantities by the plants, but the importance of any of these nutrients cannot be replaced by any other nutrient. These micronutrients are among the essential nutrients for the production of a quality tobacco leaf. The micronutrients are essential in the protein metabolism, chlorophyll formation, and alkaloid production of tobacco.

Principle

Diethylene Triamine Pentaacetic Acid (DTPA) is a chelating agent which can effectively extract all the four micronutrient cations due to its most favourable combination of stability constants for the simultaneous complexing of Zn, Fe, Mn, Cu. In this solution, the 0.005 M DTPA acts as chelating agent sufficient for large quantity of micronutrients. The 0.01 M $CaCl_2$. $2H_2O$ converts the chelate to Ca DTPA and nullify the effect of Ca^{+2} displaced from soil solution and exchange. Effect of 0.1 M TEA is to buffer soil pH and it burns cleanly during flame atomization in AAS. The pH 7.3 is selected to equilibrate with $CaCO_3$ and CO_2 (g) at about 10 times the CO_2 of the atmosphere. The deficiency of these micronutrients is mostly in calcareous soils.

Operation of AAS

Atomic Absorption Spectrophotometry (AAS) operates on the principle that free, ground-state atoms in a gaseous state absorb light at specific wavelengths characteristic of the element being analyzed. In AAS, a sample is atomized in a high-temperature flame or graphite furnace, and light from a hollow cathode lamp passes through the vaporized sample. The atoms absorb light at their characteristic wavelengths, and the amount of light absorbed is measured. This absorbance, governed by the Beer-Lambert Law, is proportional to the concentration of the element in the sample, allowing for precise quantification when compared to a calibration curve created with known standards.

Instrument is adjusted to 100% transmittance by using blank solution. The absorption for standard solutions of the element to be determined is measured and the calibration curve is drawn by ploting absorption conc. from the calibration curve conc. of test solution is measured. For each metal the curve is linear up to certain conc. in solution.

Reagents

DTPA extracting solution: The DTPA extracting solution is prepared to contain 0.005M. DTPA, 0.01 M $CaCl_2$. $2H_2O$, 0.1 M TEA and pH adjusted to 7.3. For this solution, 1.967

g of DTPA, 1.47 g of $CaCl_2$. $2H_2O$ and 13.3 ml (14.92 g) of reagent grade TEA is dissolved separately in double distilled water and then mix and make the volume to 900 ml with double distilled water. Now adjust the pH to 7.3 with 1 M HCl. Add HCl while stirring. When pH is adjusted, dilute it to one liter with double distilled water.

Preparation of standard curves

Zinc

Standard stock solution (1000 ppm Zn): Weigh exactly 1.0 g of pure Zn metal (AR grade) and dissolve it in minimum volume (about 10 ml) of dil. HCl (1:1) and make the volume to one litre.
Standard solution (50 ppm Zn): Dilute 5 ml of 1000 ppm solution to 100 ml to get 50 ppm Zn solution.
Working standard solutions: Pipette out 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 ml of 50 ppm solution into 50 ml volumetric flasks separately and make up the volume with double distilled water to get working standards containing 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 ppm Zn respectively. (The working standards should be prepared in the medium of the extracting solution after every few days as it cannot be preserved for long)

Copper

Standard stock solution (1000 ppm Cu): Accurately weigh 1 g of AR grade copper metal wire or turning and dissolve it in 50 ml of diluted HNO_3 (1:1 with double distilled water) and finally make the volume to one litre.
Standard solution (50 ppm Cu): Dilute 5 ml of 1000 ppm solution to 100 ml to get 50 ppm Cu solution.
Working standard solutions: Pipette out 0.25, 0.50, 1.0, 1.5, 2.0 and 2.5 ml of 50 ppm solution into 50 ml volumetric flasks separately and make the volume with double distilled water to get working standards containing 0.25, 0.50, 1.0, 1.5, 2.0 and 2.5 ppm Cu respectively.

Iron

Standard stock solution (1000 ppm Fe): Accurately weigh 1 g of AR grade Fe metal in about 50 ml of 1:1 diluted HNO_3 and dilute the contents to one litre with double distilled water.
Standard solution (50 ppm Fe): Dilute 5 ml of 1000 ppm solution to 100 ml to get 50 ppm Fe solution.
Working standard solutions: Pipette out 1.0, 2.0, 3.0, 5.0 and 10.0 ml of 50 ppm solution into 50 ml volumetric flasks separately and make the volume with double distilled water to get working standards containing 1.0, 2.0, 3.0, 5.0 and 10.0 ppm Fe respectively.

Manganese

Standard stock solution (1000 ppm Mn): Accurately weigh 1.583 g of AR grade MnO_2or 1 g of pure Mn metal and dissolve it in 50 ml of dilute HNO_3(AR grade). Make the volume to 1 litre with double distilled water to get 1000 ppm Mn solution
Standard solution (100 ppm Mn): Dilute 25 ml of 1000 ppm solution to 250 ml to get 100 ppm Mn solution
Working standard solutions: Pipette out 0.5, 1.0, 2.0, 2.5 and 5.0 ml of 100 ppm solution into 100 ml volumetric flasks separately and make the volume with double distilled water to get working standards containing 0.5, 1.0, 2.0, 2.5 and 5.0 ppm Mn respectively

Procedure

Weigh 10 g of soil and transfer it to 150 ml conical flask

⇓

Add 20 ml DTPA ex. solution and shake for 2 hours

⇓

Filter through whatman No. 42 filter paper

⇓

Prepare a blank

⇓

Feed the solution to AAS and record the readings

Calculation

Total ppm of each micro nutrient = AAS reading X 2.0 X dilution

Interpretation

Critical level in soil (ppm) below micronutrients which the nutrients are deficient	
Zn	0.6
Fe	4.5
Mn	1.0
Cu	0.2

SOIL AVAILABLE BORON

The boron is present in soil in anionic form and the amount extracted by hot water is considered as available to plants. This method was first developed by Berger and Truog (1939) and then modified by Wear (1965). Azomethine-H reagent method is also available for boron estimation (Keren and Bingham. 1985). Among these two, Azomethine-H reagent method is commonly followed as it is simple and accurate.

Principle

Boron in solution forms a stable colour complex with azomethine-H reagent at pH 5.1. The intensity of the colour, proportional to boron concentration, is measured at 420 nm wavelength.

Reagents

- Buffer mask solution: Dissolve 250 g of ammonium acetate and 15 g of EDTA-Na salt in 400 ml of distilled water and slowly add 125 ml of glacial acetic acid and mix
- Azomethine-H reagent: Dissolve 0.45 g of azomethine-H in 100 ml of 1% L-ascorbic acid solution. Fresh reagent should be prepared each week and stored in refrigerator
- Boron stock solution (100 ppm): Dissolve 0.571 g of H_3BO_3 in warm distilled water and make the volume to 1-L mark in a volumetric flask
- Boron standard solution (5 ppm) : Take 5 ml of 100 ppm boron stock solution and make up to 100 ml to get 5 ppm boron standard solution

Preparation of standard curve

- To a series of 25 ml volumetric flask, pipette out 0, 0.25, 0.5, 1, 2 and 4 ml of 5 ppm Boron solution to get 0, 0.05, 0.1, 0.2, 0.4 and 0.8 ppm of Boron
- To each of the flask, add 2 ml of buffer solution and mix, add 2 ml of Azomethine reagent, stir thoroughly and allow standing at room temperature for 30 minutes
- Make up the volume with distilled water and measure the absorbance at 420 nm

Procedure

Weigh 20 g soil into a reflux condenser

Add 40 ml distilled water and 0.5 g activated charcoal

Boil for 5 mins

Filter immediately through Whatman no. 40 filter paper

Pipette out 5 ml of the extract in a 25 ml vol. flask

Add 5 ml of buffer solution

Add 4 ml of Azomethine reagent

Keep it for one hour for colour development

Make up the volume

Measure the intensity of the colour at 420 nm

Calculation

$$\text{Boron Conc. (ppm)} = \frac{\text{graph ppm X vol. of water added}}{\text{Aliquot taken X weight of soil}}$$

SOIL CHLORIDES

Chloride is an essential element for plant growth. Excessive quantities of chloride in the cured leaf reduce the rate of burn and cause certain adverse effects such as increased hygroscopicity, discoloration, uneven colors and undesirable odors in cured tobacco leaves (Peele *et al*., 1960). Chloride content of the soil exceeds 0.01% (100 mg/kg or 100 ppm), the burning property of such a leaf will be very poor.

Principle

Chlorides in water extract of soil can be determined by titration with the standard silver nitrate solution using potassium chromate as an indicator. Silver chloride (AgCl), being more insoluble than the silver chromate (Ag_2CrO_4), is precipitated first. When a solution of silver nitrate is added to a mixture of chloride (Cl) and chromate (CrO_4^{-2}) ions, silver chloride separates out of the solution as a white precipitate, thereby gradually reducing the chloride ion concentration in the mixture. When all such chloride ions are removed, a slight excess of the silver nitrate solution produces silver chromate (brick red colour) indicating the end point. Ag_2CrO_4, is not stable in presence of acid. Slightly acidic solutions can, however, be neutralised with pure $NaHCO_3$, before titration.

Reagents

- Silver nitrate solution (0.1 N $AgNO_3$): Dissolve 16.99 g of pure (AR) $AgNO_3$, in water and make up to 1000 ml with distilled water
- Standard sodium chloride solution (0.1 N NaCl): Take 5.85 g of dried A.R. grade sodium chloride, dissolve and make up to 100 ml with distilled water
- Potassium chromate solution (5% K_2CrO_4): Dissolve 5 grams of pure K_2CrO_4, in 100 ml chloride free distilled water

Procedure

Take 20 grams of soil in a 150 ml conical flask and add 100 ml of distilled water

Shake the contents of the flask for 1 hour

Filter it through Whatman No.42 filter paper

Pipette out 25 ml of the soil extract into a clean, dry porcelain dish

Add 3 drops of K_2CrO_4 indicator solution (gives yellow colour)

Run down the standard (0.1 N) silver nitrate solution from the burette drop by drop into the contents of the porcelain dish with constant stirring

The titration is continued until a faint but permanent red colour just appears

Calculation

Chloride (mg/kg) = TVx200

TOTAL ORGANIC CARBON

This analytical method quantitatively determines the total amount of organic carbon in soil. The method involves pre-treating the sample with dilute acid to remove carbonate carbon and then analyzing for total carbon using an instrument that utilizes a combustion system with an induction furnace coupled with a thermal conductivity detector (TCD) system and an IR detector system. This method is based on the oxidation of the sample by "combustion" which converts all organic and inorganic substances into combustion gases (N_2, NO_x, CO_2, and H_2O).

This method is based on the Dumas dry combustion principle. The sample is burned at high temperature (between 850 and 1000 °C) in an atmosphere of pure oxygen. Under these conditions, all C-containing compounds are completely decomposed and converted into carbon oxides (mainly carbon dioxide). The Total Organic Carbon (TOC) analyzer measures and reports the TC value based on the concentration of carbon oxides present using various procedures (for example, a C gas detector and thermal differences between gas columns).

TOC analyser

a) Stable conditions for the instrument

- IR temperature = around 42 ºC
- Combustion tube temp: liquids = 850 ºC; solids = 950 ºC
- Flow = around 200 ml/min . (if above 200, then there will be a leak)
- Pressure = 1 bar

If the software shows error in temperature i.e., chamber temperature is high then press the button middle between nos. 24

b) Check list for analysis to start the analyser

Switch on power supply

⬇

Switch on instrument (wait for 30 sec)

⬇

Start software

⬇

Switch on cylinder

⬇

Observe pressure, flow, IR temperature etc., to be in limit

⬇

Select mode of operation (liquid/solid) (system mode)

⬇

Check maintenance (Options Maintenance Intervals)

⬇

Set temperature (Liquid – 850 ºC; Solid – 950 ºC)

⬇

Check feeding, standard samples etc

* Before sample analysis set coefficient (default)
* For calibration leave it vacant

Regular Analysis (liquid/solid mode)

Open new file

⬇

Check feeding (System feeding double ok)

⬇

Check standard sample (View standard sample verify)

⬇

Select Run –in (1-duplicate) (under names blanks, test samples)

⬇

Select method (TTC/TC) or TNb

⬇

Set coefficients (default)

⬇

Enter desired injection volume (Options settings & methods)

⬇

Set temperature (Liquid – 850 ºC; Solid - 950 ºC)

⬇

Select auto/ single run

⬇

Record the total carbon values in percentage

LABILE SOIL ORGANIC CARBON

Small changes in soil organic carbon (SOC) from management practices can be hard to measure but impact soil behavior and microbial processes. Labile carbon, with high turnover rates, offers early detection of SOC changes, providing a sensitive indicator of soil quality and the effects of management practices on soil health. The potassium permanganate oxidizable carbon method specifically measures the fraction of organic carbon that is readily available and susceptible to decomposition. This is crucial for understanding the dynamics of soil carbon, including its availability for microbial activity and its role in soil fertility.

Principle

In neutral to slightly alkaline solution, potassium permanganate ($KMnO_4$) acts as a powerful oxidizing agent. At pH 7.2, portions of SOC react with $KMnO_4$, and partially bleach the deep purple colour of permanganate to light pink or a clear colour. Specifically, slightly alkaline $KMnO_4$ is known to hydrolyze and oxidize simple mineralizable components of the soil organic matter (SOM). $KMnO_4$ reacts with the readily oxidizable (active) forms of soil C, converting Mn^{7+} to Mn^{2+}, and proportionally lowering the absorbance to 550 nm.

Reagents

Potassium permanganate stock solution (20 mM $KMnO_4$): Dissolve 3.16 g of $KMnO_4$, in 1000 ml distilled water. Adjust the pH to 7.2 using 0.1 M NaOH. The pH of the solution is important for maintaining stability of the stock solution for 3-6 months. The pH-adjusted 20 mM $KMnO_4$, stock solution should be kept in a dark bottle.

Standard calibration curve

Transfer aliquots of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml standard $KMnO_4$ to 50 ml volumetric flasks and make the volume with distilled water. It will give 0, 0.2, 0.4, 0.6, 0.8 and 1 mM $KMnO_4$. Read absorbance at 550 nm. Plot absorbance vs concentration graph.

Procedure (Weil *et al.* 2003)

Weigh 3 g air-dried soil in a centrifuge tube

Add 30 ml of 20 mM $KMnO_4$ and shake for 2 mins

Centrifuge the tubes at 4000-5000 rpm for 3-5 min to clear the supernatant liquid

Filter the contents in a beaker

Transfer 2 ml aliquot into 50 ml volumetric flask

Read the absorbance at 550 nm

Also record the absorbance of 20 mM $KMnO_4$ solution and consider it as a reading for blank

Calculation

$$\textbf{Active C (mg kg}^{-1}\textbf{)} = \frac{\textbf{(B-S) X 50 X vol. of KMnO}_4\textbf{ used (ml) X 1000 X 9}}{\textbf{2 X 1000 X weight of soil (g)}}$$

Where,
B = Conc. of $KMnO_4$ in blank; S = Conc. of $KMnO_4$ in the sample;
Active C (mg kg^{-1}) = (B-S) X 2250

WATER EXTRACTABLE ORGANIC CARBON

Water-Extractable Organic Carbon (WEOC) is the fraction of organic carbon in soil that dissolves in water, including compounds like dissolved organic carbon (DOC), simple sugars, amino acids, and organic acids. WEOC is crucial for soil fertility, microbial activity, and nutrient cycling, offering insights into the labile, readily available carbon pool. Its levels vary with soil type, land use, and environmental conditions.

Principle

Determining WEOC typically involve extracting soil samples with water, followed by filtration to remove soil particles, and then analyzing the filtered extract for organic carbon content using techniques such as wet digestion method. The concentration of WEOC is usually expressed in units of mass per unit volume (e.g., mg/L) or mass per unit weight of soil (e.g., mg/kg or g/kg).

Reagents

- Diacid mixture: Conc. Sulphuric acid and Phosphoric acid in the ratio of 2:1
- Potassium dichromate (0.4 N $K_2Cr_2O_7$): Dissolve 19.612 g of $K_2Cr_2O_7$ in distilled water in a 1-L volumetric flask and make up the volume to the mark
- 0.033 N Ferrous Ammonium Sulphate (FAS): Dissolve 13.071 g of ferrous ammonium sulphate in distilled water and add 21.7 ml of sulphuric acid (0.4 N) and make up the volume to 1-L with distilled water in a volumetric flask
- Ferroin indicator

Procedure (Singh *et al*. 2019)

Take 2 g of soil into 50 ml polypropylene tubes

⬇

Add 20 ml of distilled water

⬇

Shake the contents for 1 hr

⬇

Centrifuge the suspension at 3500 rpm for 20 min

⬇

Filter the supernatant solution through Whatman no. 42 filter paper

⬇

Determine the organic carbon content in the extract by the wet digestion method

⬇

Take 8ml of the extract into 150 ml conical flask

⬇

Add 2 ml of 0.4 N $K_2Cr_2O_7$

⬇

Add 70 mg of HgO

⬇

Add 15 ml of di-acid mixture

⬇

Boil the mixture gently on a hot plate at 150 ºC for 30 min under reflux conditions

⬇

Allow it to cool at room temperature

⬇

Dilute it by adding 20-25 ml of distilled water

⬇

Determine the excess dichromate by back titration with 0.033 N FAS using 5-8 drops of ferroin indicator to get brick red colour end point

Calculation

$$\textbf{WEOC } (\mu g\ g^{-1}) = \frac{\textbf{(BTV-STV) X N of FAS X vol. of extractant X 0.003 X 100}}{\textbf{Wt of soil X aliquot taken}}$$

Where,
BTV = Blank Titre Value
STV = Sample Titre Value

MICROBIAL BIOMASS CARBON

The soil microbial biomass is an important component of the soil organic matter that regulates the transformation and storage of nutrients in soils. It is a labile component of the soil organic carbon fraction containing 1-3% of the total soil organic carbon. It is the living part of soil organic matter (Brookes *et al*., 2001). The size and activity of soil microbial biomass must be assessed to fully understand the nutrient fluxes in the managed and natural ecosystems. Fumigation-incubation and fumigation-extraction methods are the most widely used and recommended methods for the measurement of C, N contents and other nutrients in the soil microbial biomass.

Assumptions

- Fumigation of soil sample kills all the organisms
- Carbon mineralization is more rapid in the dead organisms than in the living organisms and C mineralization from the dead biomass does not differ in different soils over a given period

Fumigation-Extraction method

Principle

Direct measurement of C and other nutrients in the microbial biomass is carried out. The fumigated and control samples are analyzed for mineralized C, N and P. It is a comparitively rapid and less time-consuming method.

Reagents

- Chloroform ($CHCl_3$)
- Diacid mixture: Conc. Sulphuric acid and Conc. Phosphoric acid in the ratio of 2:1
- 0.5 M Potassium sulphate (K_2SO_4): Dissolve 87.13g of K_2SO_4 in distilled water in a 1-L volumetric flask and make up the volume to the mark
- 0.4 N Potassium dichromate ($K_2Cr_2O_7$): Dissolve 19.612 g of $K_2Cr_2O_7$ in distilled water in a 1-L volumetric flask and make up the volume to the mark
- 0.033 N Ferrous Ammonium Sulphate (FAS): Dissolve 13.071 g of ferrous ammonium sulphate in distilled water and add 21.7 ml of sulphuric acid (0.4 N) and make up the volume to 1-L with distilled water in a volumetric flask
- Ferroin indicator: Dissolve 0.695 g of $FeSO_4$.$7H_2O$ and 1.485 g of ortho-phenonthroline monohydrate in 100 ml distilled water in a volumetric flask

Procedure (Singh *et al.* 2019)

Weigh seven sets of 20 g moist soil for each sample

⇩

Keep one set of soil sample in a pre-weighed empty moisture box

⇩

Keep the box in an oven at 105 ºC for 24 hr until a constant oven dried weight is achieved

⇩

Calculate moisture content of the soil

⇩

Keep three sets of soil sample in 50-ml beakers for fumigation

⇩

Fumigate these sets in a dessicator containing about 25 ml methanol $CHCl_3$ in a 50 ml beaker with a few glass beads

⇩

Apply vacuum for 2 mins until chloroform boils

⇩

Close the outlet and put the dessicator in dark for 24 hr

⇩

Keep three sets of soil samples in other dessicator for 24 hr (unfumigated control)

⇩

After 24 hr, release the vacuum for fumigated samples

⇩

Take out the beaker containing chloroform

⇩

Perform back solution 4-5 times to ensure removal of any chloroform vapours present inside the dessicator

⇩

Transfer both the fumigated and un-fumigated soils into 250-ml conical flasks

⇩

Add 80 ml of 0.5 M K_2SO_4 and shake for 30 min

⇩

Filter the extract through Whatman No. 1 filter paper

⇩

Digest the filtered extract (8 ml) with 0.4 N $K_2Cr_2O_7$ (2 ml), 70 mg HgO and 15 ml of di-acid mixture

⇩

Determine organic carbon in the extracts using wet digestion method

⇩

Boil the mixture gently on a hot plate at 150 ºC for 30 min under reflux conditions

⇩

Allow it to cool at room temperature

⇩

Dilute it by adding 20-25 ml of distilled water

⇩

Determine the excess dichromate by back titration with 0.033 N ferrous ammonium sulphate using 5-8 drops of ferroin indicator to get a brick-red end point

Calculation

Normality of FAS (x) = 0.04 X 2/Y

0.04 = Expected N of FAS
2 ml = volume of 0.4 N $K_2Cr_2O_7$
Y = Volume of FAS

$$\textbf{Soil water content (\%)} = \frac{\text{Wt. of wet soil (g) – Wt. of oven dry soil (g) X 100}}{\text{Wt. of oven dry soil (g)}}$$

$$\textbf{Extractable C (µg g}^{-1}\textbf{)} = \frac{\text{(BTV - STV) X N of FAS X 12 X 1000 X (80+water content)}}{\text{4 X 8 X wt. of dry soil}}$$

Where,
BTV = Blank Titre Value; STV = Sample Titre Value

$$\textbf{MBC (µg g}^{-1}\textbf{)} = \frac{\textbf{Extractable C in fumigated soil - Extractable C in unfumigated soil}}{\textbf{K}_{\textbf{EC}}}$$

Where, K_{EC} = 0.45 ± 0.05 (represents the efficiency of extraction)

SOIL ORGANIC CARBON POOLS AND CARBON MANAGEMENT INDEX (CMI)

Soil organic carbon pools consist of labile carbon, which is easily decomposed and influences soil fertility, and stable carbon, which persists long-term and contributes to soil structure. Labile pools, including Water-Extractable Organic Carbon (WEOC), $KMnO_4$ oxidisable carbon and microbial biomass carbon provide early indicators of soil changes, while stable pools, including total organic carbon (TOC) reflect long-term carbon storage and soil health.

Soil carbon is a major determinant of sustainability of agricultural systems and changes can occur in both total and active or labile C pools. These two indices are used to calculate a Carbon Management Index (CMI).

Calculations of CMI require samples of the soils of interest and a sample of reference site. (Use soil from undisturbed site as reference for evaluating impact of agriculture or land uses on C dynamics; and in an experiment control can be used as reference to compare effects of soil management treatments on SOC). Since continuity of C supply depends on both total pool size and lability, both are taken into account in deriving CMI.

A high Carbon Management Index (CMI) value reflects improvements in both the quantity and quality of soil organic carbon, indicating better soil quality and sustainability. CMI helps evaluate and compare management practices, cropping systems, or land uses based on their impact on soil organic carbon and quality. It monitors changes over time or with new practices, signaling whether soil conditions are improving or deteriorating. Although there is no ideal CMI value, it offers a sensitive measure of soil carbon dynamics relative to a standard reference (Blair ***et al***, 1995).

Carbon Pool Index (CPI)

The loss C from a soil with a small C pool size is of grater consequence than the loss of same quantity of C loss from a soil with a large C pool size. To account for this, a CPI (ratio of total C pool in sample to total C pool in reference) is calculated as:

$$\text{CPI} = \frac{\text{Total C in sample}}{\text{Total C in reference}}$$

Carbon Lability Index (CLI)

The loss of labile C is of greater consequence than the loss of non-labile C. To account for this, a CLI (ratio of carbon lability in sample soil to carbon lability in reference soil) is calculated as:

$$\text{CLI} = \frac{\text{Lability of C in sample}}{\text{Lability of C in reference}}$$

Where, lability of C represents the ratio of labile C (i.e., C fraction oxidized by $KMnO_4$) to non-labile C (i.e., C fraction not oxidized by $KMnO_4$) in a given soil.

Carbon Management Index (CMI)

The CMI is the product of carbon pool index and carbon lability index size.

$$\text{CMI} = \text{CPI} \times \text{CLI} \times 100$$

ENUMERATION OF SOIL MICROBIAL POPULATION

Soil is a heterogeneous matrix in which microbes are associated with organic and inorganic soil particles, forming aggregates. The goals of sample preparation for conventional enumeration techniques are to release the microbes from the matrix of a representative soil sample, then disperse them in a suitable diluent so that individual cells can be enumerated either by microscopic visualization or cultivation methods.

Principle

The pre-weighed soil sample is diluted in suitable buffered diluents which releases the microbial cells from the soil matrix in suspension to suitable cell density which can be enumerated after plating. The main assumption of this method, as a means of estimating the microbial population in soil, is that each viable cell of microbe or its propagule in a soil suspension will produce a visible colony after its inoculation in the plate containing appropriate growth medium. The viable population of a particular microorganism in a sample is found by multiplying the number of colonies formed from a particular serial dilution with the reciprocal of dilution factor.

Reagents

- Sterile buffered diluent dispensed in dilution bottles/conical flask/test tubes (9, 90, 99ml)
  [Diluents: Phosphate buffered saline (0.85% NaCl, 2.2 mM KH_2PO_4 4.2 mM Na_2HPO_4; pH 7.0) with or without 0.01% gelatine or peptone; Mineral salt medium without carbon
  source (Atlas 1995); 0.1% sodium pyrophosphate with or without 1% glycerol (Trevors and Cook 1992) or 0.1% peptone
- Filter sterilized stock solution of streptomycin (3.0 g 100 ml)
- Filter sterilized stock solution of actidion (5.0 g 100 ml)

Growth Media

The growth media can be made partially selective to favour the growth of specific groups of microorganisms by altering the pH of medium or by adding inhibitors. The setting of pH to 5.0-5.5 or addition of streptomycin (30 mg L^{-1}) and Rose Bengal (33 mg L^{-1}) checks the bacterial growth and favours fungi. Similarly, neutral to slightly alkaline pH or addition of actidion (50 mg L^{-1}) and nystatin (50 mg L^{-1}) checks the fungal growth and favours bacteria and actinomycetes.

Bacteria- Plate count agar

Tryptone-5 g; Yeast extract – 2.5 g; Glucose – 1.0 g; Agar – 15 – 20 g; Distilled water – 1000 ml;pH – 7.0

Nutrient Agar

Beef exctract – 3.0 g; Peptone – 5.0 g; NaCl – 5.0 g; Agar – 15 – 20 g; Distilled water – 1000 ml; pH – 7.0

Fungi - Rose Bengal - Streptomycin Agar

Glucose – 10 g; Peptone – 5 g; KH_2PO_4 – 1 g; $MgSO_4$. $7H_2O$ – 0.5 g
Agar – 15 – 20 g; Rose Bengal – 33 mg; Streptomycin – 30 mg; Distilled water – 1000 ml pH – 5.5, Autoclave the medium, cool to 45^0C and add filter sterilized (0.2 µm pore diameter filter) streptomycin

Actinomycetes – KenKnight and Munaiers Agar

Dextrose – 1.0 g; KH_2PO_4 – 0.1 g; $NaNO_3$ – 0.1 g; KCl – 0.1 g
$MgSO_4$. $7H_2O$ - 0.1 g; Agar – 15 – 20 g; Distilled water – 1000 ml; pH – 7.0

Procedure(Singh *et al.*, 2019)

Sample Collection

Collect the representative soil sample aseptically following the standard procedures

Keep the soil sample in fresh polythene bag/sterilized container and transport it to the lab immediately under cool conditions and store it in a refrigerator at 4 ^{o}C

The sample preferably should be processed immediately within 10-15 days

Soil should not be dried as it would reduce the number of microorganisms

Preparation of Serial Dilutions

Weigh 10 g of field moist soil accurately on a top loading balance and suspend it in to first dilution bottle or flask containing 95 ml. diluent and 15-20 g glass beads and shake it vigorously for 5 min on reciprocating shaker

⇓

Alternatively, if glass beads are not added, then mix it thoroughly in blender/ mixer for 2 min to disrupt the soil aggregates, which will 1/10 or 10^{-1} dilution

⇓

The volume of diluent may be taken either as 90 ml or 95 ml

⇓

The quantity of diluents may vary from 90 to 95 ml, but the ratio of soil and diluents will remain 1:10

⇓

Weigh a similar sample in the aluminium moisture box for determining the dry mass of soil by drying the sample at 105 °C to a constant mass (gravimetric method)

⇓

Perform 10-fold dilution by transferring 10 ml of 10 dilution with the help of a sterilized 10-ml. pipette to another

90 ml dilution bottle to get 1/100 or 10^{-2} dilution

⇓

Continue the serial dilution process by transferring either 10 ml or 1 ml of the previous dilution to the 90/9 ml dilution blank to get subsequent higher dilutions

⇓

Transfer 10/1 ml suspension from 10^{-2} dilution to the 90/ 9 ml dilution blank for subsequent dilutions, viz. 10^{-3}

⇓

Continue the process with a 10-fold serial dilution up to 10^{-8} dilution for most of the fertile soils or a lower dilution, depending on the type of soil sample

Plating

Bacteria – select 10^{-6}, 10^{-7}, 10^{-8} dilutions
Actinomycetes – select 10^{-5}, 10^{-6}, 10^{-7} dilutions
Fungi – select 10^{-4}, 10^{-5}, 10^{-6} dilutions

Spread Plate Technique

Pour 15-20 ml of sterilized appropriate medium in pre-sterilized dishes under aseptic conditions and allow it to solidify

After solidification, keep the plates half open in a laminar air-flow cabinet for drying of the excess moisture, taking utmost precaution for not to occur contamination

Incubate the poured plates for 24 hr and check the plates for any growth of microbes as contaminants

Use only the clean poured plates having no contamination for plating the sample

Prepare three replicate plates for each dilution using the spread plate method

Inoculate the surface of the solid agar with 0.1 ml of the diluted soil suspension and spread it evenly with the help of sterile L-shaped glass spreader

Use a separate glass spreader for each dilution

Appropriate dilutions should be selected for different groups of microbes

Transfer 0.1 ml of soil suspension after thorough vortex mixing with the help of a sterilized pipette from the 10^{-6}, 10^{-7}, 10^{-8} dilutions on the surface of plate count agar/ nutrient agar for bacterial enumeration

Transfer 0.1 ml of soil suspension after thorough vortex mixing with the help of a sterilized pipette from the 10^{-5}, 10^{-6}, 10^{-7} dilutions on the surface of Kenknight and Munaier's agar medium for actinomycetes enumeration

Transfer 0.1 ml of soil suspension after thorough vortex mixing with the help of a sterilized pipette from the 10^{-4}, 10^{-5}, 10^{-6} dilutions on the surface of Rose Bengal-streptomycin agar for fungi enumeration.

Incubate the plates in an inverted position (upside down) at 28 °C for 7 days

Record the observations after 48 hr for bacteria, 96 hr for fungi and a week for actinomycetes

At the end of incubation, i.e., after 7 days, check the bacterial and fungal plates for new colonies if any, and add those to the previous count

For bacteria, select the plates having colony count of 30-300

Counting of colonies can be made using colony counter

Place the plate over an illuminated platform and count the colonies looking through a 75-mm magnifier by marking on glass above each colony with tip of marker

Alternatively, the plates can be held against the light and colonies can be counted by marking above each colony

Record the count for fungal and actinomycetes colonies too by marking the colonies

Calculate the viable count of microbial group as colony forming unit (CFU) g^{-1} of oven-dried soil

Pour Plate Technique

Fixed amount of inoculum, preferably 1 ml, from a dilution/broth/sample is placed in the center of a sterile plate using a sterile pipette

The molten cooled medium agar to 45 °C (15-20 ml) is then poured into the plate containing the inoculum and mixed well by rotating clock- and anticlockwise

After solidification of the agar medium, the plate is inverted and incubated at 28 °C for 7 days

The microorganisms (bacteria, actinomycetes) will grow both on the surface and within the medium

Each (both large and small) colony is carefully counted using a magnifying colony counter, if needed and the number of cells/ propagules is ascertained as CFU g^{-1} oven-dried soil

Melt the requisite media and cool to 45 °C and keep in a water bath set at 45 °C

Take three sterilized plates per dilution to be tested and label them with dilution number on the bottom of the plate

Transfer 1 ml of soil suspension from the appropriate dilution with the help of a sterilized pipette in the centre of the plate by opening it sufficient to insert the pipette

Add 15-20 ml appropriate molten media cooled to 45 °C to each plate and mix it thoroughly by rotating the plate six times each clockwise and anticlockwise

Also, move the plate back and forth six times

Allow the medium to solidify

After solidification, incubate the plates in an inverted position (upside down) in a B.O.D. incubator at 28±0.2 °C for seven days

Record the observations for enumeration of bacteria, fungi and actinomycetes

Calculation

$$\text{No. of colony forming units (CFU) } g^{-1} \text{ of oven dried soil} = \frac{\text{(No. of average colonies) X (dilution factor) X (soil moisture correction factor)}}{\text{Volume of soil suspension plated}}$$

SOIL DEHYDROGENASE ACTIVITY

Microbes play an important role in the oxidation of organic matter and dehydrogenase is an integral part of the viable microbial cells. Assaying dehydrogenase enzyme activity is a common method to estimate microbial activity, especially in soil and water environments. Dehydrogenase is an enzyme that catalyzes the oxidation-reduction reactions involved in the transfer of hydrogen atoms from various substrates to electron acceptors, such as NAD+ or NADP+. Thus, dehydrogenase activity correlates well with microbial biomass and microbial population.

Principle

Triphenyl tetrazolium chloride (TTC) is used as a substrate in this assay, as it possesses the property of being easily transformed into an intensely coloured water-insoluble but methanol-soluble, compound, *viz*. triphenylformazan (TPF). This method is based on the extraction of triphenylformazan (formed by the reduction of TTC in soil) with methanol and its colorimetric estimation.

Reagents

- 2, 3, 5-Triphenyl tetrazolium chloride (TTC 3%): Dissolve 3 g of TTC in about 80 ml water, and make up the volume to 100-ml with distilled water in a volumetric flask and store it in dark bottle
- Methanol (A R grade)
- Standard triphenyl formazan (TPF) - stock solution: Dissolve 100 mg of TPF in about 80 ml of methanol, and make up the volume to 100-ml in a volumetric flask with methanol and mix thoroughly. This stock solution contains 1000 µg TPF ml^{-1}
- Calcium carbonate ($CaCO_3$)

Preparation of standard curve

- Take stock solution of TPF (0.1, 0.2, 0.4, 0.8 and 1.0 ml) in 10 ml volumetric flasks, and make up the volume of each flask to 10 ml by adding methanol
- Measure the intensity of pink colour at 485 nm wavelength with the help of a spectrophotometer using methanol as blank
- A standard curve may be plotted between concentrations of TPF vs absorbance at 485 nm wavelength and the concentration of TPF in unknown sample may be calculated from the graph

Procedure (Singh *et al.*,2019)

Place 6 g field moist soil sample (in triplicate) in a screw-capped tube

⇩

Add 0.1g $CaCO_3$ to each tube and mix thoroughly

⇩

Add 1 ml of 3% aqueous solution of TTC

⇩

Add 2.5 ml of distilled water to each tube

⇩

Mix the contents of each tube and stopper it

⇩

Incubate the contents for 24 hr at 30°C in dark

⇩

Remove the stopper of each tube and add 10 ml of methanol, stopper it again

⇩

Shake it for 1 min

⇩

At this stage, pink colour will appear in the tube

⇩

Unstopper and filter the suspension through What. No.42 filter paper using a glass funnel

⇩

Note down the amount of extract used

⇩

Measure the intensity of pink colour at 485 nm wavelength with a spectrophotometer using methanol as blank

⇩

Calculate the amount of TPF produced with the help of a calibration graph prepared from the standard TPF solutions

Calculation

Dividing the amount of TPF by 6, since 6 g soil is used and represent the results as µg of TPF released g^{-1} soil day^{-1}

SOIL ALKALINE AND ACID PHOSPHATASE ACTIVITY

Phosphatases are the enzymes that catalyze the hydrolysis of ester-phosphate bonds, leading to the release of P, which can be taken up by plants or microorganisms. The phosphatase enzymes are produced by the bacteria, fungi and plant roots and as a result, soil contains large quantities of intracellular and extracellular phosphatases. The activities of phosphatases depend on several factors such as soil properties, soil organism interactions, plant cover, leachate inputs and presence of inhibitors and activators.

Principle

The assay of phosphomonoesterase activities is based on the colorimetric estimation of p-nitrophenol released by the phosphatase activity when soil is incubated with a buffered solution (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphatase activity) of sodium p-nitrophenyl phosphate and toluene. The colorimetric procedure used for estimation of p-nitrophenol is based on the fact that alkaline solutions of this phenol have a yellow colour (acid solutions of p-nitrophenol and acidic and alkaline solutions of p nitrophenyl phosphate are colourless).

Reagents

- Toluene
- Modified Universal Buffer (MUB) stock solution: Dissolve 12.1 g of tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid (H_3BO_3) in 488 ml of 1 N sodium hydroxide (NaOH) and dilute the solution to 1L with distilled water in a volumetric flask. Store it in a refrigerator
- Working Modified Universal Buffer (MUB) solution (pH 11 for alkaline phosphatase): Place 200 ml of MUB stock solution in a 1000-ml beaker containing a magnetic stirring bar, and place the beaker on a magnetic stirrer. Adjust the pH of the solution to 11 with 0.1 N NaOH and make up the volume to 1-L with distilled water in a volumetric flask
- Working Modified Universal Buffer (MUB) solution (pH 6 for acid phosphatase): Place 200 ml of MUB stock solution in a 1000-ml beaker containing a magnetic stirring bar, and place the beaker on a magnetic stirrer. Adjust the pH of the solution to 6 with 0.1 N HCl and make up the volume to 1-L with distilled water in a volumetric flask

- *p*-Nitrophenyl phosphate (PNP) solution (0.025 M): Dissolve 0.42 g of disodium *p*-nitrophenyl phosphate tetrahydrate in about 40 ml of modified universal buffer (MUB) of pH 11 (for assay of alkaline phosphatase), and dilute the solution to 50-ml with MUB of the same pH. Store the solution in a refrigerator in dark bottle
- Calcium chloride solution (0.5 M $CaCl_2$): Dissolve 73.5 g of $CaCl_2$. $2H_2O$ in about 700 ml of water, and make up the volume to 1-L
- Sodium hydroxide solution (0.5 M NaOH): Dissolve 20 g of NaOH in about 700 ml of water, and make up the volume to 1-L with distilled water
- Standard *p*-nitrophenol solution (1000 ppm): Dissolve 100 mg of *p*-nitrophenol solution in about 70 ml of distilled water and make up the volume to 100 ml with distilled water. Store the solution in a refrigerator in a dark bottle

Preparation of standard curve

- To draw standard curve, dilute 10 ml of standard p-nitrophenol solution to 100 ml with distilled water in a 100 ml volumetric flask, and mix the solution thoroughly
- Then pipette out 0, 1, 2, 3, 4 and 5 ml aliquots of this solution into 10 ml volumetric flasks to get 0, 10, 20, 30, 40 and 50 µg of nitrophenol concentrations and proceed as described for p-nitrophenol analysis of the incubated soil samples. If the colour intensity of the filtrate exceeds that of the 50 µg of the p–nitrophenol standard, an aliquot of the filtrate should be diluted with water until the colorimeter reading falls within the limits of the standard curve
- Controls should be performed without soil to allow for, colour not derived from p-nitrophenol released by phosphate activity. To perform controls, follow the procedure described for the assay of phosphatase activity, but add 1 ml of p-nitrophenyl phosphate solution after the additions of 0.5 M $CaCl_2$ and 4 ml of 0.5M NaOH (just before filtration of soil suspension)

Procedure (Singh *et al.*, 2019)

Place 10 g of soil in a 100-ml conical flask

⇩

Add 0.2 ml of toluene

⇩

Add 4 ml of MUB

⇩

(of pH 11.0 for assay of alkaline phosphatase and of pH 6.0 for acid phosphatase)

⇩

Add 1 ml of p-nitrophenyl phosphate solution prepared in the same buffer

⇩

Swirl the flask for a few seconds to mix the contents

⇩

Stopper the flask, and place it in an incubator at 37°C for 1 hr

⇩

After 1 hr, remove the stopper

⇩

Add 1 ml of 0.5 M $CaCl_2$

⇩

Add 4 ml of 0.5 M NaOH

⇩

Swirl the flask for a few seconds

⇩

Filter the soil suspension through Whatman No. 1 filter paper

⇩

Measure the intensity of yellow colour of filtrate at 430 nm wavelength

Calculation

- Calculate the p-nitrophenol content, by referring to the calibration graph plotted from the results obtained using the standard solutions containing 0, 10, 20, 30, 40 and 50 µg of p-nitrophenol.
- The µg of p-nitrophenol released g^{-1} soil hr^{-1} can be calculated by referring to the standard curve

SOIL UREASE ACTIVITY

Urease is vital in soil as it converts urea into ammonia, making nitrogen available for plant uptake. This enzyme driven process is crucial for nutrient cycling, soil fertility, and the effectiveness of urea-based fertilizers. Urease activity influences nitrogen availability, impacting plant growth and overall soil health

Principle

Soil samples are incubated with a urea solution as a substrate. The ammonium released is then determined using steam distillation followed by a volumetric assay (Tabatabai and Bremmer 1972).

Reagents

- Toluene AR Grade
- Tris (hydroxymethyl) amino methane (THAM) buffer, 0.05 M, pH-9.0: Dissolve 6 g of THAM in about 700 ml of water and adjust pH of solution to 9.0 by addition of approximately 0.2M H_2SO_4 and make the volume to 1 litre with distilled water
- Urea Solution, 0.2 M: Dissolve urea (1.2 g) in about 80 ml of Tris (hydroxymethyl) amino methane buffer and dilute the solution to 100 ml with THAM buffer. Prepare this solution before usage
- Potassium Chloride Silver Sulphate (2.5M KCl-Ag_2SO_4 100 ppm) Solution: Dissolve 100 mg of reagent grade Ag_2SO_4 in about 700 ml of distilled water and dissolve 188 g of reagent grade KCl in this solution and make the volume to one litre with distilled water

Reagents for the estimation of NH_4^+- N

- Magnesium Oxide: Heavy magnesium oxide.
- Boric Acid Indicator Solution: Dissolve 20 g boric acid in about 700 ml of hot distilled water. Transfer the cooled solution to one litre volumetric flask containing 20 ml of mixed indicator solution (prepared by dissolving 100 mg bromocresol green and 50 mg of methyl red in 100 ml of ethanol). After mixing the contents of flasks dilute the solution to one litre with water and mix thoroughly
- Devarda's Alloy
- 0.005 N Sulphuric acid (H_2SO_4): Add 0.133 ml of conc. H_2SO_4 in 1 L volumetric flask and make up with distilled water

Procedure (Schinner *et al.*, 2012)

Weigh 5 gm of field-moist soil samples in to 50 ml volumetric flasks

⬇

Add 9 ml of THAM buffer

⬇

Swirl the flasks for a few seconds to mix the contents

⬇

Add 1 ml of 0.2 M urea solution

⬇

Swirl again for a few seconds to mix the contents

⬇

Stopper the flasks and incubate at 37°C for 2 hours

⬇

After 2 hr remove the stoppers

⬇

Add 35 ml of KCl- Ag_2SO_4 solution

⬇

Shake the flasks for 30 mins on a rotary shaker

⬇

Allow to stand until the contents cool down to room temperature

⬇

Make the contents to 50 ml by distilled water

⬇

Stopper the flasks and invert several times to mix the contents and filter the suspension

⬇

Pipette 20 ml of filtrate in to the distillation flask

⬇

Add 0.2 g of MgO

⬇

Distill NH_4^+-N into 10 ml boric acid indicator solution for 3.3 mins after the indicator changes colour from violet to green

⬇

Determine NH_4^+-N in the distillate by titration with diluted sulphuric acid

⬇

For blank, follow the same procedure as described for assay for urease activity except that add 1 ml of 0.2 M urea solution after the addition of 35 ml of KCl- Ag_2SO_4 solution

Calculation

$$\textbf{Urease activity } (\mu g\ NH_4^+\text{-}N\ g^{-1}2h^{-1}) = \frac{TV \times 0.07 \times 50 \times 1000 \times 100}{20 \times 5 \times \%\ dm}$$

100% dm - factor for soil dry matter

SOIL RESPIRATION BY ALKALI TRAP METHOD

Soil respiration is a measure of soil microbial activity or soil catabolic activity has been widely used for many years to quantify the influence of treatment and management inputs. The flux of CO_2 evolved as soil respiration in a soil is influenced by all the factors that govern the decomposition rate of organic matters in a soil system, including the quality and quantity of organic substrates, the efficiency and population dynamics of various decomposer groups and the soil physico-chemical conditions such as moisture, temperature, oxygen, acidity and redox potential.

Principle

This method involves absorption of CO_2 in a given period of time in known volume and strength of alkali (e.g., NaOH or KOH). When CO_2 is absorbed in NaOH it converts to Na_2CO_3. The excess of NaOH is titrated against standard HCl, few drops of saturated $BaCl_2$ solution is added to NaOH solution to precipitate the Na_2CO_3 as $BaCO_3$, otherwise CO_3^{-2} in Na_2CO_3 will consume HCl and underestimate the CO_2 evolved.

Reagents

- 1N Sodium hydroxide (NaOH): Dissolve 40 g of NaOH pellets in 800 ml of distilled water in a 1-L, volumetric flask and make up the volume to the mark
- 1N Hydrochloric acid (HCl): Add 12 ml of conc. HCl in distilled water in a 1-L volumetric flask and make up the volume to the mark. Standardize this solution against 1N Sodium carbonate (Na_2CO_3) solution to know the precise strength of HCl
- Saturated barium chloride solution
- Phenolphthalein indicator: Dissolve 0.5 g of phenolphthalein in 100 ml of 95 % alcohol

Procedure (Singh *et al.*, 2019)

Place 10 g air-dried and sieved (2-mm) soil in a conical flask

⇩

Add water to its water holding capacity, which is to be maintained throughout the incubation period

⇩

Hang a bottle or vial containing 10 ml of 1 N NaOH solution with a thread in the conical flask

⇩

Tighten the mouth with a rubber cork to ensure that there is no leakage

⇩

Incubate the flask at 30 +/- 2°C initially for two days and then estimate the evolved CO_2. Subsequent estimation can be done at weekly intervals

⇩

Set a blank in empty flask with 10 ml of 1 N NaOH in vial and tighten the mouth of flask

For estimation of evolved CO_2

Take 1 N NaOH solution contained in the bottle

⇩

Place in a 250-ml conical flask

⇩

Wash the vial with distilled water to remove any traces of CO_2 left

⇩

To this 250 ml conical flask, add a few drops of phenolphthalein indicator

⇩

Add 10 ml of saturated or 2 N $BaCl_2$ solution to precipitate the absorbed CO_2 as $BaCO_3$

⇩

Titrate the contents of conical flask against 1 N HCl till the pink colour disappears

⇩

Replace the bottle with 10 ml of 1 N NaOH in the same flask containing soil for next observation

Calculation

C or CO_2 (mg) = (B-V) X N X E

Where, B = Volume of HCl to titrate NaOH in the blank;V = Volume of HCl to titrate NaOH in soil sample; N = Normality of HCl; E = Equivalent weight (For C = 6, CO_2 = 22)

SOIL TEXTURE BY FEEL METHOD

Soil texture refers to the relative percentage of sand, silt and clay in a soil which is otherwise called the mechanical composition of the soil. The texture of the soil horizon is a permanent character. Soil texture can be measured by feel method and international pipette method. The soil texture by feel method offers several advantages *viz.,* it is cost-effective, can be carried out directly in the field, provides immediate results, is easy to use, involves simple procedures, and does not require chemical reagents and have disadvantages *viz.,*limited precision, does not provide quantitative data and limited to field conditions only.

General characteristics of soil separate

Sand: Individual particles feel gritty when the soil is rubbed between thumb and forefinger. They are non-plastic and non-sticky when moist.

Silt: In dry condition, it feels like flour or talcum powder when rubbed.

Clay: In moist condition, it feels smooth, sticky and plastic. It forms very hard clods when dried. The particles tend to remain suspended in water for long time.

Procedure (Ditzler *et al*. 2017)

Place about a tea spoon of soil in the left palm

⇩

Add few drops of water

⇩

Mould the soil in the left palm and bring consistency to dough, by adding soil/water, depending on the wetness or dryness of the soil

⇩

This wet soil mass is used for evaluating grittiness, stickyness or plasticity of the soil

⇩

Rub the soil mass between fingers to feel the predominance of grittiness or smoothness

⇩

Knead the soil mass to form balls

⇩

Look for its strength (weak, crumbles, strong, etc) and appearance (smooth or rough surface)

⇩

Observe the soil mass for stickyness and ribbon formation (forms the ribbon/rod like structure and bends them to form a ring)

⇩

The length and thickness of the ribbon/rod is used for course and fine texture analysis (good/poor or no ribbon formation)

⬇

Similarly, the ring formation of the soil mass – breaks immediately, bends to some extent and it can be moulded into a perfect ring

⬇

The observations recorded are compared with the table

Keys to determine soil texture by feel method

Sl. No	Soil textural class	Feel of moist soil	Ball formation from slightly moist soil	Stickiness (stains finger or not)	Plasticity Ribbon (wire) formation
1	**Sand**	Very gritty	Does not form ball	Does not stain fingers	No ribbon formation
2	**Loamy sand**	Very gritty	Forms very weak balls that break very easily	It stains fingers slightly	No ribbon formation
3	**Sandy loam**	Moderately gritty	Forms fairly good balls but easily broken i.e., it can bear careful handling	Stains fingers	No ribbon formation
4	**Loam**	Slightly gritty and fairly smooth	Forms fairly firm balls and can bear easy handling	Stains fingers	No ribbon formation
5	**Sandy clay loam**	Slightly gritty and fairly smooth	Fairly firm balls are slightly hard on drying	Sticks on finger	Slight tendency to form ribbon
6	**Silt loam**	Smooth butter feel	Fairly firm balls are moderately hard on drying	Sticks to both fingers	No ribbon or slight tendency to form ribbon with flaky surface
7	**Clay loam**	Smooth	Fairly firm balls are moderately hard on drying	Sticks to both fingers	Short ribbons are formed but breaks easily
8	**Silty clay loam**	Very smooth	Fairly firm balls are moderately hard on drying	Sticks to both fingers and it is somewhat flexible	Slightly longer ribbons are formed and shows flaking on ribbon surface
9	**Clay**	Very smooth	Very firm balls are very hard on drying and cannot be crushed by fingers	Sticks to both fingers and it is very flexible	Long flexible ribbons (2.5 cm - 7.5 cm) are formed

SOIL TEXTURE BY INTERNATIONAL PIPETTE METHOD

Principle

The International Pipette Method operates on the principle of Stokes law (1851). It states that the resistance offered by a liquid to the falling of rigid spherical particle varies with the circumference of the sphere, and not with its surface. The force of fall of particle is proportional to its weight and consequently, to its volume.

Stokes law: Rate of falling of spherical particle in liquid medium is directly proportional to square of the radius and inversely proportional to viscosity of the medium. As viscosity of water at a given temperature is constant, the velocity of the falling particle is directly related to the size (radius) of the particles.

$$V = \frac{2r^2 g \, X \, (D_p - D_l)}{9\eta}$$

Where,
V = settling velocity (cm/s); R = radius of the particle (cm); g = acceleration due to gravity
D_p = density of particle; D_l = density of liquid; η = viscosity of the liquid in poise (g cm^{-1} s^{-1})

Materials: Beaker (500-ml), Hot water bath, Rubber tipped glass rod, Wash bottle, Filter paper (Whatman No. 50), Electric stirrer, Rubber pestle, Sieve (70-mesh), Measuring cylinder (1-L with cap), Glass funnel, Thermometer, Pipette (10-ml cap) (preferably Robinson's pipette), An iron rod, attached to a circular metallic disc of 5-6 cm in diameter and about 0.15 cm in thickness, i.e., a plunger, Porcelain dishes or silica crucibles, Balance, and Oven.

Reagents

(i) Hydrogen peroxide (6% H_2O_2)

(ii) Hydro Chloric Acid (2 N HCl)

(iii) Silver Nitrate (0.1N $AgNO_3$)

(iv) Sodium Hydroxide (0.1 N NaOH) or Sodium Hydrogen Phosphate (1 N $NaHPO_4$)

(v) Phenolphthalein indicator

Procedure (Hinga *et al*. 1980; Singh *et al*. 2019)

A. Treatment with Hydrogen Peroxide

Take 20 g (a) of air-dried soil (ground and sieved through a 2-mm sieve) in a 500-ml beaker

Add 50-60 ml of 6% H_2O_2

Swirl the contents well

Let the reaction proceed for 5-10 min

Place the beaker over a hot water bath

Continue digestion by constant stirring with a glass rod to avoid frothing, till the reaction completely subsides

In case frothing persists, add a second lot of 30-40 ml of H_2O_2

See for effervescence, if any

Continue the step till the whole organic matter is oxidized

Cool the beaker

Rinse its sides with a rubber pestle and with a jet of distilled water

B. Treatment with Acid and Filtration

Add 25 ml of 2 N HCl

⬇

Shake to destroy $CaCO_3$ present in the soil

⬇

If the soil contains more than 2% $CaCO_3$, more HCl should be added at the rate of approximately 250 ml for each per cent of $CaCO_3$

⬇

Dilute to approximately 250 ml and thoroughly rub the soil with a rubber pestle

⬇

Allow the contents to react for about 1 h with intermittent shaking

⬇

Filter the contents through a Buchner funnel under vacuum using a What. No. 50 filter paper.

⬇

Wash the soil with distilled water till the filtrate runs free of chloride

C. Dispersion and Separation of Coarse Sand

Transfer the soil sample from the filter paper to a 500-ml beaker with a jet of distilled water

⬇

Make the volume to about 400-500 ml with distilled water

⬇

Add a few drops of phenolphthalein indicator

⬇

Add 0.1 N NaOH till the whole suspension shows a pink colour, indicating its alkaline reaction (about 7 ml of 0.1 N NaOH is just sufficient for the purpose)

⬇

Stir the contents of beaker with an electric stirrer for 10 min

⬇

Transfer the contents quantitatively to a 70-mesh (0.2 mm) sieve placed in the mouth of the funnel which is held over the top of a 1-L sedimentation cylinder

⬇

Pour the suspension onto the sieve and with a jet of distilled water

⬇

Wash the material as much as possible, through the sieve, until no more clay and silt remain on the sieve and the cylinder is about one-half full

⬇

Transfer the coarser particles, retained on the sieve, to a weighed dish(W_1) or crucible and dry it at 105 °C in the oven to a constant weight

⬇

Record the weight (W_2) nearest to 0.001 g accuracy

Separation of Silt and Clay

Make up the volume of the suspension in the cylinder to 1-L by adding more distilled water

↓

Homogenize the suspension

↓

Next day in early morning hours, place the cylinder in a temperature controlled chamber to ensure minimum variation of temperature between the two samplings

↓

Alternatively, measure the temperature of the suspension from Table 1 and find out the requisite time for sedimentation, corresponding to this temperature, for silt and clay

↓

Determination

Table 1. Sedimentation times for particle of silt (<60 µm, <20 µm and <6 µm) clay (<2 µm) settling through water for a depth of 10 cm

Temp (°C)	Silt			Clay (<2 µm)
	<60 µm	<20 µm	<6 µm	
19	32 sec	4m 44sec	52 m 38 sec	7 h 54 m
20	31 sec	4m 36 sec	52 m 09 sec	7 h 40 m
21	30 sec	4m 31 sec	50 m 06 sec	7 h 31 m
22	29 sec	4m 24 sec	48 m 55 sec	7 h 20 m
23	29 sec	4m 18 sec	47 m 44 sec	7 h 10 m
24	28 sec	4m 12 sec	46 m 42 sec	7 h 0 m
25	27 sec	4m 07 sec	45 m 40 sec	6 h 51m
26	27 sec	4m 01 sec	44 m 40 sec	6 h 42 m
27	26	3m 56 sec	43 m 42 sec	6 h 33 m

Stir the content with a plunger by moving it up and down, about 20-25 times per min so as to ensure stability of the suspension

Remove the plunger gently and note down the time at which the plunger is taken out (This is the time of commencement of sedimentation)

Insert the sampling pipette gently into the suspension

Dip it to 10-cm depth from the surface of the suspension about 20 sec before the time is up

Withdraw 10 ml suspension carefully

Deliver the content of the pipette into a weighed dish (W_3)

Dry the sample at 105 °C to a constant weight

Record the weight of the suspension (W_4)

Separation of Clay

Stir the contents of the cylinder again for 1 min as before

Sample in the same way at a depth of 10 cm immediately after the expiry of the requisite time for clay

Transfer the suspension to a weighed dish (W_5)

Dry it at 105°C

Record the weight (W_6)

Separation of Fine Sand

Decant the bulk of the suspension

↓

Transfer the sediment containing the fine sand to a 500-ml beaker with a jet of distilled water

↓

Pour away the turbid suspension each time the sediment is washed with distilled water

↓

Continue the last step until the liquid no longer stands turbid

↓

Remove quantitatively with a jet of distilled water the particles left in the beaker to a weighed dish (W_7) and record its weight (W_8)after oven-drying at 105 °C to a constant value

↓

Compare the fine sand, thus, obtained with that obtained by the difference method

↓

Take a given amount of the same soil and record its oven-dry weight for the determination of water content of the soil

Observations and Calculations

$$\text{Oven dry weight of the soil (W)} = \frac{100 \text{ X a}}{100 \text{ X b}}$$

Where, a = weight of the soil taken (g), b = water content of the soil (%)

$$\text{Coarse sand (\%), P} = \frac{W_2 - W_1}{W}$$

Where, W_1 = weight of dish (g), W_2 = weight of dish + coarse sand W = oven dry weight of the soil

$$\text{Silt + Clay (\%), } P_{sic} = \frac{(W_4-W_3) \text{ X } 1000 \text{ X } 1000}{W \text{ X } 10}$$

Where, W_3 = weight of dish (g), W_4 = weight of dish and silt + clay (g),

$$\text{Clay (\%), } P_c = \frac{(W_6-W_5) \text{ X } 1000 \text{ X } 1000}{W \text{ X } 10}$$

Where, W_5 = weight of dish (g), W_6 = weight of dish clay (g)

$$\text{Silt (\%), } P_{si} = P_{sic} - P_c$$

$$\text{Fine sad (\%), } P_{fs} = \frac{(W_8 - W_7) \text{ X } 100}{W}$$

Where, W7 = weight of dish, W8 = weight of dish + fine sand

SOIL MOISTURE BY GRAVIMETRIC METHOD

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

Procedure

Collect soil samples by tube or auger from a number of points within the experimental site and mix thoroughly

Place composite sub samples of about 50 g to 100g in soil moisture cans with tight-fitting lids

Take at least three sub samples

The moist samples are weighed immediately (W_2), dried to constant weight in an oven at 105°C (for about 24 hr) and reweighed (W_3) after cooling in a desiccator

Determine the weight of moisture cans (W_1)

Calculate the soil moisture content by determining the loss in weight on drying and the weight of the oven-dry soil

Calculation

$$W = \frac{(W_2 - W_3) \times 100}{W_3 - W_1}$$

Where, W_1 is the weight of empty container in grams; W_2 is the weight of container + wet soil in grams; W_3 is the weight of container + dry soil in grams

BULK DENSITY

Soil bulk density (BD) is a measure of the oven-dried mass per unit volume of bulk soil. Typically, sands pack more closely and values range from 1.4 to 1.9 Mg m^{-3}. The value depends on several factors like soil texture, structure, organic content, state of compaction, ***etc.*** The clays tend to bridge and cannot pack as tightly, giving BD values from 0.9 to 1.4 Mg m^{-3}.

Core Method

Principle

The core auger method involves sampling of a soil core from a desired depth under its most natural condition using a cylindrical core sampler and determining the oven-dried mass of soil per unit volume of core.

Apparatus

Core sampler, Metal cores, Aluminum can box, Oven, Weighing balance, Knife, Vernier callipers

Procedure

Measure the length (l) and diameter (2r) of the core (volume = $\pi r^2 l$) using a vernier caliper and arrange them before going to the field

In the field, place the core inside the core sampler

For collecting samples, the assembled core cutter is positioned over a clean levelled surface and pressed inside the soil by rotating the handles or by dropping a hammer over the central portion of the upper end of the thick rod until the edge of the collar comes to rest over the soil surface

Loosen the grip of core cutter from soil around it by moving the stem forward and backward

Pull out the core cutter slowly from the soil and place it horizontally on the surface. Unscrew the collar and take out the rings and core containing the soil carefully

Trim off the extra soil from both ends of the core with a sharp knife or spatula

If weighed in the laboratory, place cores containing soil samples into strong plastic bags that can be sealed tightly with minimal air space

Transfer the soil core from the sampler to a pre-weighed aluminum can and take the weight. Then, put the same in the oven at 105°C till constant weight is achieved

Take the dry weight of the soil with can (W_1)

Take out the sample and weigh the empty can again (W_2)

Calculation

$$\textbf{Bulk density of soil (Mg m}^{-3}\textbf{)} = \frac{W_1 - W_2}{V}$$

Where W_1 is the weight of oven dry soil + aluminium can; W_2 is the weight of empty can; V is the volume of soil in the core i.e., volume of core.

STCR BASED FERTILIZER RECOMMENDATION SYSTEM

This approach is aiming at obtaining a basis for precise quantitative adjustment of fertilizer doses under varying soil test values and response conditions of the farmers and for a given targeted yield levels. The fertilizers are recommended based on the following criteria.

- Fertilizer recommendations based on regression analysis approach
- Recommendations for certain % of maximum yield

In order to rationalise the usage of phosphorus and potassium and to avoid the imbalance in nutrient application the STCR based fertilizer prescription equations were developed for desired yield targets in FCV tobacco (Prasad ***et al.***, 2019) with the help of AICRP on STCR, IISS, Bhopal.

STCR based Fertiliser Recommendation in FCV Tobacco

- Evaluate the nutrient status of the soil
- Identify specific soil conditions such as alkalinity, salinity, and acidity
- Ensure balanced nutrition to achieve higher yields and improved crop quality
- Optimize the use of fertilizers, reducing excess application

SOIL HEALTH CARD SCHEME

The Soil Health Card (SHC) scheme is an initiative by the Government of India aimed at promoting soil testing and providing accurate information to farmers about the health of their soil. Launched in February 2015, the scheme is part of the government's broader efforts to improve agricultural productivity and ensure sustainable farming practices.

1. Soil Testing

Under this scheme, soil samples from farmers' fields are collected and tested in accredited laboratories to assess various soil parameters such as nutrient content, pH levels, organic carbon, micro nutrients, etc.

2. Data Analysis

Based on the results of soil testing, a Soil Health Card is prepared for each farmer. This card contains information about the current nutrient status of the soil, recommendations for appropriate doses of fertilizers and micro nutrients, and advice on soil management practices to improve soil health and crop productivity.

3. Distribution to Farmers

Once the Soil Health Cards are prepared, they are distributed directly to farmers. These cards serve as personalized advisory documents, guiding farmers on the precise fertilizer requirements and other soil management practices tailored to their specific land and crop types.

4. Periodic Updates

Soil Health Cards are not a one-time activity. They will be updated periodically, typically every 2-3 years, to reflect any changes in soil health due to farming practices or other factors. This ensures that farmers to have access to the most current and relevant information for their agricultural activities.

5. Benefits

The Soil Health Card scheme aims to empower farmers with knowledge about their soil's health, enabling them to make informed decisions regarding fertilizer use, crop selection, irrigation, and other agronomic practices. By optimizing inputs

and improving soil health, the scheme ultimately seeks to enhance agricultural productivity, reduce input costs, and promote sustainable farming practices.

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SOIL HEALTH CARD

NAME OF THE LABORATORY : ICAR - CTRI - SOIL TESTING LAB, RAJAHMUNDRY

Soil Health Card No : CTRI SHC 2 TBRGNO : 17016004 Region : NORTHERN LIGHT SOIL (NLS)

FARMER DETAILS	
Farmer Name	MAREDDI RUSHIKESHAVARAO
Village/Mandal	PEDDAPURAM
District	West Godavari
State/ Pin	Andhra Pradesh
Aadhar Number	
Mobile Number	

SOIL SAMPLE DETAILS	
Soil Sample No	
Collected Date	30.04.2021
Survey No	74/11, 74/12
Irrigated/ Rainfed	IRRIGATED

GENERAL RECOMMENDATION	
Organic manure	Sunhemp green manuring @20kg/acre (or) FYM /filter press cake @ 4 to 5 tonnes /acre

SOIL TEST RESULTS

S.No	Parameter	Test Value	Rating
1	pH	8	Modarately Alkaline
2	EC (dS/m)	0.2	Normal
3	Organic Carbon (%)	0.26	Low
4	Available Nitrogen (kg/ha)	108	Low
5	Available Phosphorus (kg/ha)	36	36
6	Available Potassium (kg/ha)	182	Medium
13	Soil Chlorides (ppm)	16	Suitable

FERTILIZER RECOMMENDATION FOR FCV TOBACCO
Nutrient Requirement:(N–P_2O–K_2O) (kg/ha):115 – 46 – 150

Application Type	Name of the Fertiliser	Dose (kg/ha)	N-P_2O_5-K_2O-Ca (kg/ha)
Basal application	Dolomite	200	0-0-0-48
I Split/ha	Di-Ammonium Phosphate	100	18-46-0-0
	Sulphate of Potash	100	0-0-50-0
II Split/ha	Ammonium Sulphate	250	51-0-0-0
	Sulphate of Potash	100	0-0-50-0
III Split/ha	Urea	100	46-0-0-0
	Sulphate of Potash	100	0-0-50-0

- Application of Dolomite is restricted in the case of soils having moderately alkaline and alkaline soil pH.
- In the case of low phosphorus soils, 25% additional phosphorus fertilizer over the recommended P fertilizer should be applied.
- In the case of low potassium soils, 25% additional potassium fertilizer over the recommended K fertilizer should be applied.
- In the case of high potassium soils, 25% of the recommended potassium fertilizer should be reduced.

Soil Health Card given by ICAR-CTRI-Soil Testing Lab, Rajahmundry

ICAR-CTRI role in distribution of Soil Health Cards

Every year on Dec 5th on the occasion of World Soil Day, soil health cards will be distributed to the tobacco farmers. Till now we have distributed 1500 soil health cards to the tobacco farmers. This manual ensures the accurate, consistent, and effective distribution of soil health cards, empowering tobacco farmers with the information needed for optimal soil management and improved crop productivity.

Chapter - 2

Plant Analysis

COLLECTION AND PROCESSING OF TOBACCO PLANT SAMPLES

Plant Analysis (Leaf, Stem and Root)

Plant sample analysis is crucial for optimizing nutrients, improving yield, and ensuring sustainable agricultural practices. It provides essential data on nutrient levels, helping diagnose deficiencies, monitor plant health, and guide precise fertilizer application**.** Whole plant analysis is to be carried out to determine the uptake of various nutrients. We can compute the fertilizer use efficiency, deficiency of any specific nutrient and fertilizer recommendation based on the analysis report.

Plant Sample Collection

Precision of chemical data depends upon the collection of representative samples. As the leaf chemistry varies with the position of leaf on the tobacco plant, it is necessary to collect samples from different plant positions which is being followed in FCV tobacco cultivated in light textured soils of Andhra Pradesh and Karnataka. In case of black soils, a composite sample is to be obtained by mixing the material from different grades received from different primings in proportion to their weights.

In case of light soil tobacco, the samples are to be collected from plant position wise i.e., primings, lugs, cutters, leaf and tips. So after collecting the samples composite of all grades from different primings, the primings samples corresponding to the particular plant position are to be mixed and designated as sample from that plant position. Sub samples of these grades from the different positions are collected in the proportion of the weights in which they are obtained and mixed to constitute a sample which should be about 1 kg.

In case of black soil tobacco, the samples are to be collected generally as composite of all primings and grades. So in this case the samples from different primings are mixed to get a composite sample.

Processing of the samples

Mid rib is removed from these leaf samples and the stripped lamina portions are dried in an air oven at a 60 ^{0}C temperature and powdered to pass through 60 mesh sieve (0.2 mm) for quality analysis. For nutrient analysis composite samples are to be powdered. After powdering, the samples are preserved in a well cleaned polythene or glass bottles, stoppered, wax sealed and stored in a cold room before taking up the analysis. For micronutrient analysis to be carried out in the samples, utmost care should be taken in handling the samples at the time of processing as well as

doing analysis. The powdering of samples should be done with a grinder having stainless steel blades or with a stainless steel pestle and mortar. If boron is to be estimated in the samples, glass bottles, glassware should not be used either in preservation or analysis of the samples. Only polythene ware should be used.If there is any time gap for processing, the samples are packed properly and preserved in cold room for analysis.

Chemical analysis

The leaf contains several organic and inorganic constituents. Determination of these constituents involves two steps; 1) Extraction 2) Analysis.

Organic constituents undergo decomposition if the extraction is vigorous and involves direct heating. Constituents like sugars, nicotine, starch, proteins ***etc.,*** are of this group. Their extraction is being carried with distilled water or mild extracting either in cold or hot condition. By extracting with methanol acetic acid mixture in cold, sugars, nicotine and chlorides are determined with the help of auto-analyzer.

Mineral constituents like P, K, Ca and Mg also occur in organic combinations. For their determination they are converted to inorganic form through oxidation. This process of oxidation is achieved in two ways i.e., by ashing in a muffle and extracting (dry ashing) or by digesting the material with powerful oxidizing agents like tri-acid mixture (mixture of HNO_3, H_2SO_4& $HClO_4$) Individual constituents can be determined from these extracts by adopting a suitably analytical technique.

Chlorides are highly soluble in water and hence their extraction is carried out with distilled water.

TOTAL NITROGEN

Principle

The determination of total nitrogen using block digestion involves heating a sample in the presence of concentrated sulfuric acid and a catalyst, which breaks down organic matter, converting nitrogen into ammonium sulfate. The mixture is then neutralized with a strong base, typically sodium hydroxide, to liberate ammonia. The ammonia is distilled and absorbed in a boric acid solution, forming ammonium borate, which is then titrated with a standard acid (usually hydrochloric or sulfuric acid) to determine the total nitrogen content. This method is commonly used in the Kjeldahl digestion process for nitrogen analysis

Reagents

- Conc. Sulphuric acid (H_2SO_4)
- Digestion mixture: Mix 110g of K_2SO_4, 5.13 g of mercuric oxide and grind it to get fine powder
- Sodium Hydroxide solution (16 % NaOH): Dissolve 16 g of NaOH in about 70 ml water and make up the volume to 100 ml
- Boric acid (4% H_3B0_3): Dissolve 4 g in warm distilled water and then make up the volume to 100 ml
- Mixed indicator: Mix 0.5 g of Bromocresol green + 0.07 g of methyl red and dissolve this mixture in 100 ml ethanol
- Standard Sulphuric acid(0.1 N H_2SO_4): Dilute 3.85 ml of conc. H_2SO_4 to 1000 ml and then standardise it against std. Na_2CO_3 solution

Procedure

Weigh 0.25 g of powdered plant sample into digestion tube

↓

Add 2.5 g of digestion mixture

↓

Add 5 ml of conc. H_2SO_4

↓

Leave it for 8-12 hours

↓

Digest the contents at 160 ºC for initial 1hr

↓

After 1hr Digest the contents at 260 ºC for 1hr

↓

Next digest the contents at 360 ºC for 1hr till white colour is obtained

↓

Cool the contents and make up the volume to 100 ml with distilled water

↓

Pipette out 10 ml of the digested sample into distillation tube

↓

Add 10 ml distilled water

↓

Take 20 ml boric acid solution in a receiving flask

↓

Distill the contents

↓

Titrate the content of the receiving flask against std. acid till colour turns to pink

Calculation

$$\% N = \frac{\text{TV X N of acid X 0.014 X Vol. of digested sample X 100}}{\text{Weight of plant sample X aliquot taken (ml)}}$$

NITRATE NITROGEN

Principle

Nitrate nitrogen estimation involves extracting nitrate from a tobacco leaf sample and then reducing the nitrate to nitrite using zinc dust in an alkaline medium. The resulting nitrite reacts with sulfanilic acid and á-naphthol in the Griess reagent to form a colored azo compound. The intensity of this color, which is proportional to the concentration of nitrate nitrogen, is measured spectrophotometrically at 475 nm. This absorbance is then used to quantify the nitrate nitrogen content in the tobacco sample.

Reagents

- A) Extraction mixture (0.5 N): Dilute 34 ml NH_4OH with distilled water to one litre
  B) Reaction mixture (0.745N): Dilute 50 ml NH_4OH with distilled water to one litre
- Manganous Sulphate solution ($MnSO_4.4H_2O$): Dissolve exactly 10g $MnSO_4.4H_2O$ (7.5772 g $MnSO_4.H_2O$) in 51 ml acetic acid and dilute to one litre with distilled water.
- Zinc metal dust
- Griess reagent (modified): Dissolve exactly one gram of sulfanilic acid in 200 ml distilled water. Add 0.8 g alpha naphthol and 752 ml acetic acid. Dilute to one litre with distilled water. Refrigerate the contents.
- Stock diluent: To 103 ml NH_4OH and 400 ml distilled water and then cautiously, with stirring, add 321 ml acetic acid. Dilute to two litres with distilled water
- Nitrate standard solution: Dissolve exactly 360.9 mg KNO_3 in 34 ml NH_4OH. Dilute to one litre with distilled water. One ml of solution is equivalent to 0.05 mg nitrate nitrogen as nitrogen.

Procedure (Broaddus *et al.*, 1965)

Place 300 mg of tobacco powder in a 250 ml conical flask

⇩

Add 100 ml of extraction mixture

⇩

Stopper and shake for one hour

⇩

Filter through whatman no. 42 filter paper

⇩

Take 9 ml of the filtrate into 50 ml vol. flask

⇩

Add one ml of $MnSO_4$ solution

⇩

Make up the volume with reaction mixture(B)

⇩

Transfer the whole solution into a 250 ml conical flask

⇩

Add 200 mg of zinc dust (weigh the exact quantity)

⇩

Stopper and shake for one hour

⇩

Filter through whatman no. 42 filter paper

⇩

Place 5 ml of griess reagent (freshly prepared) in 50 ml vol. flask

⇩

Add 10 ml of tobacco filtrate

⇩

Make up to volume with stock diluent

⇩

Allow 30 mins for the colour to develop

⇩

Read the absorbance of sample vs reagent blank using spectrophotometer at 475 nm

Preparation of standard curve

- Take 40 ml reaction mixture (B) into 50 ml vol. flasks separately
- Add 0, 1, 2, 3, 4 ml of KNO_3 standard solution into the above flasks separately
- Add 1 ml of $MnSO_4$ to each flask and make upto volume with distilled water
- Transfer the above solution into five 250 ml conical flasks
- Add 200 mg Zinc dust and shake for one hour
- Filter the solution through whatman no. 42 filter paper
- Place 5 ml of griess reagent in each of five 50 ml vol. flasks
- Add 5 ml of filtrate and make up to volume with stock diluent
- Allow 30 mins for the colour to develop

Calculation

$$\textbf{NO}_3\textbf{-N in tobacco (ppm)} = \frac{\textbf{500 x (µg of nitrate nitrogen)}}{\textbf{weight of tobacco in g}}$$

DIGESTION OF PLANT SAMPLE FOR P, K, SECONDARY AND MICRONUTRIENTS

Principle

Plant samples are to be digested by wet oxidation withoxidising acids like HNO_3-H_2SO_4-$HClO_4$ (tri-acid mixture) or HNO_3-$HClO_4$ di-acid. Use of $HClO_4$ avoids the volatilization loss of K and provides a clear solution while H_2SO_4 helps completing oxidation. $HClO_4$ on heating produces anhydrous $HClO_4$ which dissociates into nascent chlorine and oxygen, which in turn increase the oxidation efficiency at high temperature. Direct contact of $HClO_4$ with plant samples might lead to explosion and fire and hence the pre-digestion of samples in HNO_3 is preferred. Digestion with HNO_3-$HClO_4$ instead of the tri-acid mixture can be adopted especially when S is also to be determined in the same digest.

Reagent

Tri-acid mixture: Mix AR grade conc. HNO_3-H_2SO_4-$HClO_4$ in 10:1:4 ratio
Di-acid mixture: Mix AR grade conc. HNO_3-$HClO_4$ in 10:4 ratio

Procedure

Weigh 2 g of plant sample

⇓

Pre-digestion with 12 ml HNO_3 (conc) and leave it overnight

⇓

Put this on hot plate for digestion

⇓

Continue digestion for 30-45 mins till the red fume stops

⇓

Cool the contents

⇓

Add 14 ml of di-acid/Tri acid mixture

⇓

Continue the digestion till the volume reduced to 3-5 ml (When the liquid becomes colourless and appears snow white precipitate)

⇓

Add 10 ml of 6 N HCl

⇓

Dilute this residue to 100 ml and store in bottles for further analysis

TOTAL PHOSPHORUS

Principle

In presence of V^{5+} and Mo^{6+} orthophosphates form a yellow coloured phospho-vanadomolybdate complex, which shows an optimal absorption at wavelength of 420 nm. Once the absorption is measured, the absorption measurement can be related directly to the concentration of phosphorus by comparing with the measured absorption of a series of P standards.

Reagents

- Phosphate standard (50 ppm): Dry the potassium dihydrogen phosphate (KH_2PO_4) at 40 ºC and dissolve 0.2195 g in about 400 ml of distilled water present in a 1000 ml volumetric flask. Then add 25 ml of 7N H_2SO_4 and make up the volume to 1 L with distilled water
- Vanadate-Molybdate reagent: Solution A: Dissolve 25 g of ammonium molybdate in 400 ml warm water; Solution B: Dissolve 1.25 g of ammonium metavanadate in 300 ml of boiling water. Cool it and add 250 ml of conc. HNO_3. Mix solution A & B, dilute the mixture to 1 L using distilled water

Preparation of standard curve

- Take 0, 1, 2, 3, 4 and 5 ml of 50 ppm P solution in separate 50 ml volumetric flasks to get 0, 1, 2, 3, 4 and 5 ppm 'P', respectively
- Add 10 ml of vanadomolybdate reagent to each standard
- Make up the volume with distilled water and mix well
- After half an hour, record the intensity of yellow colour of these solutions at 420 nm wavelength in spectrophotometer and plot the OD Vs. Concentration of standards and draw the curve

Procedure

Pipette out 2 ml of digested sample into a 50 ml vol. flask

⇩

Add 10 ml of vanado-molybdate reagent
(yellow colour develops)

⇩

Make up the volume to 50 ml with distilled water

⇩

After half an hour, record the intensity of yellow colour at 420 nm using spectrophotometer

Calculation

$$\% P = \frac{\text{Graph ppm}}{10^6} X \frac{\text{Vol. of digested sample}}{\text{wt. of plant sample}} X \frac{\text{Volume made}}{\text{Aliquot of digest}} X\ 100$$

TOTAL POTASSIUM

Procedure

Pipette out 2 ml of digested sample into a 100 ml vol. flask

Make up the volume to 100 ml with distilled water

Measure the K concentration in filtrate using the flame photometer

*If the concentration is high, suitable dilution be followed

Preparation of standard curve

Same as given in the estimation of soil potassium

Calculation

$$\% K = \frac{\text{Graph ppm}}{10^6} X \frac{\text{Vol. of digested sample}}{\text{wt. of plant sample}} X \frac{\text{Volume made}}{\text{Aliquot of digest}} X 100$$

WATER EXTRACTION METHOD FOR LEAF POTASSIUM IN FCV TOBACCO

Principle

Potassium remains in soluble forms in the plant and is not bound to organic compounds in plant cells (Marschner, 1986), indicating its easy extractability with water. The amount of K released from plant tissue by water is related to tissue K content (Rosolem *et al.,* 2005) and depends on plant-tissue resistance in the form of a physical barrier to K release. Greater K liberation would occur with plant-tissue decomposition or with grinding plant material to a fine powder. A simple inexpensive extraction method is used for determining K concentration in tobacco plant tissue.

Preparation of standard curve

Same as given in the estimation of soil potassium

Procedure (Reddy and Krishnamurthy, 2013)

Weigh 0.5 g of finely ground (<0.5 mm) plant sample into a 250-ml conical flask

⇓

Add 50 ml of distilled water to the flask

⇓

Keep it for shaking for 20 mins

⇓

Filter through Whatman No 1 filter paper

⇓

Take 5ml of filtrate and make upto 50ml with distilled water

⇓

Measure the K concentration in filtrate using the flame photometer

Calculation

$$K\ (\%) = \frac{\text{ppm X vol. of extracant X vol. made X 100}}{10^6 \text{ X wt. of sample X Aliquot taken}} \text{ X } 100$$

CALCIUM AND MAGNESIUM

Principle

The principle given in estimation of soil calcium and magnesium holds goods in plant sample also

Reagents

- Standard EDTA solution (0.01N): Dissolve 1.861 gm of EDTA in 900 ml distilled water and make up the volume to 1000 ml
- Std. Ca solution: Dissolve 0.6005 g portion of pure dried $CaCO_3$ in 0.2 N HCl. Solution is boiled to expel the CO_2 and dilute to 1 L
- Sodium Hydroxide solution (10% NaOH) : Dissolve 10 g portion of NaOH in about 90 ml distilled water and dilute to the 100 ml
- Murexide indicator
- Buffer solution (pH 10): Add 142 ml of NH_4OH to 17.5 g of NH_4Cl and dilute to 250 ml with distilled water
- Erichrome Black T indicator

Procedure

Estimation of calcium + magnesium

Pipette out 5 ml aliquot of digested sample into porcelain dish

⇩

Add 25 ml of distilled water

⇩

Add 5 ml buffer solution

⇩

Add 3-5 drops of EBT-indicator

⇩

Titrate with EDTA solution till colour turns from wine red to sky blue

Estimation of calcium

Pipette out 5 ml aliquot of digested sample into porcelain dish

⇩

Dilute with 10 ml of distilled water

⇩

Add 5 ml of 10% NaOH

⇩

Add about 0.5 g of murexide indicator (Rose red colour)

⇩

Titrate the contents against std. EDTA with stirring until it becomes violet in colour

Calculation

$$\text{Ca (\%)} = \frac{\text{(TV for Ca) X (N of EDTA) x 0.02 X Vol. of digested sample}}{\text{Aliquot taken X weight of sample}} \text{ X 100}$$

$$\text{Mg (\%)} = \frac{\text{(TV for Ca+Mg) - (TV for Ca) X N of EDTA X 0.012 X Vol. of digested sample}}{\text{Aliquot taken X weight of sample}} \text{ X 100}$$

SULPHUR

Principle

The plant sulphur is released into solution after digestion with diacid mixture (HNO_3 + $HClO_4$). The released 'S' in the solution is precipitated by Ba^{2+} ions as $BaSO_4$. This turbidity developed by $BaSO_4$ is then determined by turbidometry. Turbidometry is a technique used to measure the turbidity or cloudiness of a liquid. Turbidity is caused by the presence of suspended particles in a solution, which scatter light passing through the liquid. The degree of light scattering is related to the concentration of the particles. In turbidometry, a light source is passed through a sample, and the intensity of the light that passes through the sample is measured on the opposite side. The higher the turbidity, the lower the intensity of the transmitted light.

Reagents

- $BaCl_2$ crystals-powdered to 20-60 mesh
- Conditioning agent: Dissolve 15 g sodium chloride in 300 ml distilled water, add 30 ml conc. HCl and 100 ml 95% ethyl alcohol and 500 ml glycerol and mix well
- Standard sulphur solution: Dissolve 0.5434 g K_2SO_4 in distilled water and make up the volume to one litre to get 100 ppm S-stock solution

Preparation of standard curve

Same as described in the estimation of sulphur in soil

Procedure (Krober and Howell, 1958)

Take 10 ml of digested sample in a 50 ml volumetric flask

⇓

Add 1 ml of conditioning agent

⇓

Add 0.5 g of $BaCl_2$

⇓

Make up the volume to 50 ml

⇓

Record the turbidity at 420 nm within 20 min

Calculation

$$S\ (\%) = \frac{\text{(Graph ppm) X (Vol. of digested sample) X (Vol. made up)}}{10^6 \text{ X (weight of sample) X (qliquot taken)}} \text{ X } 100$$

Fe, Mn, Cu AND Zn

Fe, Mn, Cu and Zn in plant sample digest can be determined by Atomic absorption spectroscopy (AAS) (Pequerul*et al*., 1993)

Standards of micronutrient cations are to be prepared as per the procedure given in soil analysis

Calculation

$$\text{\% micronutrient} = \frac{\text{Graph ppm}}{1000 \text{ X } 1000} \text{ X} \frac{\text{Vol. of plant digest}}{\text{Wt. of plant sample}} \text{ X dilution X } 100$$

LEAF NICOTINE, REDUCING SUGARS AND CHLORIDE

Principle

The colorimetric technique is to be used to assess nicotine, reducing sugars, and chlorides.

- Cyanogen Bromide reacts with nicotine in the presence of aniline buffer forms a yellow complex. The intensity of the yellow colour is proportionating to the nicotine concentration in the sample and is measured at 460 nm. Cyanogen bromide releases pyridine ring [$C_5H_5N_2$] from nicotine, which reacts with aniline forming yellow colour complex
- **Reducing sugars** in the sample reduces yellow coloured K_3 [Fe $(CN)_6$ (ferri cyanide) in an alkaline medium. The degree of decolourization will be measured at 420 nm
- **Chloride** ions release thiocyanate ions which react with ferric nitrate forming blood red coloured ferric thiocyanate complex. The intensity of colour is proportional to the original chloride concentration and is measured at 480 nm. The number of thiocyanate ions released is proportional to the chloride ions present in the sample

Reagents

- Extracting solution: Dissolve 1000 ml Acetic Acid and 4000ml Methanol in 5000 ml distilled water and make up to 20000 ml with distilled water
- Carbon Suspension: Dissolve 500 g Darco-G in 1675 ml of Glycerol and 1675 ml of distilled water. Mix the components thoroughly

Nicotine estimation

- Cyanogen Bromide (CNBr) Solution: Dissolve 100g of CNBr solid in 1000 ml Alcohol and make up the volume up to 5000 ml with filtered distilled water
- Buffer Solution: Dissolve 8.2g of citric acid and 11.24g of sodium phosphate di basic in 200 ml distilled water. Add 3 ml of Aniline drop wise and make up to 1000 ml with distilled water and add 30 drops of Brij

Reducing sugars estimation

- Potassium Ferricyanide [K_3 [Fe $(CN)_6$] Solution (0.015%): Dissolve 0.015g of K_3 [Fe $(CN)_6$] in 100 ml of 1N NaOH solution
- Sodium Chloride [NaCl] solution: Dissolve 9 g of NaCl in 1000ml distilled water.

Chlorides estimation

- Mercuric Thio Cyanate (Hg $(SCN)_2$) Solution: Dissolve 2.085g of Mercuric Thio Cyanate in 500 ml of methanol

- Ferric Nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) solution: Dissolve 500g Ferric Nitrate of in 90 ml of Conc. HNO_3 and make the volume up to 2.5 litres with distilled water
- Colour Reagent: 6.5 ml of Mercuric Thio Cyanate solution and 6.5 ml of Ferric Nitrate solution are mixed and made up to the volume of 100 ml with distilled water

Calibration

- Stock Standard Solution: 0.5120g of Nicotine Hydrogen Tartarate, 1.25g of Dextrose and 0.2749g of NaCl is taken in 250 ml volumetric flask and made up to 250 ml with extracting solution
- Working Standards: 15ml, 7.5ml and 3.75 ml of stock standard solution is pipetted out in three individual 50ml volumetric flasks and made upto the volume with extracting solution to get 200ppm, 100ppm and 50ppm of Nicotine, 1500ppm, 750ppm and 375ppm of Reducing Sugars, 200ppm, 100ppm and 50ppm of Chlorides respectively

Procedure (Harvey *et al.*, 1969)

Sample preparation

Take 0.25 g of tobacco leaf lamina powder in 150 ml conical flask

⇓

Add 2 ml of carbon suspension

⇓

Add 48 ml extracting solution and shake well for 15 minutes at 170 rpm

⇓

The solution is filtered and taken into cuvettes

Estimation by Auto Analyser

Pass all the reagents to the instrument till it acquires a stable baseline

⇓

Then switch on the sampler

⇓

Reagents when mixed with samples produce colour complexes

⇓

The intensity of the colour is measured at 460nm, 420nm and 480nm, respectively for Nicotine, Reducing Sugars and Chlorides

The samples are compared with known standards

The peak values are noted

Calculation

$$\text{Nicotine (\%) or Reducing Sugars (\%) or Chlorides (\%)} = \frac{0.005 \times \text{ppm}}{\text{weight of the sample}}$$

ACETIC ACID METHOD AS AN EXTRACTANT

Reagents

Extractant - 10 % acetic acid (CH_3COOH) + 4 cc active charcoal suspension

Procedure (Prasad *et al*., 2022)

250 mg of tobacco leaf lamina powder sample was taken in 150 mlconical flask and 50 ml of extractant was added to get a sample to extractant ratio of 1:200 (w/v)

The contents in the flask were shaken using rotary shaker for15 minutes at 170 rpm for homogenous mixing

The extract was filtered into cuvettes using Whatman no. 5 filter paper

Estimation by auto analyser

Flow process of nicotine channel was fixed at 0.23 ml/min for sample, 0.32 ml/min for cyanogen bromide and 1.00 ml/min for buffer. While rate of flow was 0.10 ml/min for sample, 1.40 ml/min for $K_3 Fe(CN)_6$, 1.00 ml/min for sodium salt solution, and 0.32 ml/min for air flow in case of reducing sugars. The sample, color reagent, diluent, and wash flow rates for chlorides were 0.10 ml/min, 1.00 ml/min, 1.40 ml/min, and 2.00 ml/min fixed, respectively.

Advantages over methanol based extraction: Methanol is a hazardous and more expensive solvent compared to other polar solvents. Acetic acid, with a polarity index of 6.2, is a non-hazardous alternative.

Chapter - 3

Water Analysis

COLLECTION OF WATER SAMPLE FOR IRRIGATION ANALYSIS

Irrigation of tobacco is an essential input for attaining high yields, but the quality of the irrigation water is also equally critical especially in tobacco cultivation. Chloride rich water will generally deteriorate the quality of leaf by reducing its burning quality. Excess of the soluble salts in waters leads to their accumulation in the surface particularly in heavy textured or in poorly drained soils. Many areas in the country are facing a serious problem of not only scarcity of water but also of critical poor quality of irrigation water. Tube well or well waters generally pose such problems more than canal waters. It is, therefore, advisable to get the water tested for quality for sustainable tobacco production.

Water sampling techniques

Water samples for irrigation quality assessment are to be analysed for chemical constituents. Therefore, utmost care should be exercised to avoid the possibility of any external contamination. The samples can be collected in glass or plastic bottles thoroughly cleaned and rinsed 3-4 times with the water to be tested. For collection of water sample, the following method may be adopted.

Surface water (e.g., ponds, lakes, rivers): Collect the sample from a location that is representative of the water being used. Avoid areas with obvious contamination or stagnation. Hold the bottle near its base, facing downwards. Submerge the bottle below the water surface (around 15-30 cm deep) while turning it upright to fill, avoiding contact with the bottom or surface.

Ground water: Run the tube well or hand pump for about 15-20 min. to drain out the water retained in the pipe. Wash the bottle repeatedly with the water before taking about 500 ml of sample. The bottle should not be washed with detergents or soaps. Collecting a water sample for an irrigation system is essential for analysing water quality and ensuring it is suitable for crop production.

WATER pH

pH is defined as the negative logarithm of Hydrogen ion activity of a solution.

Procedure (Yadav *et al.*, 2015)

Take 50 ml of irrigation water sample into 100 ml beaker

Record the pH using standardized pH meter

Interpretation

S.N.	pH	Class	Interpretation
1	< 6.5	Acidic	Can be used after treatment with amendments
2	6.5– 7.5	Normal	Can be used safely
3	>7.5	Alkaline	Can be used after treatment with amendments

ELECTRICAL CONDUCTIVITY (EC)

Principle

Water is a poor conductor of electricity. The presence of salts in the aqueous media enhances conductivity. EC used as an index of salt content. Higher the salt content lower is the resistance to flow of current and higher is the electrical conductance. EC is a reciprocal of resistance and this, increases with increase in salt concentration.

Note: 1 m.mhos cm^{-1} = 1 dS m^{-1}

Procedure (Yadav *et al.*, 2015)

Take 50 ml of irrigation water sample into 100 ml beaker

⬇

(Same water prepared for pH estimation can be used)

⬇

Measure the EC using EC meter

Interpretation

S.N.	Salinity classes	EC (µ mhos/cm)	Remarks
1	Low (C1)	< 250	Can be used safely
2	Medium (C2)	250 - 750	Can be used with moderate leaching practices
3	High (C3)	750 - 2250	Cannot be used for irrigation
4	Very High (C4)	>2250	Cannot be used for purposes

CHLORIDES

Principle

Silver chloride (AgCl), being more insoluble than the silver chromate (Ag_2CrO_4), is precipitated first. When a solution of silver nitrate is added to a mixture of chloride (Cl) and chromate (CrO_2^{-2}) ions, silver chloride separates out of the solution as a white precipitate, thereby gradually reducing the chloride ion concentration in the mixture. When all such chloride ions are removed, a slight excess of the silver nitrate solution produces silver chromate (brick red colour) indicating the end point. Ag_2CrO_4, is not stable in presence of acid.

Reagents

- Silver Nitrate solution (0.1 N $AgNO_3$): Dissolve 16.89 g of pure (A.R) $AgNO_3$, in water and make upto 1000 ml with distilled water
- Potassium chromate solution (5 % K_2CrO_4): Dissolve 5 grams of pure K_2CrO_4, in 100 ml chloride free distilled water

Procedure (Yadav *et al.*, 2015)

Take 10 ml of water sample in a porcelain dish

Add 3 drops of K_2CrO_4 solution

Titrate against 0.1 N $AgNO_3$ solution until the red precipitate appears

Calculation

$$\textbf{Chloride (meq/L)} = \frac{\textbf{TV X N of } AgNO_3 \textbf{ X 1000}}{\textbf{Volume of aliquot taken}}$$

*Irrigation water containing > 50ppm is unsuitable for tobacco cultivation

CARBONATE AND BICARBONATE

Principle

Carbonate and bicarbonate in irrigation water can be determined by titrating against standard acid using phenolphthalein and methyl red respectively as indicators. When the colour of the phenolphthalein is discharged, it indicates half the neutralization of the carbonate. At this stage methyl red indicator is added and titration is to be continued. When colour changes from yellow to rose red, it is the end point for complete neutralization of bicarbonate.

Reagents

- Sulphuric acid (0.1 N H_2SO_4): Take 3 ml of conc. H_2SO_4 and dilute to 1 L of distilled water. Standardize the 0.1 N H_2SO_4 with 0.1 N sodium carbonate
- Sodium carbonate (0.1 N Na_2CO_3): Dissolve 5.29 g of AR dry sodium carbonate in 1L of distilled water
- Phenolphthalein (0.5%): Dissolve 0.5 g of phenolphthalein in 100 ml of 95% alcohol
- Methyl red (0.5%): Dissolve 0.5 g of methyl red in 100 ml of 95% alcohol

Procedure (Yadav *et al.*, 2015)

Carbonate

Take 10 ml of water sample into a porcelain dish

⇩

Add 3 drops of phenolphthalein (Appearance of pink colour indicates the presence of carbonates)

⇩

Titrate against 0.1 N H_2SO_4 till the solution becomes colourless

⇩

Record the reading (TV 1)

Bicarbonate

Add few drops of methyl red indicator

⇩

Continue the titration with 0.1 N H_2SO_4 till yellow colour changes to red

⇩

Record the reading (TV 2)

Calculation

$$CO_3 \text{ (meq/L)} = \frac{\text{2 X TV 1 X N of Acid X 1000}}{\text{Volume of aliquot taken}}$$

$$HCO_3 \text{ (meq/L)} = \frac{\text{[TV2 - (2 X TV1)] X N of Acid X 1000}}{\text{Volume of aliquot taken}}$$

SODIUM

Sodium (Na) is a common element, the sixth most abundant, and present to some extent in most natural waters. Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). Sewage, industrial effluents, sea water intrusion in coastal area, and the use of Na compounds for corrosion control and water-softening processes all contribute to Na concentration in water because of the high solubility of sodium salts and minerals. Sodium levels in groundwater vary widely but normally range between 6 and 130 mg L^{-1}.

Reagents

- Standard stock solution (100 ppm): Dissolve 5.845g of AR grade dried NaCl in distilled water and make up the volume to 1 L

Preparation of standard curve

- Working standard solution: Dilute 5, 10, 15, 20, 30, 40 and 50 ml portion of the stock solution (containing 100 ppm) to 100 ml in volumetric flask to get working standards of 5, 10, 15, 20, 30, 40, and 50 ppm concentrations.

Procedure (Banerjee and Prasad, 2020)

Filter a portion of water sample through Whatman filter paper No. 1

Feed the sample to the flame photometer

Calculation

$$\text{Na (meq L}^{-1}) = \frac{\text{Na (ppm) from calibration curve}}{23}$$

POTASSIUM

Although potassium (K) is a relatively abundant element, its concentration in natural fresh waters is usually less than 20 mg L^{-1}. Brines and seawater, however, may contain as much as 400 mg L^{-1} K or more. Potassium in water can be determined by flame photometry.

Principle

The estimation of K is based on the emission spectroscopy, which deals with excitation of electrons from ground state to a higher energy state and coming back to its original state with the emission of light.

Reagents

Standard Stock Solution: Dissolve 1.907 g dried KCl in distilled water, and bring to 1-L volume. This solution contains 1000 ppm K (Stock Solution).

Preparation of standard curve

Same as given in the determination of soil potassium

Procedure (Banerjee and Prasad, 2020)

Filter a portion of water sample through Whatman filter paper No. 1

Feed the sample to the flame photometer

Calculation

$$\text{K (meq L}^{-1}) = \frac{\text{K (ppm) from calibration curve}}{39.1}$$

CALCIUM AND MAGNESIUM

Calcium (Ca) is dissolved easily out of almost all rocks and is, consequently, detected in most waters. Magnesium (Mg) are relatively abundant in the earth's crust and hence a common constituent of natural water. Waters associated with granite or siliceous sand usually contain less than 10 mg of calcium per litre and less than 5 mg magnesium per litre. Many waters from limestone areas may contain 30-100 Ca per litre, and those associated with gypsiferous shale may contain several hundred milligrams per litre. But for the water in contact with dolomite or Mg-rich limestone may content 10-50 mg L^{-1} and several hundred milligrams per liter may be present in water that has been in contact with deposits containing sulfates and chlorides of magnesium. Calcium and Mg contribute to the hardness of water, it should be noted that the difference between total hardness and the Ca concentration can be used to calculate the magnesium concentration. However, some $CaCO_3$ is desirable for domestic waters because it provide a coating in the pipes which protects them against corrosion.

Principle

EDTA-disodium salt solution is used to chelate Ca^{2+} + Mg^{2+}. Calcium is separately estimated by the versenate method using ammonium purpurate (Murexide) indicator, when the pH is made sufficiently high, the Mg is largely precipitated as hydroxide and an indicator is used that combines with Ca only. Thus, Mg can be obtained by deduction of Ca from Ca+Mg content. Both cations can also be estimated using atomic absorption spectrophotometer (Dehghani ***et al***., 2012).

Reagents

- Sodium hydroxide solution (10 % NaOH): Weigh 100 g of pure sodium hydroxide and dissolve it in distilled water and makeup the volume to 1 L mark in a volumetric flask
- EDTA solution (0.01 N): Weigh 1.86 g of EDTA in distilled water and make up the volume to 1L mark in a volumetric flask. Titrate this solution with standard solution of 0.01 N Ca solution for standardization
- Murexide indicator powder: Take 0.2 g of murexide and mix it with 40 g of powdered potassium sulphate
- Ammonium chloride-ammonium hydroxide (NH_4Cl-NH_4OH) buffer solution: Weigh 67.5 g of ammonium chloride and dissolve it in 250 ml. distilled water, add 570 ml. of concentrated ammonium hydroxide, and make up the volume to 1L with distilled water

- EBT indicator: Take 100 ml of ethanol and dissolve 4.5 g of hydroxylamine hydrochloride in it. Add 0.5 g of EBT indicator and prepare the solution

Procedure (Yadav *et al.*, 2015)

Calcium

Pipette 10 ml of water sample into a porcelain dish

⇩

Add 3 ml of 10 % NaOH solution

⇩

Add 50 mg murexide indicator

⇩

Titrate with 0.01 N EDTA

⇩

The color change is from red to lavender or purple

Calcium + Magnesium

Pipette 10 ml of water sample into a porcelain dish

⇩

Add 3-5 ml buffer solution

⇩

Add few drops of eriochrome black indicator

⇩

Titrate with 0.01 N EDTA until the color changes from red to blue

Calculation

$$\text{Ca (meq L}^{-1}) = \frac{\text{TV X N of EDTA X 1000}}{\text{Volume of water taken}}$$

$$\text{Ca + Mg (meq L}^{-1}) = \frac{\text{TV X N of EDTA X 1000}}{\text{Volume of water taken}}$$

$$\text{Mg (meq L}^{-1}) = [(\text{meq of Ca + Mg})-(\text{meq of Ca})]$$

BORON

In most natural waters boron (B) is rarely found in concentrations greater than 1 mg L^{-1}, but even this low concentration can have deleterious the effects on certain agricultural products. Water having B concentrations in excess of 2 mg L^{-1} can adversely affect many common crops. However, where levels are greater than 5 mg L^{-1}, toxicity may occur. Groundwater may have a greater B concentration, particularly in areas where the water comes in contact with igneous rocks or other B-containing strata. The hot-water procedure is still the most popular method for measuring B, and it was introduced by Berger and Truog (1939), and was modified by later researchers. The B is measured calorimetrically using Azomethine-H (Bingham, 1983).

Reagents

- Buffer Solution: Dissolve 250 g ammonium acetate (NH_4OAc), and 15 g EDTA disodium (ethylene diamantine-tetraacetic acid, disodium salt) in 400 ml distilled water. Slowly add 125 ml glacial acetic acid (CH_3COOH), and mix well

- Azomethine-H Solution ($C_{17}H_{12}N.NaO_8S_2$): Dissolve1g L-ascorbic acid in 100 ml distilled water and then add 0.45 g Azomethine-H, and mix well

- Standard Stock Solution: Dissolve 0.114 g boric acid (H_3BO_3) in distilled water, and bring to 1-L volume. This solution contains 20 ppm B (Stock Solution)

Preparation of standard curve

- Prepare a series of working Standard Solutions from the stock solution by diluting 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ml stock solution to 100 ml numbered flasks by adding distilled water, and then bring to volume. These solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm, respectively

Procedure (Mohammed *et al*., 2014)

Pipette 2 ml aliquot of the natural water sample in to a 10 ml polypropylene tube

⇩

Add 4 ml buffer solution

⇩

Add 4 ml Azomethine-H solution, and mix well

⇩

Prepare a standard curve by pipetting 2 ml of each standard (0.5 – 3.0 ppm)

⇩

Proceed as for the samples

⇩

Also make a blank with 2 ml distilled water

⇩

Proceed as for the samples

⇩

Read the absorbance of blank, standards, and samples after 30 minutes on the spectrophotometer at 420-nm wavelength

⇩

Prepare a calibration curve for standards, plotting absorbance against the respective B concentrations

⇩

Read B concentration in the unknown samples from the calibration curve

Calculation

$$\mathbf{B\ (mg\ L^{-1}) = (S\text{-}A)}$$

Where S =B (mg L^{-1}) from standard curve in sample
A = B (mg L^{-1}) from standard curve in blank

Water class	Boron Concentration			Remarks
	Sensitive crops	Semi tolerant crops	Tolerant crops	
Very low	<0.33	<0.67	<1.00	Can be used safely
Low	0.33-0.67	0.67-1.33	1.00-2.00	Can be used with management practices
Medium	0.67-1.00	1.33-2.00	2.00-3.00	Unsuitable for
High	1.00-1.25	2.00-2.50	3.00-3.75	irrigation
Very high	>1.25	>2.50	>3.75	purposes

WATER QUALITY INDICES AND SUITABILITY

After determination of water samples for different parameters like total salts (EC), cations and anions, it is imperative to calculate some indices in order to assess water quality and its subsequent effect on soil as well as plant growth.

Sodium Adsorption Ratio (SAR): It indicates the sodicity or alkalinity hazards of irrigation water.

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{+2} + Mg^{+2}}{2}}}$$

Sodium hazard	Class	SAR	Suitability
Low	S_1	<10	Can be used on all soils with little hazard of accumulation of exchangeable sodium
Medium	S_2	10 – 18	Can be used in all soils with good permeability; Appreciable sodium hazard in soils of high clay and low organic matter
High	S_3	18 – 26	Causes harmful sodium accumulation in most soils: needs good drainage, high leaching and organic matter addition
Very high	S_4	>26	Not suitable for irrigation

Residual sodium carbonate (RSC): The RSC may be calculated simply by subtracting the quantity of Ca + Mg from the total of the carbonates and bicarbonates determined in different samples and expressed in me L^{-1}.

$$RSC = (CO_3^{-2} + HCO_3^{-}) - (Ca^{+2} + Mg^{+2})$$

Water class	RSC	Remarks
Low RSC	<1.25	Can be used safely
Medium RSC	1.25 – 2.50	Can be used with certain management practices
High RSC	>2.50	Unsuitable for irrigation purposes

IV. LABORATORY SAFETY

Safety is a paramount concern in soil, plant, and water analysis laboratories, yet it's often overlooked. Chemical laboratories require special attention to safety considerations, including both the design and construction of the lab and the handling of chemicals.

Chemical operations pose risks such as the release of gases and fumes, which are controlled through mechanisms like fume hoods or by trapping in acidic/alkaline solutions and washing through flowing water. Additionally, improper handling of chemical reactions during analysis can lead to explosions.

Temperature variations can also impact analytical processes, potentially altering results. Therefore, maintaining consistent temperature and humidity levels in the laboratory and working rooms is crucial. Fluctuations in these conditions can affect the storage stability of chemicals and the accuracy of analyses. Ideally, the air temperature should be kept constant at around 20-25°C, with humidity maintained at approximately 50%. These measures help ensure a safe and reliable working environment for laboratory staff. All staff, irrespective of grade, technical skill, or employment status, should be briefed on all aspects of safety upon work commencement. Periodic reminders of such regulations should be given to encourage familiarity concerning regulations. Ideally, posters related to laboratory safety should be prominently displayed in the laboratory. While rules pertaining to safety can be extensive, we have endeavoured to concisely list the more important ones within different categories of concerns.

Here is a breakdown of the guidelines for safety, instrument operation, accidents, chemical handling, gas handling, equipment maintenance, eating and drinking policies, protective equipment, waste disposal, continuing education, contamination prevention, and technical considerations.

General approach

- Maintain a positive attitude towards safety
- Adhere to standard laboratory safety practices
- Ensure a safe and clean work environment
- Avoid working alone
- Wear appropriate protective gear including lab coats, gloves, masks, safety glasses, and footwear

Accidents

- Be prepared for emergencies like fires or chemical spills
- Provide readily accessible fire-fighting equipment and first aid supplies
- Seek medical attention promptly in case of chemical exposure

Instrument control

- Follow manufacturer's safety instructions while instrument operation
- Ensure stable power supply, especially for critical equipment
- Monitor instruments during operation
- Ensure proper ventilation for instruments like the Atomic Absorption Spectrophotometer
- Exercise caution with equipment like centrifuges and balances

Chemicals

- Use fume hoods for handling hazardous chemicals
- Never pipette by mouth; always use suction bulbs
- Follow proper procedures for diluting acids
- Handle toxic chemicals with care and wash hands thoroughly afterward
- Promptly clean chemical spills and label reagent bottles clearly

Gases

- Secure compressed gas cylinders and maintain gas facilities properly
- Conduct routine checks on equipment like fume hoods

Dining and drinking

- Prohibit eating, drinking, or storing food in the laboratory
- Designate specific areas for breaks
- Avoid using laboratory glassware for eating or drinking

Waste handling

- Dispose of liquid waste carefully and follow local regulations
- Dispose of broken glassware safely

Continuing education

- Display safety posters prominently and ensure staff is trained in safety procedures
- Identify a safety officer or responsible staff member
- Identify and eliminate sources of contamination, such as external dust, cross-contamination and improper storage of reagents
- Consider environmental factors like temperature and humidity in sample analysis
- Ensure the quality of tap water supplied to the laboratory
- Design drainage systems to handle effluents from soil laboratories effectively

By following these guidelines, laboratory staff can work safely and efficiently, minimizing risks and ensuring accurate results.
These components ensure the laboratory operates efficiently, maintains sample integrity, and produces accurate results.

V. FERTILIZER RECOMMEDATIONS TO FCV TOBACCO

Manuring in FCV Tobacco

Manuring is essential in FCV tobacco cultivation, particularly in light soils with low organic matter content. It is recommended to apply 5 tons per hectare of well-decomposed farmyard manure (FYM) by thoroughly mixing it into the topsoil at least 20 days before transplanting. Additionally, growing a green manure crop like Sunhemp for 6 to 7 weeks and incorporating it into the soil is an effective practice. Gliricidia *spp.* also serves as a valuable green leaf manure for improving soil quality in these light soil areas.

Fertilizer recommendation for Northern Light Soils of AP

Fertilizers are to be applied in 3 splits *viz.*, 7-10 days, 25-30 days and 40-45 days. Dollop method of application at 10 cm depth on both sides of plant is to be followed. The type, dose and the time of application of fertlizers is given below :

Time of application	Name of theFertilizer	Dose(kg/ha)	N -P_2O_5-K_2O-Ca (kg/ha)
Basal application	Dolomite	200	0-0-0-48
I Split/ha	Di-Ammonium Phosphate	100	18-46-0-0
	Sulphate of Potash	100	0-0-50-0
II Split/ha	Ammonium Sulphate	250	51-0-0-0
	Sulphate of Potash	100	0-0-50-0
III Split/ha	Urea	100	46-0-0-0
	Sulphate of Potash	100	0-0-50-0
	Total		**115-46-150-48**

In very light soils, apply potassium sulphate 300 kg/ha in 4 splits (75 kg each) and apply the 4th split on 70 DAT for increasing the quality and yield.

Fertilizers recommended for Fertigation in NLS

	Name of the Fertiliser	Dose (kg/ha)	N -P_2O_5 -K_2O - Ca (kg/ha)
Basal /ha	Di-Ammonium Phosphate	75 kg	13.5 – 34.5 – 0
	Sulphate of Potash (soil)	100 kg	0 – 0 – 50
Top dressing/ha	Urea	80 kg	36.8 – 0 - 0
	Ammonium sulphate	50 kg	10.5 – 0 – 0
	Calcium nitrate	50 kg	7.75 – 0 – 0 - 9.25
	Potassium Nitrate	160 kg	20.8 – 0 – 72
Total			**90 - 35 - 120 - 9.25**

Fertilizer recommendation for Southern Light Soils of AP

Name of the Fertilizer	Dose (kg/ha)	$N\text{-}P_2O_5\text{-}K_2O\text{-}Ca$ (kg/ha)
Di-Ammonium Phosphate	75	15.0-34.5-0
Calcium nitrate	50	8-0-0-9.5
Ammonium Sulphate	180	37.8-0-0-0
Sulphate of Potash	120	0–0-60-0
Sulphate of Potash (Podili)	150	0–0-80-0
Total		**60- 35- 60 to 80 -9.5**

Apply the fertilizers in the plant row plough furrow method 15 days before planting to ensure nutrient availability near the root zone.

Fertilizer recommendation for SBS

Recommendation 1

Name of the Fertilizer	Dose (kg/ha)	$N\text{-}P_2O_5\text{-}K_2O\text{-}Ca$ (kg/ha)
20-20-0	250	50-50-0
Ammonium Sulphate	50	10-0-0
Sulphate of Potash	100	0-0-50
Total		**60 - 50 - 50**

Recommendation 2

Name of the Fertilizer	Dose (kg/ha)	$N\text{-}P_2O_5$ - K_2O (kg/ha)
Ammonium Sulphate	200	40-0-0
Di-Ammonium Phosphate	100	18-46-0
Sulphate of Potash	100	0-0-50
Total		**58-46-50**

Fertilizer recommendation for NBS

Recommendation 1

Name of the Fertilizer	Dose (kg/ha)	N-P_2O_5 - K_2O (kg/ha)
20-20-0	125	25-25-0
Ammonium Sulphate	100	20-0-0
Sulphate of Potash	50	0-0-25
Total		**45-25-25**

Recommendation 2

Name of the Fertilizer	Dose (kg/ha)	N-P_2O_5 - K_2O (kg/ha)
Ammonium Sulphate	170	35-0-0
Di-Ammonium Phosphate	55	10-25-0
Sulphate of Potash	50	0-0-25
Total		**45-25-25**

Fertilizer recommendation for KLS

- The fertilizer doses recommended for Karnataka Light Soils is 60- 40-120 (N-P_2O_5-K_2O/ha). Fertilisers are to be applied in two splits at 10 and 30-35 days after transplanting.

Fertiliser schedule for KLS			N-P_2O_5-K_2O-Ca (kg/ha)
1st split (30:40:60) 10 days after planting	DAP AS SOP	100 kg 60 kg 120 kg	18-46-0-0 12-0-0-0 0-0-60-0
2nd split (30:0:60) 30-35 days after planting	AS SOP	150 kg 120 kg	30-0-0-0 0-0-60-0
	Total		**60-46-120-0**

- For calcium supply, either dolomite at 75 kg/ha or calcium nitrate at 75 kg/ha can be applied to light textured, acidic soils. If calcium nitrate is chosen, the nitrogen doses from other fertilizers should be adjusted accordingly
- A starter dose of calcium nitrate at 25 kg N/ha, followed by foliar spray with nitrogen and potassium through a 2.5% solution of potassium nitrate at 45 and 55 days after transplanting enhances productivity under moisture stress conditions

FERTILIZER RECOMMEDATIONS TO NON FCV TOBACCO

	Organic Manures (tonnes/ha)	Fertiliser dose ($N:P_2O_5:K_2O$ kg/ha)
Bidi Tobacco Gujarat	Green manuring or FYM @12.5 or poultry manure or Azolla	160:0:0
Bidi Tobacco Karnataka	FYM @10	125:60:40
Pikka Tobacco Orissa	FYM @10	80:40:40
Burley Andhra Pradesh	FYM @ 10	125:50:50
Irrigated Natu Andhra Pradesh	FYM @ 10 –12 or green manuring	350:50:100
RainfedNatu Andhra Pradesh	FYM @ 15	80:50:50
Lanka Andhra Pradesh	FYM @10	300:50:50
Cigar & Cheroot - Tamil Nadu	FYM @ 25 or sheep - penning	100:50:100
Chewing Bihar, Tamil Nadu & West Bengal	FYM @ 25	Bihar:250:60:60 Bengal: 120:50:75 T.N:100:50:0
Hookah West Bengal	FYM @ 20	120:50:75
HDBRG Andhra Pradesh	FYM @ 10	100:50:50

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VII. APPENDICES

Appendix - I. Molecular and equivalent weights of some important compounds

Compound	Formula	Mol.Wt.(g)	Eq.Wt.(g)
Ammonium acetate	CH_3COONH_4	77.08	77.08
Ammonium chloride	NH_4Cl	53.49	53.49
Ammonium flouride	NH_4F	37.04	37.04
Ammonium nitrate	NH_4NO_3	80.04	80.04
Barium acetate	$(CH_3COO)_2Ba$	255.43	127.72
Barium chloride	$BaCl_2.2H_2O$	244.28	122.14
Boric acid	H_3BO_3	61.83	20.61
Calcium acetate	$(CH_3COO)_2Ca$	158.00	79.00
Calcium carbonate	$CaCO_3$	100.09	50.05
Calcium chloride (dihydrate)	$CaCl_2.2H_2O$	147.02	73.51
Calcium hydroxide	$Ca(OH)_2$	74.00	37.00
Calcium nitrate	$Ca(NO_3)_2$	164.00	82.00
Calcium sulphate	$CaSO_4.2H_2O$	172.17	86.08
Ferrous ammonium sulphate	$(NH_4)_2SO_4.FeSO_4.6H_2O$	392.13	392.13
Ferrous sulphate	$FeSO_4.7H_2O$	278.01	139.00
Magnesium chloride	$MgCl_2.6H_2O$	203.30	101.65
Magnesium nitrate	$Mg(NO_3)_2.6H_2O$	256.41	128.20
Potassium chloride	KCl	74.55	74.55
Potassium dichromate	$K_2Cr_2O_7$	294.19	49.04
Potassium hydroxide	KOH	56.10	56.10
Potassium permanganate	$KMnO_4$	158.03	31.60
Potassium nitrate	KNO_3	101.10	101.10
Potassium sulphate	K_2SO_4	174.27	87.13
Potassium hydrogen phthalate	$COOH\ C_6H_4\ COOK$	204.22	204.22
Oxalic acid	$C_2H_2O_4\ 2H_2O$	126.00	63.00
Silver nitrate	$AgNO_3$	169.87	169.87
Sodium acetate	CH_3COONa	82.04	82.04
Sodium bicarbonate	$NaHCO_3$	84.01	84.01
Sodium carbonate	Na_2CO_3	106.00	53.00
Sodium chloride	NaCl	58.45	58.45
Sodium hydroxide	NaOH	40.00	40.00
Sodium nitrate	$NaNO_3$	84.99	84.99
Sodium oxalate	$Na_2C_2O_4$	134.00	67.00
Sodium sulphate	Na_2SO_4	142.04	71.02
Sodium thiosulphate	$Na_2S_2O_3.5H_2O$	248.18	248.18

Appendix - II. Important conversion factors

N X 1.286 = NH_4

N X 4.43 = NO_3

Organic carbon X 1.724 = Organic matter

P X 2.29 = P_2O_5

K X 1.20 = K_2O

Ca X 1.40 = CaO

Ca X 1.85 = $Ca(OH)_2$

S X 3 = SO_4

Acre X 2.471 = Hectare = 2.24 X 10^6 kg soil (plough layer)

Hectare X 0.404686 = Area in Acres

Ib $acre^{-1}$ X 1.121 = kg ha^{-1}

me L^{-1} X equivalent wt. = ppm

ppm = µg ml^{-1}= mg L^{-1}

1 mm sieve = 16 mesh per inch

Appendix – III. Procedures for preparing 1000ppm standard solutions

1. **Aluminium** : Dissolve 1 g of Al metal in 25 ml of conc. HCl and a few drops of conc. HNO_3
2. **Calcium** : Dissolve 2.4973 g of $CaCO_3$ (dried at 150 ºC) in 25 ml of 1N HCl
3. **Cadmium** : Dissolve 1 g of Cd metal (99.99%) in 50 ml of 1+1 HCl
4. **Cobalt** : Dissolve 1 g of Co metal (99.99%) in minimum of 6N HNO_3 or dissolve 2.630 g of $CoSO_4$ (dried at 250-300 ºC for 6-8 hr) in about 100 ml of double distilled water and 1 ml of conc. H_2SO_4(A R grade)
5. **Chromium** : Dissolve 1 g of Cr metal (99.99%) in 50 ml of conc. HCl or Dissolve 2.8282 g of $K_2Cr_2O_7$ in double distilled water
6. **Copper** : Dissolve 1 g of Cu metal (99.99%) in 50 ml of 1+1 HNO_3
7. **Iron** : Dissolve 1 g of Fe metal (99.99%) in 100 ml of 3.5 N H_2SO_4
8. **Potassium** : Dissolve 1.9067 g of dried KCl in double distilled water
9. **Magnesium** : Dissolve 1.0000 g of Mg metal ribbon or turning (99.99%) in 50 ml of 5N HCl
10. **Manganese** : Dissolve 1.0000 g of Mn metal wire or foil (99.99%) in 50 ml of 6N ÇÍÏ$_3$ or dissolve 3.076 g of manganous sulfate monohydrate ($MnSO_4.H_2O$) in 200 ml of double distilled water and add 1.5 ml of conc. ÇÍÏ$_3$ or dissolve 1.5824 g of manganese dioxide (MnO_2) in minimum quantity of conc. HCl, evaporate to dryness, dissolve residue in double distilled water
11. **Molybdenum** : Dissolve 1.5003 g of molybdenum trioxide (MoO_3) in 10 ml of conc. HCl
12. **Nickel** : Dissolve 1.0000 g of Ni metal (99.99%) in 50 ml of 1+1 ÇÍÏ$_3$
13. **Lead** : Dissolve 1.0000 g of Pb metal (99.99%) in 20 ml of 6N ÇÍÏ$_3$ or Dissolve 1.5982 g of $Pb(NO_3)_2$ in DDW and add 20 ml of conc. ÇÍÏ$_3$
14. **Selenium** : Dissolve 1.0000 g of Se metal (99.99%) in 20 ml of aqua-regia (15 ml conc. HCl + 5 ml conc. ÇÍÏ$_3$)
15. **Silicon** : Fuse 4.278 g of silicon dioxide (SiO_2) with 20 g of sodium carbonate in a platinum crucible and dissolve in distilled water
16. **Zinc** : Dissolve 1.0000 g of Zn metal (99.99%) in 50 ml of 1+1 HCl or dissolve 4.5490 g of zinc nitrate hexahydrate [$Zn(NO_3)_2$ $6H_2O$) in double distilled water

Appendix – IV. Preparation of indicator solutions

1. **Cochineal**: Digest 3.0 g of pulverised Cochineal in a mixture of 50 ml 95% alcohol and 200 ml of water for 1 or 2 days at the ordinary temperature with frequent shaking and then filter

2. **Diphenylamine**: Dissolve by warming 0.5 g of Diphenylamine in 100 ml of concentrated H_2SO_4. It is a redox indicator, blue when oxidised and colourless when reduced

3. **Ferric ammonium sulphate:** A cold saturated solution of A.R. Ferric ammonium sulphate in water (about 40%) to which a little 6 N. Nitric Acid has been added. One ml of this solution to be employed for each titration

4. **Methyl red:** Dissolve 0.5 g Methyl Red in 100 ml of 95% alcohol

5. **Methylene blue:** 1% aqueous solution. A redox indicator, blue colour when oxidised and colourless when reduced

6. **Methyl orange:** This indicator is obtainable either as free acid or as its sodium salt. Dissolve 0.5 g of the sodium salt in 1 litre of water and add 15.2 ml of 0.1 N HCl; filter if necessary when cold

7. **Phenolphthalein (0.5%)**: Dissolve 0.5 g of Phenolphthalein in 100 ml of 95% alcohol. It is colourless in acidic medium and pink in alkaline medium

8. **Potassium ferricyanide**: Used as an external indicator in the estimation of iron by the potassium dichromate method. By spot test, it indicates the complete conversion of ferric iron into ferrous state by the absence of blue colour when placed in contact with the drop of solution. A freshly prepared solution of less than 1.0% strength is to be used

9. **Potassium chromate**: Dissolve 5.0 g of AR potassium chromate in 100 ml of water and use 1 ml of the indicator so that the indicator concentration in the actual titration is 0.005-0.0025 molar

10. **Potassium or ammonium thiocyanate**: A freshly prepared solution of roughly 1% strength is to be used as an external indicator to determine the complete reduction of ferric iron to the ferrous condition. Absence of red colour indicates the completion of the reduction

11. **Bromo-cresol green mixed indicator solution:** Dissolve 0.5 g of Bromo-cresol green and 0.1 gram of Methyl red in 100 ml of 95% Ethyl alcohol. Adjust this solution with dilute NaOH or HCl to bluish purple mid-colour

Appendix - V. Strength of aqueous solutions of the common acids

Reagent	Specific gravity	% by weight	Normality	Volume (ml) required to make 1 litre of 0.1 N solution
HCl	1.18	35	11.3	8.9
HNO_3	1.42	70-71	16.0	6.3
H_2SO_4	1.84	96	36.0	3.0
H_3PO_4	1.69	85	14.7	2.3
CH_3COOH	1.05	99.5	17.4	5.8

Appendix - VI. Different solution concentrations used in analysis

Type of solution	Abbreviation	Definition
Molar	M	Gram-molecular weight (mole of solute) per liter of solution
Molal	m	Gram-molecular weight (mole of solute) per kilogram of solvent
Formal	F	Gram-formula weight of solute per liter of solution
Normal	N	Gram-equivalent weight of solute per liter of solution
Weight per volume, percent	w/v (%)	Number of grams of solute in per 100 volume of solvent (ml)
Volume percent	Volume % or v/v (%)	Volume of solute in per 100 volume of solution (ml)
Weight percent	Wt % or w/w (%)	Weight of solute in per 100 weight of solution
Parts per million	ppm	Milligrams of solute in per kilogram of solution or, milligrams of solute in per liter of solution
Parts per billion	ppb	Micrograms of solute in per kilogram of solution or, micrograms of solute in per liter of solution

Appendix - VII. Quality and soil properties of Indian Tobacco

Quality Parameter	Maximum	Minimum
Northern Light Soils (NLS) ofAndhraPradesh		
Nicotine	1.50	3.50
Reducing Sugars	9.00	21.00
Total Nitrogen	2.00	3.00
Total Potassium	2.00	3.50
Chlorides	0.50	1.50
Southern Black Soils (SBS) of Andhra Pradesh		
Nicotine	1.00	2.00
Reducing Sugars	10.00	20.00
Total Nitrogen	2.00	2.50
Total Potassium	1.50	2.30
Chlorides	0.50	1.00
Southern Lights Soils (SLS) of Andhra Pradesh		
Nicotine	1.20	2.50
Reducing Sugars	10.00	25.00
Total Nitrogen	1.50	2.50
Total Potassium	1.70	2.50
Chlorides	0.20	0.50
Karnataka Light Soils (KLS) of Karnataka		
Nicotine	1.34	2.54
Reducing Sugars	12.0	23.0
Total Nitrogen	1.40	2.50
Total Potassium	1.50	2.30
Chlorides	0.30	0.70

Nicotine Content (%) in Non-FCV tobacco

Tobacco Type	Nicotine (%)
Bidi tobacco (Anand)	7.00
HDBRG (Guntur)	3.89
Natu tobacco (Black soils)	2.79
Natu tobacco (Light soils)	3.50
Burley tobacco	1.26
Chewing tobacco (Tamil Nadu)	2.93
Chewing tobacco(Bihar)	3.70
Jati-Chama (West Bengal)	3.69
Jati-Podali (West Bengal)	4.02
Motihari-Hemti (West Bengal)	4.83
Motihari-Bitri (West Bengal)	6.64

Type	Major soil group	Soil properties
FCV tobacco		
Northern Light Soils (NLS)	Red Sandy and sandy loam Soils	Alfisols; pH: 4.36-8.87; Low OC, N High P and low to mediumK
Traditional Black Soils (TBS)	Heavy Black Soils	Vertisols pH: 7.5 Low OC, N High P and K
Southern Light Soils (SLS)	Red Sandy Loams and Sandy Clay loams	Alfisols/ Oxisols pH: 7.10 Low OC, N High P and medium to high K
Southern Black Soils (SBS)	Medium Black Soils (silt loams)	Inceptisols/ Entisols Vertisols pH: 8.3 Low OC, N High P and K
Karnataka Light Soils (KLS)	Red Sandy Loams	Alfisols pH: 5.5-5.8 Low OC, N High P and medium to high K
Non FCV tobacco		
Burley Tobacco	Red gravelly soil, Sandy Loam (Surface) Loam (Sub-Surface)	pH: 5.5-6.5 Low OC, NPK
Natu	Medium to Heavy Black soils Sandy to sandy loam	pH: 7.0-8.5 Low OC, N, Medium to high P and K Low OC, NPK
Lanka	Sandy to loam	Low OC, NPK
Bidi	Sandy to sandy loam/ Black Silt loams/ Silt loam to Clay	Low OC, N, Medium to high P &K
Jati and Motihari	Sandy loams and silt loams	pH 5.1-6.4, high in available P, low to medium in available K
Chewing Tobacco	Sandy loam to Clayey	pH 7.5-8.5, low in OC low to medium in available P & K