

**ACHARYA N.G.RANGA AGRICULTURAL UNIVERSITY
RAJENDRA NAGAR, HYDERABAD**



PRACTICAL MANUAL

Soil Fertility, Soil Chemistry and Nutrient Management

C.No. SSAC 221

Credits: 2 + 1 = 3

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CERTIFICATE

Certified that this is a bonafide record of practical work done by

_____ I.D.No. _____ in course No. : SSAC 221

Manures, Fertilizers and Agrochemicals during the *First/ Second* Semester

of 20__ to 20__ .

Signature of Course-in-charge

Particulars of the experiments conducted

Expt. no.	Title of the experiment	Date of submission	Page no.	Signature	Remarks
1.					
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LIST OF THE EXPERIMENTS:

- 1 Preparation of soil extracts for available nutrients and introduction to colorimetry and flame photometry
- 2 Estimation of pH and EC of soils
- 3 Estimation of available N in soils
- 4 Estimation of available P in soils
- 5 Estimation of available K in soils
- 6 Estimation of available S in soils
- 7 Estimation of available Zn in soils
- 8 Assessment of quality of irrigation water (a) pH (b) EC
- 9 Estimation of carbonates, bicarbonates and chlorides in irrigation water
- 10 Determination of calcium and magnesium in irrigation water by EDTA method
- 11 Estimation of K and Na in irrigation water
- 12 Quick tests and interpretation of soil tests and irrigation water analysis data -
Determination of lime requirement of acid soil
- 13 Determination of Gypsum requirement of alkali soil
- 14 Estimation of N in plant samples
- 15 Estimation of P in plant samples
- 16 Estimation of K in plant samples

Ref: Methods of Analysis of Soils, Plants, Waters and Fertilisers by Tandon HLS

Expt. No.: 1

Date:

**PREPARATION OF SOIL EXTRACTS FOR AVAILABLE NUTRIENTS AND
INTRODUCTION TO COLORIMETRY AND FLAME PHOTOMETRY**

Plants draw their nutrients from air, water and soil. The bulk of mineral nutrients come from soil. Soil available form of nutrient is that fraction which is distributed in different discrete chemical forms, which often exist in a state of dynamic equilibrium and constitute the pool from which plants draw it. Soil available form of a nutrient is also that fraction whose variation in amount is responsible for significant changes in yield and responses. The nutrient available to biological organisms is termed as bio available nutrient. Available nutrients in the soil are water soluble and exchangeable. The reagent used for the extraction of this pool simulates the plant roots and extracts the same amount of available nutrient as the plant is able to take in. The following are various extractants for the available fraction of nutrients.

I. Preparation of soil water extract for water soluble nutrients.

The water soluble nutrients, which consist of cations and anions in different proportion are Ca^{2+} , Mg^{2+} , K^+ , Na^+ , NH_4^+ , Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} , SO_4^{2-} , NO_3^- , Cl^- , MoO_4^{2-} , and BO_3^{3-} . These are nutrients that can be extracted by using distilled water with soil water ratio of 1:5.

Procedure :

1. Weigh 100 gm of soil passed through 2 mm sieve into one litre reagent bottle with a cork.
2. Add 500 ml of distilled water and small quantity of charcoal (decolourising agent) and shake the contents by end over end for 30 minutes to get a clear extract.
3. Filter the contents through Whatman No. 1 filter paper.
4. Store the extract in a clean bottle for the estimation of water soluble nutrients in the soil.
5. Add 2 to 3 drops of TEA (Tri Ethanol Amine) to this extract to prevent algal growth and place in a refrigerator.

II. Preparation of chemical extracts for water soluble + exchangeable nutrient

There are a variety of extractants used for extracting available nutrients from soils.

1. **Available P** : Available P content in the soil is extracted by using two extractants depending on soil pH.
 - a) Olsen's reagent: 0.5 M NaHCO_3 solution of pH 8.5. This reagent is used for extraction of P from slightly acidic, neutral, alkaline and calcareous soils.
 - b) Bray's reagent: 0.03 M NH_4F + 0.025 N HCl solution. This is used as an extractant for soils with acidic pH.
2. **Available K** : Available K is extracted by using neutral normal ammonium acetate ($\text{CH}_3\text{COONH}_4$) solution.
3. **Available S** : It is extracted by using CaCl_2 (0.15 %) solution.
4. **Available micronutrients** : The available micronutrient (cationic or anionic).
 - a) **For extracting cationic micronutrients (Zn, Cu, Fe and Mn):** DTPA (Diethylene Triamine Penta Acetic acid) reagent is used. DTPA extractant contains 0.005 M DTPA + 0.01 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ + 0.1 M TEA. Weigh 1.967g DTPA salt and 1.47g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ salt in a beaker. To this add 20 – 25 mL of glass distilled water and 13.3 mL of triethanolamine followed by 100 mL of glass distilled water. Transfer to 1 L volumetric flask, adjust the pH to 7.3 with dilute HCl (1:5) and make the volume to one liter with glass distilled water.
 - b) **For anionic nutrients:**
 1. Available 'B' is extracted by hot water.
 2. Available Mo – Ammonium oxalate solution (or) Tamm's reagent.
 3. Available Cl^- - By water.

Introduction to colourimetry

Colorimetry is the determination of concentration of a substance by the measurement of the relative absorption or transmittance of light in the visible region with respect to a known concentration of the substance.

Instrument used for colorimetric measurement is called colorimeter. This instrument has been named as spectrophotometer since photoelectric cells are generally used to measure the light transmitted by the solution.

When a beam of radiant energy passing through a medium, the energy of the beam is partly altered by reflection, refraction, diffraction or absorption and the reminder of the energy may be transmitted through the substance.

Spectrophotometry or colorimetry is based on Lambert's and Beer's law which states that the decrease in the intensity of light is directly proportional to the concentration of the solution if the thickness of the absorbing medium is kept constant.

$$\text{Transmittance } T = I/I_0$$

Where,

I_0 is the intensity or energy per cm^2 per second before passing through substance.

I is the intensity of energy after passing through the medium

$$\begin{aligned} \text{Absorbance (A)} &= \log 1/T \\ &= \log I_0/I \\ &= \log I_0 - \log I \\ &= \log 100 - \log T \\ A &= 2 - \log T \end{aligned}$$

The light from the source travels through the field lens, entrance slit, and collimating mirror. Collimating mirror allows the light to fall on the grating chamber which disperses the light. The required wavelength of the light is adjusted by adjustable light control (wave length adjustment). The monochromatic light passes through the exit slit, filter and the sample. Some of the light energy is absorbed by the sample and the remaining is transmitted to the photocell which converts light energy into the electrical energy. This transmittance is measured by galvanometer.

Selection of wave length range

Wave length range (nm)	Colour of the sample solution	Complement	Wave length range (nm)
400 -500	Violet	Yellowish green	560 – 575
450 – 480	Blue	Yellow	575 - 590
480 – 490	Greenish blue	Orange	590 – 625
490 – 500	Bluish green	Red	625 - 750
500 – 560	Green	Purple	---
560 – 575	Yellowish green	Violet	400 -500
575 - 590	Yellow	Blue	450 – 480
590 – 625	Orange	Greenish blue	480 – 490
625 - 750	Red	Bluish green	490 – 500

Introduction to flamephotometry

The use of flame emission spectroscopy for the estimation of the alkali metals is an important application in routine chemical analysis. For this, low temperature flame photometry provides the most reliable and convenient method. This exploits the fact that compounds of the alkali and alkaline earth metals are thermally dissociated at the temperature of a burner flame and some of the atoms produced are further excited to a higher energy level. When these excited atoms return to the ground state, they emit radiation which, for the alkali and alkaline earth elements, lies mainly in the visible region of the spectrum.

The wavelength of light emitted from the flame is characteristic of the particular element. The intensity of this light is proportional to the amount of the salt solution volatilised in the flame at any moment.

Hence, a flame photometer essentially consists of the following components.

- A flame that can be maintained in a constant form and at a constant temperature
- A means of transporting a homogenous solution in the flame at a steady rate

- A means of separating the light of the wavelength to be measured from that of extraneous emission with a monochromator or an optical filter
- A means of measuring the intensity of the radiation emitted from the flame.

The two characteristics of the alkali metals chiefly facilitating their determination by flame photometry are:

- (i) Their spectra are excited at a temperature lower than that at which most other elements are excited. This low excitation temperature permits ordinary bottled gas to be used as fuel for the flame
- (ii) Their characteristic wavelengths are widely separated from each other and from those of most other elements. This wide separation of the characteristic wavelengths makes it possible to use simple optical filters rather than the more expensive monochromators.

Alkali & Alkaline Earth metals & their Characteristic Wavelengths (nm)	
Barium	553.6
Calcium	623.0
Cesium	455.6
Lithium	671.0
Potassium	766.0
Rubidium	780.0
Strontium	460.7
Sodium	589.0

Expt. No.:2

Date:

ESTIMATION OF pH AND EC OF SOILS

I) ESTIMATION OF pH OF SOILS

The pH value is A measure of hydrogen ion concentration of the soil water system and expresses the acidity and alkalinity of soil. pH is very important property of soil as it determines the nutrient availability, microbial activity and physical condition of the soil.

The concept of pH was given by Sorensen in 1901. The pH of solution has been defined as negative logarithm of hydrogen ion activity which in very dilute solution is expressed as g ions L⁻¹ or g mol L⁻¹.

$$\text{pH} = -\log_{10} [\text{H}^+] \quad \text{or} \quad [\text{H}^+] = 10^{-\text{pH}}$$

It is found that in aqueous system at 22°C

$$[\text{H}^+] [\text{OH}^-] = 10^{-14}$$

Which is called ionic product of water.

In soil water system, some of the adsorbed hydrogen ions dissociate from the surface of the soil colloids into soil solution. These dissociated H⁺ give rise to soluble acidity or active acidity. pH is a sort of voltage measurement and to cover the entire range of 0 – 14, a potential measurement in the range of + 420 to - 420 mV is needed. A potential difference of 59.1 mV is developed for a difference of one pH unit. Instrument used for pH determination is a glass electrode pH meter with a calomel or reference electrode introducing salt bridge. Most digital pH meters now a days have a single or combined electrode assembly.

Apparatus required: pH meter, beaker (100 mL), measuring cylinder, glass rod and a balance.

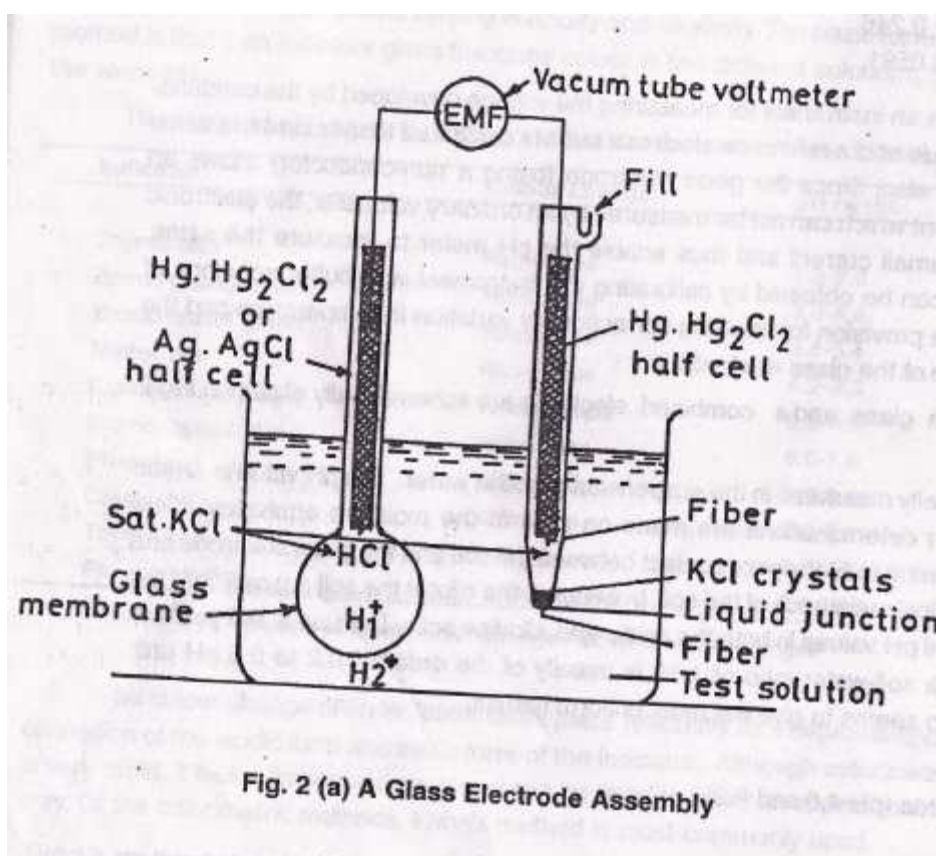
Reagents: Buffers of different pH values (4.0, 7.0 and 9.2) for standardization or calibrating the pH meter. Dissolve one tablet in 100 mL of solution.

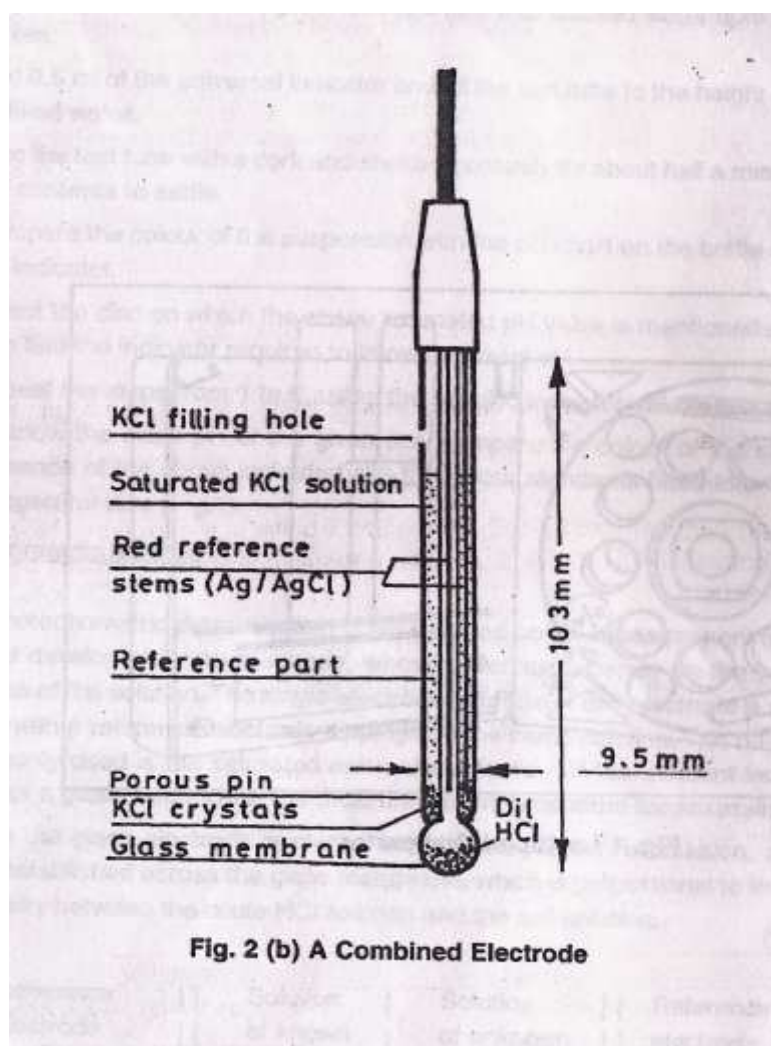
Description of pH meter:

a) **Glass electrode (indicator electrode):** This electrode is a glass membrane electrode consisting of hydrogen ion responsive thin walled soft glass bulb sealed to a stem of non

hydrogen responsive high resistance glass. Both the inner and outer surfaces of the bulb are sensitive to the changes in H^+ ion concentration. The inside bulb is filled with dilute HCl. When the glass electrode bulb is dipped in a solution having H^+ concentration different from the inside electrode, an electrical potential develops across the membrane and this potential is proportional to the difference in pH between the two cells.

b) **Calomel electrode (reference electrode)** : It consists of Hg in contact with KCl saturated with Hg_2Cl_2 . This electrode is having a constant potential. The two electrodes are connected to a potentiometer and a galvanometer which reads the pH.





Procedure

1. Weigh 20 g of air dried soil into a 100 mL beaker.
2. To this add 50 mL of distilled water.
3. Stir the contents with a glass rod intermittently for 30 minutes.
4. Switch on the pH meter and allow for warming.
5. Calibrate with the known buffers.
6. Immerse the electrodes in soil water suspension and record the pH reading.

Interpretation of soil pH

S.No	pH range	Rating	Remarks
1	<4.5	Very strongly acidic	Needs liming
2	4.5 – 5.5	Strongly acidic	Needs liming
3	5.5 – 6.5	Slightly acidic	Needs liming
4	6.5 – 7.5	Neutral	Suitable for crop growth
5	7.5 – 8.5	Slightly alkaline	---
6	8.5 – 10.0	Strongly alkaline	Needs gypsum application
7	>10.0	Very strongly alkaline	

Result: The pH of soil water suspension (1:2.5) is ----- and it is rated as -----
-----.

II) ESTIMATION OF EC OF SOILS

Aim: To determine the electrical conductivity and soluble salt content of soil.

Soil salinity, which is caused due to the total soluble salt content, is determined by measuring the electrical conductivity of soil. EC is a measure of the ability of soil solution to carry electric current by the migration of ions under the influence of an electric field. Like metallic conductors, solutions also obey ohm's law.

Electric potential (E)

Resistance (R) = _____

Current (I)

R in 'ohms'

E in 'volts'

I in 'amperes'

The reciprocal of resistance is called conductance

Conductance (C) = $1/R$; the units are 'mhos'.

Specific conductance or electrical conductivity is the conductance 1 cc of solution measured between two electrodes placed 1 cm apart. It is expressed in mho cm^{-1} or mmho cm^{-1} or dS m^{-1} .

$$1 \text{ dS m}^{-1} = 1 \text{ mS cm}^{-1}$$

Principle:

Pure water offers maximum resistance to the passage of electricity. If any soluble salts are present in water then they dissociate thus increasing the flow of current. The soil salinity in relation to plant growth is generally measured in terms of conductivity of the soil water suspension or saturation extract of the soil. The percentage of soluble salts in soils can then be computed from the following relationship

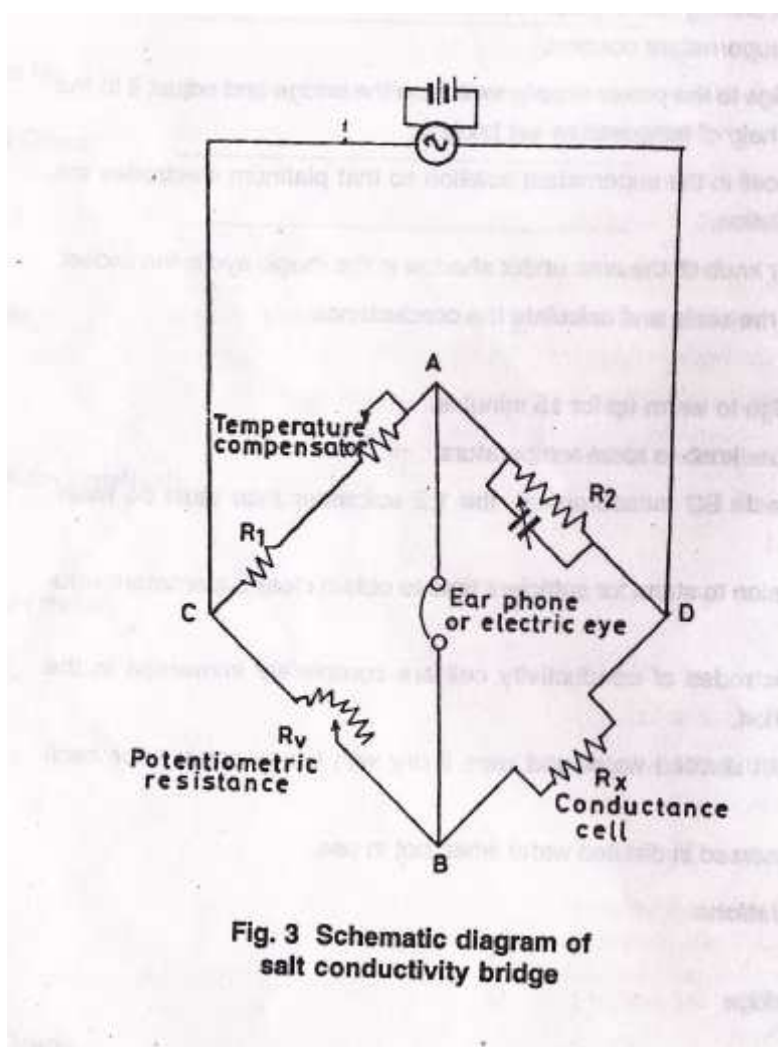
$$\text{Percentage of soluble salts in soil} = 0.064 \times \text{EC} \times \text{SP}/100$$

Where SP the saturation percentage of soil and EC is in dS m^{-1}

Principle of conductivity meter

The electrical conductivity of a solution is measured with a conductivity meter known as 'salt bridge' or 'solubridge'. It is based on the principle of Wheatstone bridge in which alternate current is used.

Two fixed resistances R_1 and R_2 ; a variable resistance R_v in a branched circuit with the cell having resistance R_x (Fig.). The current passes through R_v and the reading is recorded. R_v is adjusted till no current passes through the cell. R_v is then calibrated in terms of conductance.



Apparatus: Balance, beaker (100 mL), measuring cylinder, a glass rod, a conductivity meter.

Reagents:

Potassium chloride (0.01 N): Weigh 0.7456 g of potassium chloride and make the volume to 1 L with distilled water. This solution gives an electrical conductivity of 1.41 dS m^{-1} at 25°C .

Procedure:

1. Weigh 25 g of air dried soil into 100 mL beaker and add 50 mL of distilled water.
2. Carry out intermittent stirring with a glass rod for 30 minutes and then allow to stand for obtaining clear supernatant solution.
3. Connect the salt bridge to the power supply, switch on the bridge and adjust it to room temperature with the help of temperature setting knob.
4. Calibrate the conductivity bridge with the help of standard KCl solution.
5. Dip the conductivity cell in the supernatant solution so that platinum electrodes are completely immersed in solution.
6. Note down the EC value.

Result: The Electrical Conductivity (1 : 2) of the given soil is ----- dS m^{-1} .

Date:

- NaOH (2.5 %) – Dissolve 25 g of NaOH in distilled water making the volume to 1 litre.
- Boric acid (H_3BO_3) – (2.5 %) Weigh 25 g of boric acid and dissolve in warm water and dilute to 1 litre.
- Mixed indicator –0.5 g bromocresol green is mixed with 0.1 g methyl red in 100 ml of 95 % ethanol.
- Standard H_2SO_4 (0.01 N) - Dissolve 0.3 ml of concentrated H_2SO_4 in 1 L solution and standardize against 0.01 N Na_2CO_3 using methyl red as indicator.

Procedure : (With Kjeldplus)

- Place 5 g soil sample into kjeldahl tube.
- Add 30 ml of 0.32 % KMnO_4 .
- Place a 250 ml conical flask containing 25 ml of 2.5 % boric acid with mixed indicator at the end of delivery tube; care is taken to see that the tip of the tube is completely dipped in boric acid;
- Tap water is allowed to run through condenser unit.
- To the contents of the kjeldhal flask, add 30 ml of NaOH (2.5 %) automatically and keep the flask in place.
- Start the distillation process and continue until about 100 ml of distillate is collected into the conical flask. The completion of the distillation can be confirmed by moist red litmus paper test.
- After completion of distillation, take out the conical flask containing the distillate from the unit and titrate the contents against standard H_2SO_4 (0.01 N) till the bluish green colour turns light red.
- Run a blank distillation without soil and note down the blank titre value.



With macro kjeldahl distillation unit:

1. Weigh 20 g of soil sample into a 800 mL Kjeldahl flask.
2. Moisten the soil with about 10 mL of distilled water, wash down the soil adhering to the neck of flask, if any.
3. Add 100 mL of 0.32 % KMnO_4 solution.
4. Add a few glass beads or broken pieces of glass rod.
5. Add 2 to 3 mL of paraffin liquid, avoiding contact with upper part of the neck of the flask.
6. Measure 20 mL of 2.5 % 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.
7. Run tap water in condenser.
8. Add 100 mL of 2.5 % NaOH solution and immediately attach to the rubber stopper fitted in the alkali trap.
9. Switch the heaters on and continue distillation until 100 mL of distillate is collected.
10. First remove the conical flask containing distillate and then switch off the heater to avoid back suction.
11. Titrate the distillate against 0.01 N H_2SO_4 taken in burette until light red colour appears.
12. Run a blank without soil with each set of samples.
13. Carefully remove the kjeldahl flask after cooling and clean the flask.

Calculations

Sample titre value	=	S ml of 0.01 N H_2SO_4
Blank titre value	=	B ml of 0.01 N H_2SO_4
Actual titre value	=	$(S-B)$ ml
Weight of soil taken	=	5 g

As per the law of equivalence or equilibrium

$$1 \text{ g. equivalent of } \text{H}_2\text{SO}_4 = 1 \text{ g. equivalent of N}$$

$$1000 \text{ ml of } 1 \text{ N H}_2\text{SO}_4 = 14 \text{ g of N}$$

$$(S-B) \text{ ml of } 0.01 \text{ N H}_2\text{SO}_4 = ?$$

$$= \frac{(S-B) \times 14 \times 0.01}{1000} \text{ g of N}$$

$$\text{So, 5 g of soil contains } \frac{(S-B) \times 14 \times 0.01}{1000} \text{ g of N}$$

1000 g of soil contains

$$= \frac{(S-B) \times 14 \times 0.01 \times 1000}{1000 \times 5} \text{ g of N}$$

$$1 \text{ hectare soil contains } \frac{(S-B) \times 14 \times 0.01 \times 2.25 \times 10^6}{5} \times 10^{-3} \text{ kg of N}$$

$$= (S-B) \times 63 \text{ kg of N/ha.}$$

Soil ratings for available N content :

Soil rating	Available N kg ha ⁻¹
Low	< 280
Medium	280 – 560
High	> 560

Result

The available N content in the soil is ----- kg ha⁻¹ and the soil is rated as --
-----.

Expt. No.: 4

Date:

ESTIMATION OF AVAILABLE P IN SOILS

Introduction : Phosphorus occurs in soil both in organic and inorganic form. More than 50 per cent of P is present in inorganic form as Ca, Fe, and Al phosphates. Plant absorbs P in the form of the H_2PO_4^- and HPO_4^{2-} (orthophosphates).

For the estimation of available P, different extractants such as water, dilute acid, alkali and salt solution are used. The available P in neutral to slightly alkaline soil will be extracted with Olsen's extractant which is 0.5 M NaHCO_3 (pH 8.5).

Principle : Solubility of $\text{Ca}_3(\text{PO}_4)_2$ is controlled by the activity of Ca^{2+} in the solution and pH of the extractant. The bicarbonate of the extractant decreases the activity of Ca and prevents the release of phosphates from apatites and also releases the orthophosphates into the extract. The soluble phosphates form heteropoly complexes with molybdate forming ammonium phosphomolybdate which gives blue colour in the presence of ascorbic acid. The intensity of blue colour in the solution is proportional to the concentration of phosphates. The intensity of blue colour of both standards and sample solution will be measured in spectrophotometer at a wavelength of 660 nm which corresponds to red region. The concentration of 'P' in soil extract is calculated from the standard graph.

Working principle of the instrument

When light of a particular color (or wavelength range) is directed through a solution, some light is absorbed by the solution and some of it is transmitted.

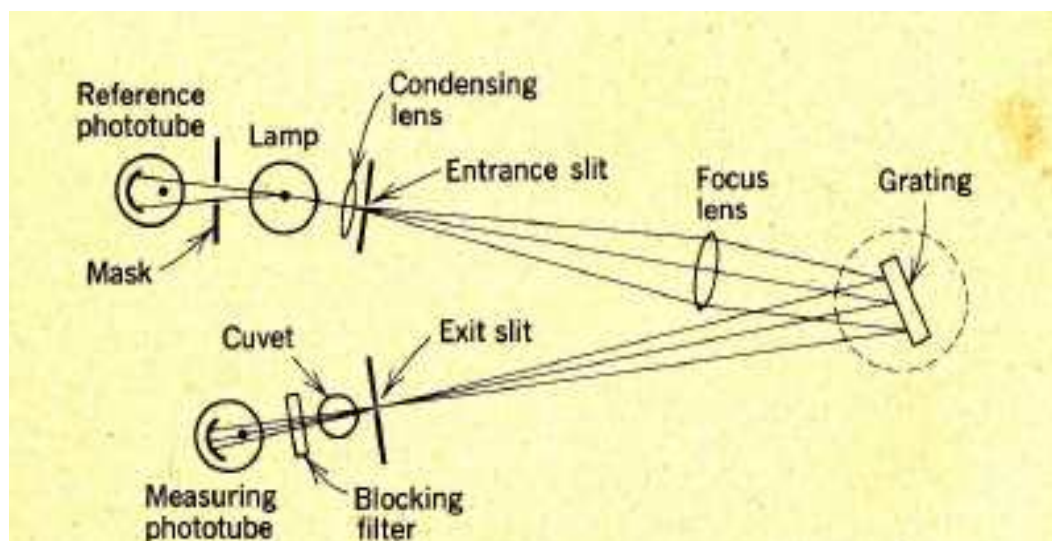
Beer and Lambert's law states that "when a ray of monochromatic light passes through a solution the intensity of radiation decreases exponentially as the concentration of absorbing medium of fixed thickness increases arithmetically.

If I_0 is the intensity of initial radiation before passing through the medium and I is the intensity of radiation after passing through the medium.

$$t = \frac{I}{I_0}$$

$$\text{Absorbance, } A = \log 100 - \log T\% = 2 - \log_{10} T\%$$

Fig.



Schematic diagram showing the working principle of spectrophotometer

The major component of colorimeter are :

1. Electric bulb – Source of light.
2. Filter – it selects the light radiation of desired wavelength.
3. Sample holder or cuvette. It holds the sample solution.
4. Photocell – Converts light energy into electric energy.
5. Galvanometer – it records the electrical energy as absorbance.

Reagents

1. **Olsen's extractant** i.e., NaHCO_3 (0.5 M) of pH – 8.5 : Dissolve 84 g of NaHCO_3 in distilled water, adjust the pH to 8.5 and dilute to 2 L.
2. **Reagent A** : Dissolve 12 g ammonium molybdate in 200 ml of distilled water, dissolve 0.2908 g of antimony potassium tartarate in 100 ml distilled water and add the two solutions to 1000 ml of 2.5 M H_2SO_4 and dilute to 2 litres.
3. **Reagent B** : Dissolve 1.056 g of ascorbic acid in 200 ml of reagent A. This has to be prepared afresh.
4. **H_2SO_4 (2.5 M)** : Dilute 140 ml of concentrated H_2SO_4 to 1 litre.
5. **Standard stock P solution**. Dissolve 0.439 g potassium dihydrogen orthophosphate

(KH_2PO_4) in 1 litre solution. This contains 100 ppm P. Prepare 2 ppm P solution by diluting 50 times.

Preparation of Standard curve

To prepare the standard curve; take 0, 1, 2, 3, 4 and 5 ml aliquots of 2 ppm P solution into a series of 25 ml volumetric flasks. To this add 5 ml of extracting solution followed by 1 drop of para nitrophenol indicator that gives yellow colour. Discolor the contents by adding dilute H_2SO_4 (2.5 M) drop wise to bring down the pH to around 5 at which pH, the formation of heteropoly complex of phosphomolybdates is perfect. Add 4 ml of reagent B and make up the volume to 25 ml with distilled water. Shake the contents and measure the intensity of blue colour in spectrophotometer at a wavelength 660 nm wave length. Plot the standard graph for P taking absorbance values on Y – axis and concentration of P on X – axis.

Procedure for sample preparation

1. Weigh 2 g of soil sample into a 150 ml conical flask.
2. To this add 40 ml of Olsen's extractant and a pinch of charcoal.
3. Shake the contents for 30 minutes and filter through whatman No. 42 filter paper.
4. Pipette out of 5 ml of extract into a 25 ml volumetric flask and add one drop of P-nitrophenol.
5. The contents are acidified with 2.5 M H_2SO_4
6. Add 4 ml of reagent B and make up the volume to 25 ml with distill water.
7. Shake the contents and measure the intensity of blue colour (absorbance) in a spectrophotometer after 10 minutes at 680 nm wave length.
8. Incorporate the absorbance value in the standard graph and obtain the concentration of P in the colored solution.

Calculations

Concentration of P in the coloured solution = X ppm

i.e., 1 mL of the coloured solution contains X μg P

25 ml of coloured solution contains $25 X$ μg of P

This $25 X$ μg of P is present in the 5 ml of soil extract

$$\text{P content in 40 mL of soil extract} = \frac{40}{5} \times 25 \times \mu\text{g of P}$$

Which has come from 2 g soil

$$\text{When 2 g of soil contains} \quad \frac{40}{5} \times 25 \times \mu\text{g of P}$$

$$1000 \text{ g of soil contains} \quad \frac{1000}{2} \times \frac{40}{5} \times 25 \mu\text{g of P}$$

$$\text{When 1 kg of soil contains} \quad \frac{1000}{2} \times \frac{40}{5} \times 25 \mu\text{g of P}$$

One hectare soil i.e., 2.25×10^6 kg of soil contains

$$= \frac{2.25 \times 10^6}{1} \times \frac{1000}{2} \times \frac{40}{5} \times 25 \mu\text{g of P}$$

$$= \frac{2.25 \times 10^6}{1} \times \frac{1000}{2} \times \frac{40}{5} \times 25 \times 10^{-9} \text{ kg of P}$$

$$= \quad \mathbf{X \times 225} \quad \text{kg ha}^{-1} \text{ of P}$$

Soil ratings for available P

Rating	Available P (kg ha ⁻¹)
Low	< 10
Medium	10 – 19
High	> 20

Results : The available P content of the soil is ----- kg ha⁻¹ and it is rated as -----
-----.

Expt. No.: 5

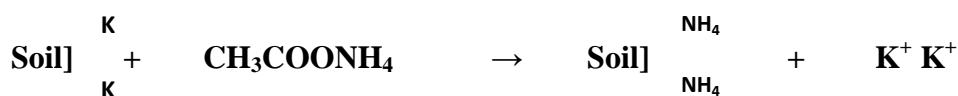
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ESTIMATION OF AVAILABLE K IN SOILS

Aim : To estimate the available 'K' content in soil by flame photometry.

Introduction : About 90-98 % of K in the soil is present in mineral forms such as feldspars, muscovite, biotite and illite. Potassium from these minerals is released slowly by weathering and usually is not of much significance to meet the crop requirements. The readily available K constitutes about 1-2 % of total K in mineral soils. It consists of soil solution and exchangeable K. The neutral normal $\text{CH}_3\text{COONH}_4$ solution which extracts both water soluble and exchangeable K is most commonly used for determination of available K in soil.

Principle: A known weight (5 g) of soil is taken and shaken with neutral normal ammonium acetate solution (25 ml). Ammonium exchanges with K^+ on the soil colloids and the extract contains exchangeable and water soluble K, the amount of which is determined by using a flame photometer by emission spectroscopy.



Exchangeable K

K^+ in the extract

Glassware and Apparatus required : Balance, mechanical shaker, conical flask, volumetric flasks, measuring cylinder, beaker, funnel and flame photometer.

Reagents

- 1. Neutral normal ammonium acetate:** Dissolve 77.09 g of ammonium acetate in 800 ml of distilled water, adjust the pH to 7.0 with ammonia solution or acetic acid and dilute to 1 L with distilled water.
- 2. Standard solution of K (1000 ppm).** Dissolve 1.907 g of KCl in distilled water and make the volume to 1 L.
- 3. Preparation of standard graph for K.**

From the stock solution (1000 ppm), different working standards are prepared as given below

Take aliquots of 0, 1, 2, 3, 4 and 5 mL of 1000 ppm K solution into a series of 100 mL volumetric flasks and make up to the mark to prepare 0, 10, 20, 30, 40 and 50 ppm K solutions respectively.

Working principle of flame photometer

Flame photometer is based on the principle of emission spectroscopy. When a liquid sample containing a metallic salt solution is introduced into a flame, the solvent is vapourised leaving the particles of the solid salt. The salt is converted into the gaseous state. A part or all of the gaseous molecules are progressively dissociated to give free neutral atoms or radicals. These neutral atoms are excited by absorbing the thermal energy of the flame. The excited atoms which are unstable return to normal or ground state by quickly emitting the absorbed energy in the form of photons. The emitted radiation is collected by a concave mirror from the flame and is then permitted to pass through a prism and slit. The radiation of the approximately selected wave length strikes a photo detector and the magnitude of the electrical signal developed is read out on a meter.



BASIC COMPONENTS OF FLAME PHOTOMETER

- Burner
- Mirrors
- Slits
- Monochromators
- Filters
- Detectors

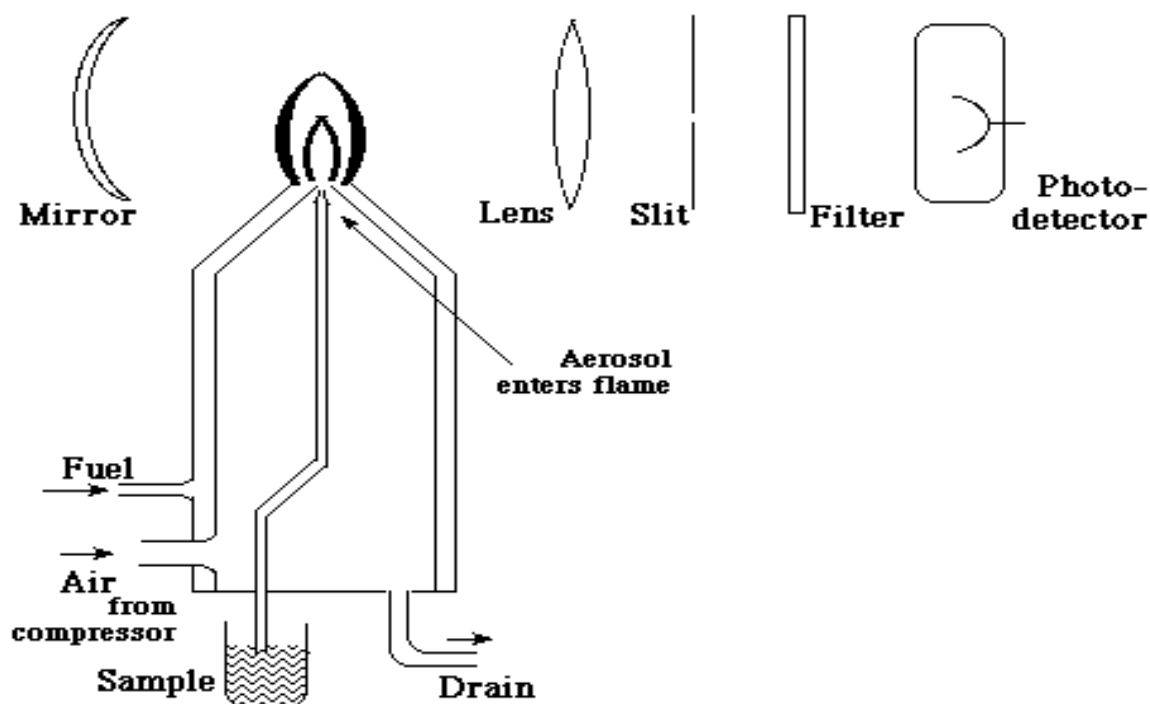


Fig. Schematic diagram showing the working principle of flame photometer

Procedure :

1. Weigh 5 g of soil sample into a 250 ml conical flask.
2. To this add 25 ml of ammonium acetate (1N, pH = 7).
3. Shake the contents for 5 minutes and filter through whatman No. 1 filter paper.
4. The extract is aspirated to the flame and the flame photometer reading is noted down.

Observations

Weight of the soil taken = 5 g
Flame photometer reading = X ppm
Volume of the extract = 25 mL

Calculations

Concentrations of K in the soil extract = X ppm
1 ml of the soil extract contains = X µg of K
25 ml of the extract contains = 25 x X µg of K
5 g of the soil = 25 x X µg of K
1 g of the soil = ?

$$\begin{aligned} & \frac{1}{5} \times 25 \times X \text{ } \mu\text{g of K} \\ &= 5 \times X \text{ } \mu\text{g of K/g of soil} \\ &= 5 \times X \text{ ppm} \end{aligned}$$

Available K in ppm = 5 x

$$\begin{aligned} \text{Available K in kg/ha} &= \text{ppm} \times 2.24 \\ &= 5 \times X \times 2.24 \\ &= \mathbf{11.2 \times X \text{ kg ha}^{-1}} \end{aligned}$$

Soil ratings for available potassium

Soil rating	Available K kg ha ⁻¹
Low	< 108
Medium	108-280
High	> 280

Result : The available K in the given soil sample is ----- kg K ha⁻¹ and is rated as -----
-----.

Expt. No.: 6

Date:

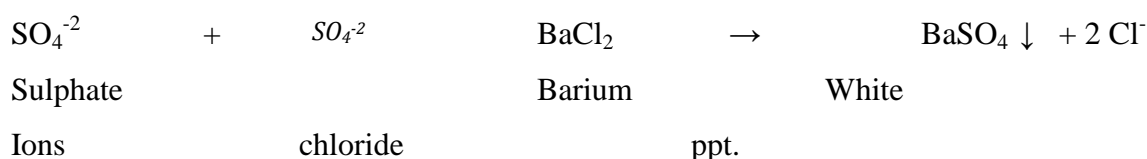
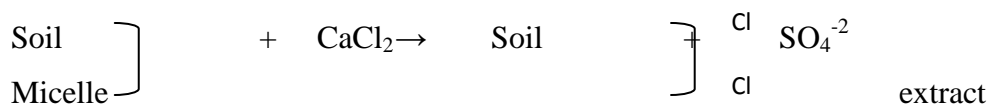
ESTIMATION OF AVAILABLE S IN SOILS

Introduction : Sulphur is present in organic and inorganic forms. The amount depends upon the parent material, soil organic matter status and texture of the soil. Plants absorb sulphur in the form of sulphate ion (SO_4^{-2}). A large number of extractants like H_2O ; monocalcium phosphate; CaCl_2 ; a mixture of ammonium acetate and acetic acid and NaCl have been used for extraction of available S. Among the different extractants CaCl_2 (0.15 %) was found to be the best extractant as suggested by Williams and Steinbergs in 1959.

Aim : To estimate the available 'S' content in the soil.

Principle : When the soil solution is shaken with CaCl_2 (0.15 %), the chloride ions displace the adsorbed sulphate during extraction. The filtrate is analysed for sulphur by turbidimetry method as outlined by Chesin and Yien (1950), in which turbidity produced due to the precipitation of SO_4^{-2} as BaSO_4 is measured on a spectrophotometer at a wavelength of 420 nm or corresponding to blue filter. The conditioning reagent is added to stabilize or suspend the BaSO_4 precipitate uniformly in the solution.

Equations



Glassware and Apparatus required : Balance, mechanical shaker, spectrophotometer, conical flask, volumetric flask, measuring cylinder, beaker, funnel, burette.

Reagent required:

- a) CaCl_2 (0.15 %). Dissolve 1.5 g of CaCl_2 dihydrate in distilled water and make the volume to 1 litre.
- b) Stabilizing agent or conditioning agent : Dissolve 75 g NaCl in 250 ml of distilled water in a 500 mL volumetric flask and add 30 mL of concentrated HCl followed by 100 ml ethanol and 50 ml glycerol with constant stirring. Make the volume to 500 mL.
- c) $\text{BaCl}_2 \cdot 2 \text{H}_2\text{O}$
- d) Standard sulphate solution: Dissolve 0.5434 g of AR grade K_2SO_4 in distilled water and dilute to 1 L. This is 100 ppm S solution.

Standard preparation : Pipette out 0, 0.5, 1.0, 1.5, 2.0, 2.5 ml of 100 ppm sulphur solution into 50 ml volumetric flask and to this add 5 ml of conditioning agent and a pinch of BaCl_2 . Make the volume to the mark to prepare the working standard of 0, 1, 2, 3, 4 and 5 ppm respectively. After 10 minutes, the turbidity developed in the standards is measured in a spectrophotometer at a wave length of 420 nm.

Procedure for sample preparation:

1. Take 5 g of soil into a 250 ml of conical flask.
2. To this add 25 ml of CaCl_2 (0.15 %) solution and shake for 30 min.
3. Filter through whatman No. 1 filter paper.
4. Pipette out 5 ml of extract into a 25 ml volumetric flask, add little amount of distilled water followed by 2.5 ml stabilizing agent and a pinch of BaCl_2 . Shake the contents and make up the volume to the mark with distilled water.

Calculations

Concentrations of S in the turbid solution = X ppm

i.e., 1 ml of the turbid solution contains X μg of S

25 ml of the turbid solution has = $25 \times X$ μg of S

→ present in 5 ml of soil extract

When 5 ml of soil extract contains = $25 \times X$ μg of S

25 ml extract contains = ?

$$= \frac{25}{5} \times 25 \times X \mu\text{g of S}$$

$$= 125 \times X \mu\text{g of S}$$

→ Present in 5 g soil

Available sulphur content in 1 g soil = ?

$$\frac{1}{5} \times 125 \times X \mu\text{g of S}$$

$$= 25 \times X \mu\text{g of S}$$

→ $\mu\text{g of S/g soil}$ is ppm

$$\text{Available S in ppm} = 25 \times X$$

$$\text{Available S in kg ha}^{-1} = \text{ppm} \times 2.25$$

$$= 25 \times X \times 2.25$$

Soil ratings for available sulphur

Soil rating	Available sulphur (ppm)
Deficient	< 10 ppm
Sufficient	> 10 ppm

Result: The available S content in the given soil is ----- ppm and is rated as -----.

Expt. No.: 7

Date:

ESTIMATION OF AVAILABLE ZN IN SOILS

In atomic absorption spectrometry radiation from an external light source, emitting the spectral lines that correspond to the energy required for an electronic transition from the ground state to an excited state is passed through the flame. The flame gases are treated as a medium containing free unexcited atoms capable of absorbing radiation from an external source when the radiation corresponds exactly to the energy required for a transition of the test element from the ground electronic state to an upper excited level. Unabsorbed radiation then passes through a monochromator that isolates the exciting spectral line of the light source and into a detector. The absorption of radiation from the light source depends on the population of the ground state which is proportional to the solution concentration sprayed into the flame. Absorption is measured by the difference in transmitted signal in the presence and absence of the test element.

Instrumentation

All atomic absorption spectrometry units have the following basic features in common.

- 1) A light source that emits resonance line radiation i.e., hollow cathode lamp
- 2) A sampling cell, usually a flame, into which the sample is fed as an aerosol
- 3) A monochromator that is used to isolate the absorbing resonance line from other non-absorbing lines.
- 4) A detector that measures the amount of absorption.
- 5) Electronic amplification of the absorption signal.
- 6) A read out system that normally is a strip chart recorder, a digital display, a meter, or a printer.

It is an established fact that DTPA is the most useful chelate to use as an extractant for simultaneous measurement of available Zn, Fe, Mn and Cu.

The method employs DTPA as a chelating agent. Since Fe deficiencies are more prevalent in calcareous soils the extractant is designed to avoid excessive dissolution of CaCO_3 with the release of occluded Fe. This is done by buffering the extractant in a slightly alkaline pH

range and including soluble Ca. Triethanalamine (TEA) is used as a buffer because it burns cleanly during use in AAS.

In the extracting solution, DTPA is fully associated with Ca. At the selected pH of 7.3, approximately three fourths of the TEA is protonated. Upon addition of the extractant to soils, additional Ca and Mg go into solution largely because the protonated TEA exchanges with Ca and Mg from soil exchange sites. This exchange usually increases the concentration of ionic Ca by two to three times and suppresses the dissolution of CaCO_3 in calcareous soils.

Zinc standard solution : Dissolve 0.439 g AR grade $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in 200 mL glass distilled water in a beaker. Add 5 mL of 1:5 H_2SO_4 . Transfer to 1 L volumetric flask and make the volume to the mark to have a solution of 100 ppm Zn. Dilute this solution 10 times to obtain 10 ppm solution. Prepare the working standards from this solution.

Extraction Procedure

1. Weigh 10 g of air dried soil into a 150 ml conical flask.
2. Add 20 ml of the DTPA extraction solution.
3. Cover each flask with polyethylene stoppers.
4. Shake the contents for 2 hrs on a horizontal shaker.
5. After 2 hours of shaking, filter the suspensions by gravity through Whatman No.42.
6. The filtrate is analysed for the trace metals using AAS.

Expt. No.: 8

Date:

ASSESSMENT OF QUALITY OF IRRIGATION WATER (A) PH (B) EC

Water is the most universal solvent and all the naturally occurring sources bound to containing variable quantities of dissolved solids and gases and sometimes suspended organic and inorganic colloids materials.

- Different types of laboratory tests of water are carried out depending on its use from the point of industry, public health and agriculture.
- Water considered as a poor quality for industries or domestic purpose may be quite suitable for irrigation.
- In general the total soluble salts, nature and concentration of various ions, the proportion of monovalent cations which has specific relevance in judging the quality of water is taken into consideration.

Quality of water is an important consideration in any appraisal at salinity or alkali conditions in an irrigated area.

- Salinity is one of the most important criteria for assessing quality of irrigation water especially in arid and semi arid region.
- Ground water contributes to about 40 % of all irrigation.
- The quality of irrigation water depends on the amount and kind of salt dissolved in it.

Harmful effects of poor quality irrigation water

- If water containing excessive soluble salts is applied to the field, soil turns saline or alkaline.
- Harmful effects due to poor quality water are due to increased osmotic pressure of soil solution, thus inhibiting uptake of water and nutrients from soil to plant roots.
- Excessive Na adsorbed by clay causes deflocculation destroys the soil structure resulting in poor circulation of air and water in the soil.

Classification of irrigation water (or) criteria for judging the quality of irrigation water :

I. Classification based on electrical conductivity

C₁ : Low salinity water can be used for most crops. Electrical conductivity ranges from 0 to 250 $\mu\text{ mho cm}^{-1}$ (or) 0 to 0.25 dS m^{-1}

C₂ : Electrical conductivity of water ranges from 250-750 $\mu\text{ mho cm}^{-1}$ or 0.25 – 0.75 dS m^{-1} , medium salinity water can be used with moderate leaching.

C₃ : 750 – 2250 $\mu\text{ mho cm}^{-1}$ (or) 0.75 – 2.25 dS m^{-1} . High salinity water and this water cannot be used on soil with restricted drainage.

C₄ : > 2250 $\mu\text{ mho cm}^{-1}$ (or) > 2.25 dS m^{-1} very high salinity water not suitable for irrigation.

II. Classification based on the SAR (Sodium Adsorption Ratio)

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

S₁ : Low Na water can be used on all soils with little danger of development of normal level of exchangeable Na. SAR < 10.

S₂ : SAR = 10-18. Medium Na water, can produce hazards with fine textured soils which have high CEC especially with low leaching conditions.

S₃ : SAR = 18-26. High Na water, may produce harmful level of exchangeable Na in most soils. It requires special management practices such as good drainage, high leaching and organic matter additions. Exchangeable Na should be replaced by the use of chemical amendments.

S₄ : SAR > 26; Very high Na water not suitable for irrigation this water can be used with the addition of gypsum or other amendments.

III. Classification based on residual sodium Carbonate (RSC)

$$\text{RSC in me L}^{-1} = (\text{CO}_3^{2-} + \text{HCO}_3^-) - (\text{Ca}^{2+} + \text{Mg}^{2+})$$

- ✓ Irrigation waters with RSC < 1.25 water can be used safely.
- ✓ Irrigation waters with RSC between 1.25 and 2.5 can be used with certain management.
- ✓ Irrigation waters with RSC > 2.5 are not suitable for irrigation.

IV. Classification based on 'B' concentration

Boron class	Boron concentration (ppm)			Remarks
	Sensitive crops	Semi tolerant crops	Tolerant crops	
Very low	< 0.33	< 0.67	< 1.0	Can be used safely
Low	0.33 – 0.67	0.67 – 1.33	1.00 – 2.00	Can be used with management
Medium	0.67 – 1.0	1.33 – 2.0	2.0 – 3.0	Unsuitable for irrigation
High	1.0 – 1.25	2.0 – 2.5	3.0 – 3.75	
Very high	> 1.25	> 2.5	> 3.75	

V. Classification based on chloride content (me L^{-1})

Chloride concentration
(me L^{-1})

Water quality

< 4

Excellent water

4-7

Moderately good water

7-12

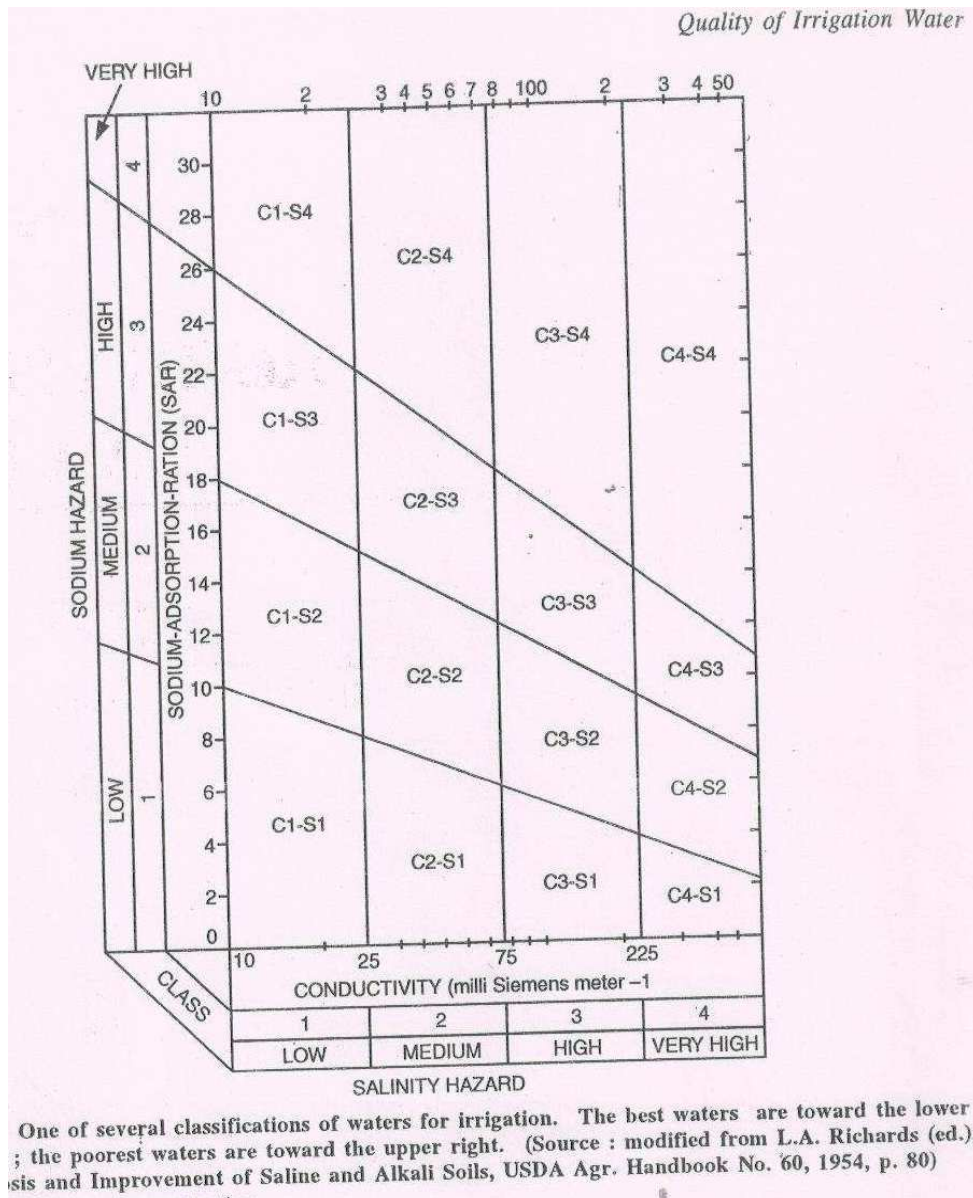
Slightly usable

12-20

> 20

}

Not suitable for
irrigation purpose



Collection of irrigation water sample

- ❖ The sample of irrigation water collected is about 500 ml.
- ❖ The sample should be collected in glass or plastic bottle with stopper and preferably transparent.
- ❖ The container must be thoroughly cleaned and should be rinsed several times with water from which samples are to be drawn.
- ❖ The source of irrigation water may be a tank, river, canals; sample should be drawn from the spots, away from the sides or from the mid stream.

- ❖ This can be easily managed with help of a small bucket tied at the end of a long pole.
- ❖ In case of tube well, either fitted with a hand pump or electric motor, the water should run for about 10 min prior to sampling.
- ❖ In case of open well, several buckets of water have to drawn out first and then the sample is collected.
- ❖ After the water sample is collected, it should be labelled properly giving details like name of farmer, well number, survey number of the field, village, mandal and the district.
- ❖ Sample then should be immediately sent into the laboratory to avoid any change or deterioration.

Laboratory analysis

- ❖ After receiving the water sample in the laboratory, it should be entered into a register taking details given on the label.
- ❖ Then water should be analyzed (as soon as the water is received, it should be analysed for carbonate and bicarbonate content).

Physical properties

- ❖ Physical properties of water to be analyzed are colour, odour, and suspended materials.
- ❖ When the water contains suspended material it should be filtered through ordinary filter paper, and water is analyzed for chemical groups.

Chemical properties

pH, electrical conductivity, Cl^{-1} , CO_3^{-2} , HCO_3^{-} , Ca^{+2} and Mg^{+2}

I) ESTIMATION OF pH OF IRRIGATION WATER SAMPLE

The pH of irrigation water is an important property. For saline irrigation water, determination of pH is not very important since the presence of neutral salts keep the pH of water at around 7.0. However, the pH of bicarbonate waters is usually more than 7.5 and its determination may reflect the degree of sodicity in the sample.

Principle:

The pH is defined as the negative logarithm of the hydrogen ion concentration.

$$\text{pH} = -\log_{10}[\text{H}^{+}]$$

For pH below 7.0, the H^+ concentration exceeds OH^- concentration and the range is acidic. When the OH^- concentration is more than H^+ ; pH lies between 7 and 14 and the range is alkaline. The pH is a sort of voltage measurement and to cover the entire range of 0 – 14, a potential measurement in the range of + 420 to – 420 mV is needed since a potential difference of 59.1 mV is developed for a difference of one unit pH.

Reagents : Buffer solutions (pH 4.0, 7.0, 9.2)

Apparatus : 100 ml beaker, a measuring cylinder, pH meter and an ordinary filter paper.

Procedure

1. Take 50 mL of irrigation water sample into a 100 ml beaker.
2. Switch on the pH meter and allow for 20 minutes for warm up.
3. Calibrate the instrument using the buffer solutions of pH either 4.0 or 7.0 or 9.2.
4. Rinse the glass and calomel electrodes or combined electrode with distilled water and lower them back into a beaker of distilled water.
5. After rinsing, wipe off the electrodes dry with a filter (or) tissue paper.
6. Lower the electrode into the water sample.
7. Record the pH of the sample.

Result : pH of the irrigation water sample is -----.

II) DETERMINATION OF ELECTRICAL CONDUCTIVITY OF IRRIGATION WATER SAMPLE

EC is determined by electrical conductivity meter.

Principle : The electrical conductivity of irrigation water is measured with a conductivity meter known as salt bridge (or) 'Solu' bridge. It is based on the principle of wheat stone bridge in which alternate current is used instead of direct current to eliminate the polarization of electrodes and to prevent electrolysis of the solution. Two fixed resistances R_1 and R_2 and a variable resistance R_v are connected on a branched circuit with the conductance as having resistance R_x . The variable resistance R_v is adjusted till no current passes and the reading is recorded. R_v calibrated in terms of resistance or conductance.

Apparatus : 100 ml – beaker, a measuring cylinder, a glass rod, a conductivity meter.

Procedure

1. Take the irrigation water sample into a beaker.
2. Connect the salt bridge to the power supply, switch on the bridge and adjust it to the room temperature with the help of temperature setting knob.
3. Rinse the conductivity cell with distilled water and then with the sample.
4. Dip the conductivity cell on the irrigation water sample in such a way that the platinum electrodes are completely immersed in the solution.
5. Record the Electrical conductivity of the sample.

Result : EC of the irrigation water sample is ----- dS m^{-1} . The water comes under ----- class.

Expt. No.: 9

Date:

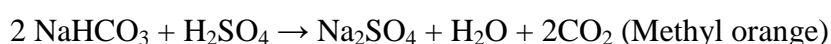
**ESTIMATION OF CARBONATES, BICARBONATES AND CHLORIDES IN
IRRIGATION WATER SAMPLE**

I. Estimation of carbonates and bicarbonates in irrigation water

Aim : To estimate carbonates and bicarbonates in irrigation water sample.

Principle:

Carbonates and bicarbonates in irrigation water sample can be determined by titrating against standard H_2SO_4 (0.1 N). When phenolphthalein indicator is added to the water sample, pink color appears if carbonates are present. Then the contents are titrated against standard sulphuric acid till color disappears. That means the carbonates are converted to bicarbonates. The standard acid utilized for conversion of CO_3^{2-} to HCO_3^- is equal to the acid needed for conversion of HCO_3^- to CO_2 and H_2O . Hence, the titre value of carbonates will be obtained by multiplying the titre value of phenolphthalein with two. When one or two drops methyl orange indicator is added to the same contents, straw yellow color appears, which then be titrated against standard sulphuric acid till the color changes to rose red. Here, the bicarbonate derived from carbonates and the bicarbonates originally present in the sample are completely neutralised to CO_2 and H_2O . So, the titre value for HCO_3^- is obtained by subtracting the phenolphthalein titre vale from methyl orange titre value.



Glassware : Burette, pipette and conical flask

Reagents:

- 1) H_2SO_4 (0.1 N) : Dilute 2.8 mL of conc. Sulphuric acid to 1L and standardize against 0.1 N Na_2CO_3
- 2) Phenolphthalein: Dissolve 0.25 g of indicator powder in 100 mL of 60 % ethanol
- 3) Methyl orange indicator: Dissolve 0.5 g of indicator powder in 100 mL of 95% ethanol

Procedure :

- ❖ Pipette out 10 ml of irrigation water sample into a conical flask.
- ❖ Add 1 or 2 drops of phenolphthalein indicator; the solution will turn pink.

- ❖ Titrate the contents against the 0.1 N H_2SO_4 mounted in burette; the end point is indicated by the disappearance of pink colour. Record the burette leading.
- ❖ If on addition of phenolphthalein the sample does not turn pink, it indicates the absence of CO_3^{2-} . In such case add methyl orange indicator to the same aliquot and titrate against H_2SO_4 till the straw yellow colour changes to rose red.
- ❖ To the same contents, add 2-3 drops of methyl orange indicator; solution turns straw yellow colour.
- ❖ Again titrate the contents against H_2SO_4 mounted in burette. The end point of titration is indicated by the development of rose red colour. Note down the reading of burette.
- ❖ Repeat the titrations till two consecutive and concurrent titre values are obtained.

Observations

S.No.	Aliquot taken (ml)	Burette readings					
		Phenolphthaleis			Methyl orange		
		Initial	Final	TV (x) Final - Initial	Initial*	Final	TV (Y) Final - Initial
1.	10						
2.	10						
3.	10						

* Final burette reading of phenolphthalein becomes the initial burette reading for methyl orange.

Titre value with phenolphthalein = X mL of 0.1 N H_2SO_4 .

Titre value for carbonates = 2X mL of 0.1 N H_2SO_4

Titre value with methyl orange = Y mL of 0.1 N H_2SO_4

Titre value for bicarbonates = Y – X mL of 0.1 N H_2SO_4

Calculations:

$$\text{TV} \times \text{N} \times 1000$$

$$\text{me/L} = \text{-----}$$

V

TV= Titre value

N = Normality of standard solution

V = Volume of sample taken for titration

Result

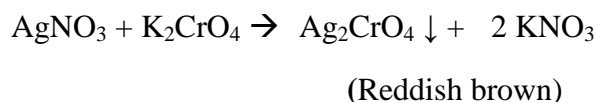
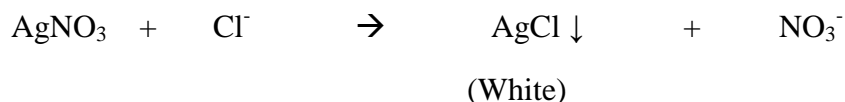
Irrigation water sample contains CO_3^{-2} and HCO_3^- to the extent of -----
and ----- m e L^{-1} .

II) Estimation of chloride content in irrigation water sample

Aim : To find out the amount of Cl^- present in the irrigation water sample by Mohr's method.

Principle :

Chloride ions present in irrigation water sample can be determined by titrating standard AgNO_3 using potassium chromate, as indicator, the silver of AgNO_3 first reacts with Cl^- in the irrigation water sample forming AgCl precipitate. Later, Ag^+ starts reacting with chromate ion (CrO_4^{-2}) to form a Reddish brown precipitate of Ag_2CrO_4 . Among AgCl and Ag_2CrO_4 , the solubility of AgCl is low hence it precipitates out first later Ag_2CrO_4 . The very first drop of AgNO_3 which forms Ag_2CrO_4 is the end point of the titration.



Apparatus : Burette, Pipette, Porcelain basin and glass rod.

Reagents

- (a) AgNO_3 (0.01 N): Dissolve 1.7 g of AgNO_3 in distilled water and make the volume to 1 lit.
- (b) K_2CrO_4 (5 %) - Dissolve 5 g of K_2CrO_4 in 100 ml solution.

Procedure :

- ✓ Pipette out 10 ml of irrigation water sample into a porcelain basin.
- ✓ To the contents, add 1 or 2 drops of K_2CrO_4 indicator and stir with a glass rod.
- ✓ Titrate the contents against standard $AgNO_3$ mounted in the burette till reddish brown tinge appears.
- ✓ Record the titre value and repeat the titrations till consecutive and concurrent titre values are obtained.

Observations

S.No.	Aliquot taken	Burette readings		TV mL (Final - Initial)
		Initial (ml)	Final (ml)	
1	10 ml			
2	10 ml			
3	10 ml			

TV for chlorides = ----- ml of 0.01 N $AgNO_3$

Calculations

$$\frac{TV \times N \times 1000}{V} = \text{me/L}$$

TV= Titre value

N = Normality of standard solution

V = Volume of sample taken for titration

Result : The given irrigation water sample contains chlorides to the extent of -----
----- meq L^{-1} .

Expt. No.: 10

Date:

ESTIMATION OF Ca AND Mg IN IRRIGATION WATER SAMPLE

Principle

This method was outlined by Schwarzenbach in 1946 using EDTA as chelating agent or complexing agent

To the irrigation water containing metallic cations, if the indicator is added either in the form of powder or as a solution, it combined with the cations forming a cation - indicator complex and imparts certain colour to the solution. When the solution is titrated against strong standard chelating agent like EDTA (ethylene diamine tetra acetic acid) solution, the cation indicator complexes are dissociated forming stable complex of cation with EDTA releasing the indicator free in the solution, which expresses a characteristic colour which is taken as the end point.

Magnesium forms insoluble $\text{Mg}(\text{OH})_2$ precipitate at pH 12.0 and allows only calcium to be titrated and to increase the pH to 12.0 NaOH buffer is used. Both calcium and magnesium can be titrated at a pH 10.0 using eriochrome Black T-as indicator. To increase the pH to 10.0 ammonia buffer is used.

Apparatus and Glassware Required : Burette stand, Burette (50 mL), Pipette 5 mL , China dish (125 mL) and glass rod.

Reagents Required

1. EDT A 0.01 N : Dissolve 2 g of EDT A and 0.05 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (AR) in volumetric flask of one litre capacity and make up the volume with distilled water. Standardise the solution against 0.01 N CaCl_2 solution using each of the indicators, EBT and ammonium purpurate separately.
2. Standard CaCl_2 0.01 N : Dissolve 5 g of pure CaCO_3 (calcite) in 10 ml of approximately 3N (1+3) HCl and add 100 mL of distilled water and boil to expel CO_2 . The boiling should be done by covering the beaker with a watch glass. Rinse the watch glass and dilute to a volume of exactly one litre. This solution remains stable for many days.

3. Ammonium purpurate (Murexide) indicator: Grind very thoroughly 0.5 g of ammonium purpurate with 100 g of powdered K_2SO_4 (AR) in an agate mortar and pestle till it becomes fine powder. This should be stored in a ground glass stoppered bottle.
4. Eriochrome black T (EBT) indicator: Dissolve 0.5 g EBT and 4.5 g of Hydroxylamine hydrochloride in 100 mL of 95% methanol. Store in a ground glass stoppered bottle.
5. NaOH approximately 4 N or 16% : Dissolve 160 g NaOH (AR) in one litre of solution and store in ground glass bottle.
6. Ammonium chloride-ammonium hydroxide buffer: Dissolve 67.5 g NH_4Cl (AR) in 570 mL of NH_4OH (AR) and make up to one litre.

Procedure

Estimation of Calcium

1. Pipette 10 mL of water sample into a dry clean 125 mL China dish.
2. Dilute with distilled water to about 25 mL.
3. Add 1 mL (15 drops) of 4N NaOH solution.
4. Add approximately 50 mg of ammonium purpurate powder. Stir the contents. The content will become vine red in colour.
5. Titrate the contents against standard EDTA (0.01 N) solution until the colour changes to violet. Since the colour change is not instantaneous, the EDTA at the end point should be added slowly at the rate of one drop for every 5 to 10 seconds. At the end point, addition of a drop of EDTA should not change the colour. To judge the end point accurately, keep a blank titrated for comparison.
6. If the sample is over titrated with EDTA, it may be back titrated with 0.01 N $CaCl_2$ till the desired end point obtained.
7. Note the volume of EDTA and repeat the titrations till consecutive and concurrent titre values are obtained.

II. Estimation of Calcium plus Magnesium

1. Pipette 10 mL of water sample in a dry clean 125 mL China dish.
2. Dilute approximately to 25 mL with distilled water.
3. Add 0.5 mL (10 drops) of $\text{NH}_4\text{Cl-NH}_4\text{OH}$ solution.
4. Add 3 to 4 drops of EBT indicator. The colour changes to vine red.
5. Titrate against standard EDTA (0.01 N), till the colour changes to sky blue. Note the titre value (B mL).

Observations

For Ca :

S.No.	Aliquot taken	Burette readings mL		TV (X)
		Initial	Final	Final - Initial
01	10 ml			
02	10 ml			
03	10 ml			

Titre value for calcium = **X** mL of 0.01 N EDTA

Calculations

$$\frac{\text{TV} \times \text{N} \times 1000}{V}$$

me/L=-----

$$\frac{\text{TV} \times \text{Fx} \times 1000}{V}$$

g/L=-----

TV= Titre value

N = Normality of standard solution

V = Volume of sample taken for titration

F=Factor = 1ml of 0.01N EDTA solution is chemically equivalent to 0.0002g Ca

Or

0.00012g Mg

For Ca + Mg:

S.No.	Aliquot taken	Burette readings mL		TV (Y)
		Initial	Final	Final - Initial
01	10 ml			
02	10 ml			
03	10 ml			

Titre value for Ca + Mg = Y mL of 0.01 N EDTA

Titre value for Mg = Titre value of (Ca + Mg) – Titre value of Ca = Y - X

Calculations

$$\text{me/L} = \frac{\text{TV} \times \text{N} \times 1000}{\text{V}}$$

$$\text{g/L} = \frac{\text{TV} \times \text{Fx} \times 1000}{\text{V}}$$

TV= Titre value

N = Normality of standard solution

V = Volume of sample taken for titration

F=Factor = 1ml of 0.01N EDTA solution is chemically equivalent to 0.0002g Ca

Or

0.00012g Mg

Result : The irrigation water sample contains calcium and magnesium to the extent of

	Strength	Ca	Mg
1.	g L ⁻¹		
2.	me L ⁻¹		

Expt. No.: 11

Date:

ESTIMATION OF POTASSIUM AND SODIUM CONTENTS IN IRRIGATION WATER

i) Estimation of sodium content in irrigation water

Aim : To estimate the K and Na contents in the irrigation water sample.

Principle : Flame photometer is based on the principle of flame emission spectroscopy. Atoms / ions when subjected to high temperature, their valence electrons jump to high energy level absorbing energy, the absorbed energy is emitted as radiation energy of discrete wavelengths, characteristic of each element when the e^- returns to its ground state, the intensity of radiation depend upon concentration of atoms in the solution. The emitted energy in the forms of photons is detected by phototube and amplified by amplifier and measured with galvanometer.

Reagents :

1. Standard solution of Na (1000 ppm). Dissolve 2.54 g of NaCl in 1 L of solution.
2. Irrigation water sample.
3. Working standards of Na.

The working standards are prepared from stock solution as follows.

Working standard (ppm)	Volume prepared (100 ml)	Stock solution	Volume of stock solution required (ml)	Flame photometer reading
0	100 ml	100 ppm	0	0
10	100 ml	100 ppm	10	
20	100 ml	100 ppm	20	
30	100 ml	100 ppm	30	
40	100 ml	100 ppm	40	100

Preparation of standard graph

Working standards are aspirated to the instrument. Flame photometer reading is adjusted to zero with 0 ppm and to 100 with the highest standard. A curve is plotted with the concentration of working standards on X-axis and flame photometer reading on Y-axis.

Procedure : Aspirate the irrigation water sample to the flame and record the flame photometer reading.

Calculations

Concentration of Na in irrigation water sample = X ppm

i.e., 1000 mL of irrigation water sample contains X mg of Na

1000 mL of irrigation water contains $1000 \times X \mu\text{g}$ of Na

$$\text{me L}^{-1} = \frac{\text{mg L}^{-1}}{\text{Eq. Wt. of Na}}$$

$$\text{meq / lit} = \frac{\text{mg L}^{-1}}{23}$$

Since, equivalent weight of sodium is 23.

Result : The given irrigation water sample contains sodium to the extent of -----
----- me L⁻¹ of Na.

ii) Estimation of potassium content in irrigation water

Aim : The estimate the potassium content in the given irrigation water sample.

Principle : Flame emission spectroscopy.

Reagents :

1. Standard solution of K (1000 ppm). Dilute 1.908 g of KCl to 1 L of water.
2. Irrigation water sample
3. Working standards of K.

The working standards are prepared from stock solution as follows.

Working standard (ppm)	Volume prepared (100 ml)	Stock solution	Volume of stock solution required (ml)	Flame photometer reading
0	100 ml	100 ppm	0	0
10	100 ml	100 ppm	10	
20	100 ml	100 ppm	20	
30	100 ml	100 ppm	30	
40	100 ml	100 ppm	40	100

Working principle of flame photometer : Flame photometer is based on the principle of flame emission spectroscopy. Atoms / Ions when subjected to high temperature, their valency i.e. jump to the high energy level absorbing energy. The absorbed energy is emitted as radiation energy of discrete wavelength characteristics of each element when there is returns to ground state, the intensity of radiation depend upon concentration of atoms in the solution, the emitted energy in the forms of photons is detected by phototube and amplified by amplifier and measured with galvanometer.

Procedure : The irrigation water sample (1 ml) is aspirated to the flame and the flame photometer reading is noted down.

Preparation of standard graph : Working standards are aspirated to the instrument a curve is plotted with the concentration of working standards on x-axis flame photometer reading on y-axis.

Calculations :

Concentration of K in the irrigation water sample = X ppm

1 L of the sample consists of X mg K

$$\text{Concentration of K in } \text{mg L}^{-1} = \frac{\text{mg L}^{-1}}{\text{eq. wt. of K}} = \frac{X}{39}$$

Result : The given irrigation water sample contains potassium to the extent of -----
----- mg L^{-1} .

Expt. No.: 12

Date:

Quick tests and interpretation of soil tests and irrigation water analysis data -

Determination of lime requirement of acid soil

i) Quick tests: Rapid chemical tests are analogous to chemical tests.

Rapid plant tissue tests:

Rapid tests for the determination of nutrient elements in the plant sap of fresh tissue. In these tests, the sap from ruptured cells is tested for unassimilated nitrogen, phosphorus and potassium. They are semiquantitative tests mainly intended for verifying or predicting deficiencies of N, P or K. The underlying assumption of the rapid tissue test is that the adequate supply of an element is indicated by an abundance of it in the plant sap and vice versa. The results are read as low, medium or high. Through the proper application of tissue testing it is possible to anticipate or forecast certain production problems which still in the field. The concentration of the nutrients in the cell sap is usually a good indication of how well the plant is supplied with nutrients at the time of testing.

(1) Plant Part to be selected: In general the conductive tissue of the latest mature leaf is used for testing.

(2) Time of Testing: The most critical stage of growth for tissue testing is at the time of bloom or from bloom to early fruiting stage. Nitrates are usually higher in the morning than in the afternoon if the supply is short.

Test for nitrates : Diphenylamine test:

Reagent :

Diphenyl amine (1%): Dissolve 1 g of Diphenyl amine powder in 100 mL of conc. H₂SO₄ and store in glass stoppered dropper bottle. Prepare the solution freshly. This reagent is very corrosive.

Procedure:

1. Cut a very thin vertical section of the specified plant part freshly in the field.
2. Add 2 or 3 drops of the diphenyl amine reagent on the freshly cut section.
3. Note blue colour intensity after 30 sec.
4. Results can be interpreted.

Interpretation of the observation

Color intensity	Level of nutrient and inference	Recommendation
No blue color	The plant is severely deficient in NO_3^- - N	There is urgent need for the application of N fertiliser
Light blue	Plant is deficient in NO_3^- - N	Need to apply N
Medium blue	Plant is slightly deficient	N fertilization may be given to increase the yield
Dark blue	Plant is abundant in NO_3^- - N	No need of N application.

B. Test for phosphorus: The test is molybdate reagent test. The leaf base without mid rib is cut into small pieces. About one tea spoon full of leaf tissues is taken into a test tube followed by 10 mL of molybdate reagent. The contents are shaken vigorously for one minute. The extract is collected into another test tube. One crystal of stannous chloride (SnCl_2) is added and shaken well and the color is observed. The interpretation is made as follows

Color	Level of P in plant and inference	Recommendation
No color	The plant is highly deficient in P	There is urgent need for application of P fertilizer.
Greenish blue	Plant is medium in P level	Yields can be increased by the application of P fertiliser
Medium blue	Plant is adequate in P	No need of application of P fertilisers
Dark blue	Plant is abundant in P	No need of P application.

C). Test for potassium : The test is Sodium cobalti nitrite test. The leaf tissue is cut into small pieces with a sharp knife. About one tea spoon full of the tissue is taken into a test tube. To this add about 10 mL of reagent and shake thoroughly for one minute. Take 5 mL of the extract into another test tube and add 5 mL of ethanol. After few minutes observe the turbidity in the extract at 20°C temperature.

Turbidity	Level of K in the plant or inference	Recommendation
Less t	Plant is deficient in K	Need for K application
Medium	Plant is doubtful in K sufficiency	K fertilisers may be applied to increase the yield.
Dense or thick	The plant is adequate in K	No need for K fertilisation.

Soil tests

Tests for $\text{NO}_3^{-1} - \text{N}$:

Soil and Morgan's reagent are taken in 1:2 ratio. Shake the contents well and filter. Take one drop of extract on a spot plate and add 4 drops of diphenyl amine. Mix with a glass rod and observe the intensity of blue color

Color	Level of nutrient or inference	Recommendation
No blue	The plant is severely deficient in $\text{NO}_3^{-1} - \text{N}$	There is urgent need to apply N fertilisers.
Medium blue	Plant is slightly deficient in $\text{NO}_3^{-1} - \text{N}$	N fertilisation may be given to increase the yield
Dark blue	Plant is abundant in $\text{NO}_3^{-1} - \text{N}$	No need of N fertilisation.

Test for phosphate:

The test is molybdate reagent test. Take about a half tea spoonful of soil. Add 10 mL of molybdate reagent. Shake the contents well for one minute. Collect the extract in a test tube and add a crystal of stannous chloride. Observe the intensity of blue color

Color	Level of P in soil	Recommendation
No color	The soil is highly deficient in P	There is urgent need for the application of P
Greenish blue	Plant is deficient in P	Need for application of P
Light blue	Plant is medium in P	Application of P may be done to increase the yields.
Medium blue	Plant is adequate in P	No need of application of P
Dark blue	Plant is abundant in P	No need of P application

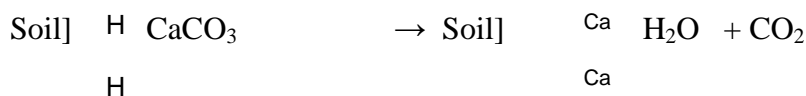
Test for potassium: The test is sodium cobalti nitrite test. Take about one tea spoonful of soil into a test tube and add 10 mL of the reagent. Shake the contents for one minute and filter the contents. Add 2.5 mL of Iso propyl alcohol at 20° C and shake well. Observe the turbidity in the extract.

Turbidity	Level of K in the soil	Recommendation
Less turbid	Soil is deficient in K	Need for K application
Medium in turbidity	Soil is doubtful in K sufficiency	K fertiliser may be applied.
Dense or thick	Soil is adequate in K	No need of application of K

ii) Determination of lime requirement of acid soil

Acid soil is one whose pH is less than 6.5. But according to USDA, the soils having less than a pH of 5.5 are practically considered as acid soils. Reclamation of acid soils by liming is

usually practised for keeping the soil pH in the optimum range essential for making nutrients available in appropriate quantities. The amount of lime or liming material required depends on the nature of the soil. Through liming (addition of lime), the exchangeable H^+ of soil is neutralised. The $CaCO_3$ equivalence of the exchangeable H of the soil is called “lime requirement of soil”. On liming, Ca^{+2} and Mg^{+2} replace both H^+ and Al^{3+} from the colloidal surface. By way of replacement of H^+ and Al^{3+} , the Ca^{2+} raises the percentage base saturation, which leads to a corresponding raise in soil pH.



Aim : To estimate the lime requirement of the given acid soil by adopting buffer method proposed by Shoemaker *et al*, 1961.

Apparatus : pH meters, balance, beaker, glass rod.

Reagents :

1. Extractant pH buffer solution:

Dissolve 1.8 g of nitrophenol, 2.5 ml of Triethanol amine, 3 g of potassium chromate, 2 g of calcium acetate and 53.1 g of calcium chloride dehydrate in 800 mL of distilled water, adjust the pH to 7.5 and make up the volume to 1 L.

2. pH buffer solutions of pH 4.0, 7.0 and 9.2

Procedure

1. Weigh 5 g of air dry soil in 50 ml beaker.
2. Add 5 ml of distilled water and 10 mL of extractant buffer solution.
3. Stir the contents continuously for 10 minutes or intermittently for 20 minutes with a glass rod.
4. Measure the pH of the soil buffer suspension with the pH meter after standardising with known buffer solutions of pH 4.0, 7.0 and 9.2.
5. Against the measured pH, find out the lime required to bring the soil pH to a desired level (eg., 6.0, 6.4 or 6.8) as given in the table.
6. Make necessary corrections to get the value of agricultural lime based on purity percentage.

Lime requirement in t ha⁻¹ of pure CaCO₃ for different pH targets

Measured pH of soil buffer suspension	Lime requirement in t ha ⁻¹ of pure CaCO ₃ for		
	pH 6.0	pH 6.4	pH 6.8
6.7	2.43	2.92	3.40
6.6	3.40	4.13	4.62
6.5	4.37	5.35	6.07
6.4	5.59	6.56	7.53
6.3	6.56	7.78	8.99
6.2	7.53	8.99	10.21
6.1	8.50	10.21	11.66
6.0	9.48	11.42	13.12
5.9	10.69	12.64	14.58
5.8	11.66	13.85	15.79
5.7	12.64	15.07	17.25
5.6	13.61	16.28	18.71
5.5	14.58	17.50	20.17
5.4	15.79	18.71	21.63
5.3	16.77	19.93	22.84
5.2	17.98	20.90	24.30
5.1	18.95	22.11	25.76
5.0	19.93	23.33	27.22
4.9	20.99	24.54	28.67
4.8	22.11	25.76	30.13

Results : The lime requirement of the given acid soil is ----- t ha⁻¹.

Expt. No.: 13

Date:

DETERMINATION OF GYPSUM REQUIREMENT OF ALKALI SOIL

Alkali soils contain excessive amounts of sodium and alkalinity (CO_3^{2-} & HCO_3^-). Their soil Na saturation is sufficiently high to interfere with the growth of several crops. To improve their productivity, these soils have to be reclaimed with the immediate objectives of neutralisation of excessive alkalinity and replacement of Na on the exchange complex with Ca in order to improve the physico-chemical and biological properties of the alkali soils. These objectives can be achieved by suitable amendment. Gypsum, being the cheapest and easily available, is commonly used as reclaiming material. The gypsum requirement of soils is determined by using the method proposed by **Schoonover (1952)**.

Aim : To estimate the gypsum requirement for reclamation of the alkali soils

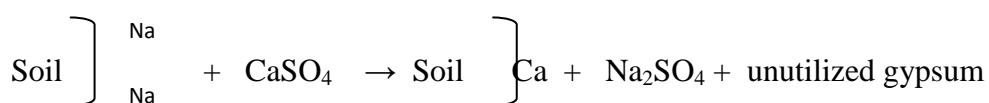
Apparatus required : Conical flask, burette and stand, pipette, filter paper, china dish, glass rod and mechanical shaker etc.

Chemicals required :

1. Saturated gypsum solution : Add 5 g of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ to 1 L of distilled water. Shake manually several times over a period of 1 hr for 10 minutes on a mechanical shaker and filter.
2. Eriochrome Black T indicator: Dissolve 0.5 g of Eriochrome Black T indicator and 4.5 g hydroxyl amine hydrochloride in 100 mL of 95 % ethanol.
3. $\text{NH}_4\text{Cl} - \text{NH}_4\text{OH}$ buffer: Dissolve 67.5 g of NH_4Cl in 570 mL NH_4OH and dilute to 1 L.
4. EDTA solution (0.01 N) : Dissolve 2 g of di sodium salt of ethylene diamine tetra acetate and dilute to 1 L. Standardise this solution against 0.01 N calcium chloride solution.

Principle:

A known weight of the soil is shaken with a known excess volume of nearly saturated gypsum solution. The unutilised calcium left out in the solution is determined by titrating against standard EDTA solution using EBT indicator in the presence of $\text{NH}_4\text{Cl} - \text{NH}_4\text{OH}$ buffer. The amount of gypsum utilised / required is calculated from the titre value.



Micelle Gypsum Sodium in the
 (saturated) sulphate extract

Procedure

- Weigh 4 g of air dried soil into a 250 mL Erlenmeyer flask.
- Add 100 ml of saturated gypsum solution to remove the Na from exchangeable complex to bring down to a reasonable level.
- Shake for 5 minutes on mechanical shaker or by hand for several times for 30 min
- Filter the contents with whatman No.1 filter paper.
- Take 5 ml of extract into a china dish.
- Dilute to approximately to 25 mL with distilled water.
- Add 1 ml of $\text{NH}_4\text{Cl} - \text{NH}_4\text{OH}$ buffer solution followed by 2 to 3 drops of EBT indicator.
- Titrate against the 0.01 N EDTA solution till the color changes from purple to blue.
- The titre value will give the volume of the EDTA required to react with the unutilized gypsum.
- Also run a blank titration with 5 mL of saturated gypsum solution to know the blank titre value.

Observations

S.No.	Aliquot taken	Burette readings		TV mL Final - Initial
		Initial (mL)	Final (mL)	
Blank	5 ml			B
Sample	5 ml			S

Blank titre value = B mL of 0.01 N EDTA

Sample titre value = S mL of 0.01 N EDTA

Actual titre value = $B-S$ ml

Calculations :

- Wt of the soil taken = 4 g
- Volume of saturated gypsum added = 100 ml
- Volume of 0.01 N EDTA required to react with 5 ml of Gypsum solution = **B** ml of 0.01 N EDTA
- Volume of EDTA required to react with unutilized gypsum solution = **S** ml of 0.01 N EDTA
- Volume of EDTA required to react with utilized gypsum = (**B-S**) ml 0.01 N EDTA

As per the law of equilibrium

- 1000 ml of 1N EDTA = 86 g of gypsum

(B-S) ml of 0.01 N EDTA = ?

$$= \frac{(B-S) \text{ ml} \times 0.01}{1000} \times 86 \text{ g of gypsum}$$

$$\text{➤ 5 ml of extract requires} = \frac{(B-S) \text{ ml} \times 0.01}{1000} \times 86 \text{ g of gypsum}$$

100 ml extract → ?

$$= \frac{(B-S) \times 0.01}{1000} \times 86 \times \frac{100}{5} \text{ g of gypsum}$$

$$= (B - S) 0.0172 \text{ g gypsum}$$

(B - S) 0.0172 g gypsum is required for 4 g soil

1 kg soil requires → (B - S) 0.0172 x 1000/4 g of gypsum

2.25×10^6 kg soil requires $\rightarrow ?$

$$= \frac{2.25 \times 10^6}{1} \times \frac{(B - S) 0.0172 \times 1000}{4} \text{ g of gypsum}$$

$$= \frac{2.25 \times 10^6}{1} \times \frac{(B - S) 0.0172 \times 1000}{4} \times 10^{-6} \text{ t of gypsum}$$

$$= 9.675 \times (B - S)$$

Result : The given alkali soil require ----- t ha⁻¹ of gypsum for its reclamation.

Expt. No.: 14

Date:

**COLLECTION AND PROCESSING OF PLANT SAMPLE FOR ANALYSIS –
ESTIMATION OF N CONTENT IN PLANT SAMPLES**

Collection and processing of plant samples

1. For analysis of seasonal crop plants, pick up few representative plants at random from each plot. Remove the shoot (aerial part) with the help of a sharp stainless steel knife or blade or scissors for whole shoot analysis or the desired part for analysis of specific plant parts.
2. If roots are to be included, uproot the whole plant carefully from the soil, retaining even the fine or active roots. Gently dip the plant roots in water several times to remove adhering soil as far as possible.
3. Wash the plant parts with water several times.
4. Wash the sample thoroughly with about 0.2 % detergent solution to remove the waxy / greasy coating on the leaf surface, which is often present.
5. Wash with 0.1 N HCl followed by thorough washing with plenty of water. Give final rinse with distilled water.
6. Rinse with double distilled water, specially if micronutrient analysis is to be carried out.
7. Keep the sample with good quality tissue paper.
8. Air dry the sample on a perfectly clean surface at room temperature for atleast 2-3 days in a dust free atmosphere away from any kind of contaminants.
9. Place the samples in an electric oven and dry at 60°C for 48 hours.
10. Powder the dried plant samples in rustless mortar and pestle.

II) ESTIMATION OF N IN PLANT SAMPLES

Aim : To determine the nitrogen content in the given plant sample.

Materials required : Kjeldplus (microkjeldhal apparatus), Bunsen burner, glassware etc.

Procedure

i) Digestion of plant sample for N

1. Take 0.1 g of the powdered plant sample into a 100 mL microkjeldahl flask .
2. Add the 3 ml of concentrated H₂SO₄ to the plant sample.

- Kept it for 24 hours for cold digestion. The contents turn char.
- Place the flask on the flame of a bunsen burner till the contents start boiling.
- Take it out and add slowly 1 mL of H_2O_2 (30%) along the walls of the flask and again place the flask on flame.
- Repeat the steps 4 and 5 till the contents turn into clear transparent solution.

ii) Distillation of the digest for N content

- Transfer the digest into a kjeldahl distillation flask quantitatively.
- Place a 250 ml conical flask containing 25 ml of 4 % boric acid with mixed indicator at the end of delivery tube; care is taken to see that the tip of the tube is completely dipped into boric acid;
- Tap water is run through condenser unit.
- Into the contents of the kjeldhal flask, run 15 ml of NaOH (40 %) automatically and keep the flask in place.
- Start the distillation process and continue until about 10 ml of distillate is collected into the conical flask. The end of the distillation can be determined by using red litmus paper. It takes 9 minutes with 'kjelplus' for the completion of distillation.
- After completion of distillation, take out the conical flask containing the distillate from the unit and titrate the contents against standard H_2SO_4 (0.01 N) till the bluish green colour turns pink.
- Run a blank distillation without the plant digest and note down the blank titre value.

Observations

Weight of the plant sample taken = 0.1 g

Blank titre value = B mL of 0.01 N H_2SO_4

Sample titre value = S mL of 0.01 N H_2SO_4

Actual titre value = (S – B) mL

Calculations

1000 mL of 1 N H₂SO₄ = 14 g N

$$(S - B) \text{ mL of } 0.01 \text{ N H}_2\text{SO}_4 = \frac{(S - B) 0.01 \times 14}{1000}$$

→ Present in 0.1 g plant sample

$$100 \text{ g plant sample contains} = \frac{(S - B) 0.01 \times 14 \times 100 \text{ g of N}}{1000 \times 0.1}$$

$$(S - B) \times 0.14 \quad \% \text{ N}$$

Result : Total N content of the plant sample is _____ % .

Expt. No.: 15

Date:

ESTIMATION OF P IN PLANT SAMPLES

Aim: Estimation of P content in the plant sample.

Digestion of the plant samples : For nutrients other than N, the plant material can be digested in a diacid mixture or a triacid mixture or dry ashed and dissolved in acid.

Diacid digestion : It is carried out using 5:1 of HNO_3 : HClO_4 if the sample is high in fats / oils, pre-digestion using 25 ml HNO_3 /g sample is recommended to avoid explosion.

Triacid digestion : It is carried out using a mixture of HNO_3 : H_2SO_4 : HClO_4 in the ratio of 9 : 4 : 1.

The sample digestion is carried out as described under

- Weigh 1 g ground plant material in 100 ml conical flask.
- To this add 10 ml of acid mixture and swirl the content of the flask gently.
- Keep for overnight for predigestion.
- Place the flask on low heat hot plate in a digestion chamber then heat the contents at higher temperature until the production of red N_2O fumes ceases.
- Evaporate the contents until 3 to 5 ml digest is obtained but not to dryness. The completion of digestion is confirmed when the liquid become colourless.
- After cooling the flask, add 20 ml of deionized or glass distilled water to the plant digest. Transfer the digest quantitatively into a 100 mL volumetric flask .
- Make up the volume with deionized water.
- Filtered through whatman No. 1 filter paper.
- Aliquots of this diluted digest are used for the determination of P, K, Ca, Mg, S, Fe, Mn, Zn and Cu.

Principle : When vanadomolybdate and phosphate radical reacts in nitric acid medium a heteropoly compound is formed which is yellow in colour. The intensity of yellow colour is measured in a spectrophotometer at 420 nm. The amount of P in the plant sample is determined from the absorbance values.

Reagents

1. Ammonium molybdate – ammonium vanadate in HNO_3 (Barton's reagent)

- Dissolve 22.5 g of ammonium molybdate in 400 ml of warm distilled water.
- Also dissolve 1.25 g of ammonium metavanadate in 150 ml of boiling water.
- Add the two solutions and cool to room temperature.
- Add 250 ml of concentrated nitric acid (HNO_3) and dilute to 1 L.

2. Standard P solution : Dissolve 0.2195 g of analytical grade KH_2PO_4 and dilute to 1 L.

This solution contains 50 $\mu\text{g P / mL}$.

Procedure

Preparation of standard curve:

- ✓ Transfer 0, 1, 2, 3, 4 and 5 mL of standard solution into 50 mL volumetric flasks and add 10 mL of vanado molybdate reagent to each flask. Make up the volume with distilled water to obtain 0, 1, 2, 3, 4 and 5 ppm P standards respectively. Read the absorbance after 30 minutes.
- ✓ Plot the absorbance (Y axis) against concentration of working standards (X axis). Linear calibration curve is established.

Sample preparation

- Pipette out 5 ml of plant digest into a 50 ml of volumetric flask.
- Add 10 ml of Barton's reagent, shake well and make up the volume.
- Allow to stand for 30 minutes for yellow colour development.
- Read the intensity of the colour at 420 nm in a spectrophotometer.
- Plot the absorbance value in the standard graph to obtain the concentration of P in the colored solution (X ppm).

Calcultaions

Concentration of P in colored solution = X ppm

i.e., 1 mL of colored solution contains $X \mu\text{g P}$

50 mL of colored solution contains $50 \times X \mu\text{g P}$

→ Which is present in 5 mL of the plant digest

100 mL of plant digest consists of $50 \times X \times 100/5 = X \times 1000 \mu\text{g P}$

→ Which is obtained from 1 g plant sample

100 g of plant sample consists of $X \times 1000 \times 100/1$

= $X \times 10^5 \times 10^{-6}$ g P

= $X \times 0.1\%$

Result : The P content in the given sample is _____. %

Expt. No.: 16

Date:

ESTIMATION OF POTASSIUM IN PLANT

Aim: To estimate the total **K** content in the plant sample.

Principle : When liquid samples containing potassium is burnt in the flame, K emits photons characteristic of its wave length. The intensity of the emission is proportional to the K content. By measuring the intensity in flame photometer the K content is determined.

Materials required : Flame photometer, volumetric flask (25 ml), pipette.

Preparation of working standards : Dissolve 1.907 g of AR grade KCl in 1 litre of distilled water. This gives 1000 ppm K. From this different working standards are prepared. Dilute 0, 1, 2, 3, 4 and 5 mL of the stock solution 100 mL to obtain 0, 10, 20, 30, 40 and 50 ppm K working standards.

Procedures

- Pipette out 5 ml of plant digest into a 25 ml volumetric flask and dilute to 25 mL with distilled water.
- Aspirate the standards and then the sample to the flame and note the meter reading and calculate the K content by referring to the standard curve prepared.

Calculations

Concentration of K in the plant digest = X ppm

1 ml of the plant digest $\rightarrow X$ μg of K

100 ml of the plant digest $\rightarrow ?$

$= 100 \times X$ μg of K

1 g of plant sample $\rightarrow 100 \times X$ μg of K.

100 g of plant sample $\rightarrow ?$

$= 100/1 \times 100 \times X$ μg of K.

$= X \times 10^4 \times 10^{-6}$ g K = $X \times 0.01$ %

Critical limits

< 1 % - Deficit.

1-5 % - Normal

> 5 % - Toxic.

Result : The given plant sample contains _____ % of K which is -----.
