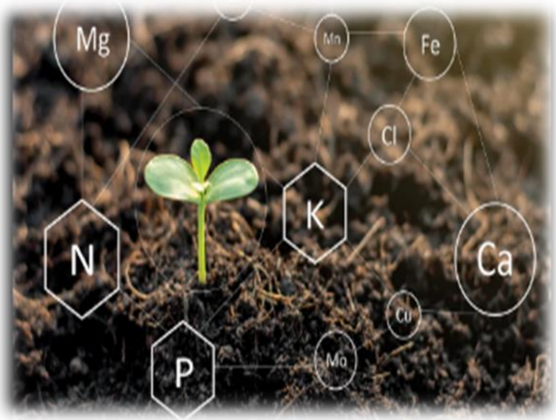
Manures, Fertilizers and Soil Fertility Management

Practical Manual

Course No.: CC-AGP-536 Credits: 3(2+1)



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Exercise No 1: Introduction of Analytical Instruments

Objective: Principles and application of the instruments

Analytical lab instruments encompass a wide range of instrumentation whose principle purpose is to qualitatively and quantitively analyze samples, the chemical makeup of a sample and the quantity of each component within a sample.

1. pH meter:

pH meter, electric device used to measure hydrogen-ion activity (acidity or alkalinity) in solution. Fundamentally, a pH meter consists of a voltmeter attached to a pH-responsive electrode and a reference (unvarying) electrode. The pH-responsive electrode is usually glass, and the reference is usually a mercury–mercurous chloride (calomel) electrode, although a silver–silver chloride electrode is sometimes used. When the two electrodes are immersed in a solution, they act as a battery. The glass electrode develops an electric potential (charge) that is directly related to the hydrogen-ion activity in the solution (59.2 millivolts per pH unit at 25 °C [77 °F], and the voltmeter measures the potential difference between the glass and reference electrodes. A pH meter provides a value as to how acidic or alkaline a liquid is. The basic principle of the pH meter is to measure the concentration of hydrogen ions. Acids dissolve in water forming positively charged hydrogen ions (H^+). The greater this concentration of hydrogen ions, the stronger the acid is.

2. Electrical Conductivity meter (EC meter):

The Electrical Conductivity (EC) of a solution is a measure of the ability of the solution to conduct electricity. The EC is reported in either meter. When ions (salts) are present, the EC of the solution increases. If no salts are present, then the EC is low, indicating that the solution does not conduct electricity well. The EC indicates the presence or absence of salts but does not indicate which salts might be present. The instrument is used for measuring conductivity is also known as conductivity bridge. A typical system consists of an alternating current Wheatstone bridge, a primary element of conductivity cell and a null balance indicator in the conductivity meter.

pH meter

EC meter

Basic Instrumental Analysis:

Colorimetry:

Colorimetry, measurement of the wavelength and the intensity of electromagnetic radiation in the visible region of the spectrum. It is used extensively for identification and determination of concentrations of substances that absorb light. Higher the concentration of the substance being determined, greater is the intensity of the colour. The intensity of colour is measured using an instrument called photoelectric colorimeter.

Photoelectric Colorimeter:

The photoelectric colorimeter is an instrument which helps to determine the extent of absorption of light of particular wavelength through a coloured solution. It consists of:

1) A light-source

2) Collimating lens

3) Filter to isolate the required band of wavelength

4) Sample holder/cuvette

5) Photoelectric cell – It convert light energy into electric energy

6) Colorimeter – measures the current output

Operations:

1) See that a proper filter is in position

2) Check whether the galvanometer needle is on central line of galvanometer scale. If not bring it to null position by adjusting the mechanical zero adjustment knob located on top of the instrument (without switching on the instrument)

3) Switch on the instrument (main lamp) and allow it to warm up for 30 minutes (after filling with blank or distilled water) in the holder. Clear the outside with tissue paper and place it in a sample holder.

4) Place the potentiometer needle at zero reading of the scale & see that galvanometer arresting switch (side switch) is in ON position

5) Adjust the reference knob (which adjust the slit across the reference beam) till the deflected needle is brought to null position

6) Switch off the galvanometer side switch remove the cuvette with reagent blank, clean it with distilled water & fill it (appr.5 ml) with standard solution, wipe the outer surface & place it in the instrument

7) The galvanometer side switch is ON & needle of galvanometer deflects from central line to right

8) Rotate the potentiometer knob (located in front of the instrument) anticlockwise until the deflected needle is brought back to central line / null position of the galvanometer. Note down the reading Switch OFF the galvanometer side switch & potentiometer knob is brought back to zero position.

9) Take further readings of all standards using above steps

10) When all the readings are over, clean the sample folder & fill it with distilled water. Before it is inserted, switch OFF the main lamp

11) Plot the readings in a graph paper & connect the points by a straight line (standard curve)

12) Find out concentration of sample from the standard curve

Flame Photometry:

Flame photometry (more accurately called Flame Atomic Emission Spectrometry) is a branch of spectroscopy in which the species examined in the spectrometer are in the form of atoms. A photoelectric flame photometer is an instrument used in inorganic chemical analysis to determine the concentration of certain metal ions among them sodium, potassium, calcium and lithium. Flame photometry is based on measurement of intensity of the light emitted when a metal is introduced into flame.

Essential parts of a Flame Photometer:

1) Pressure regulator for the fuel, gas &air

2) Atomizer: - Aspirates the solution & atomize it in the form of mist

3) Burner: - Produce desired flame in which atoms are excited

4) The optical system: - Collects light energy, renders it monochromatic by a filter or monochromator 5) Photosensitive detectors: - usually a barrier layer cell converts light energy into electrical energy

6) Galvanometer: - Measure electric output

7) The fuel gas ordinary LPG gas – air combination can excite only elements like Li, Na, and K while Ca, Mg requires higher flame temperature (3000^{o}C) for excitation and hence acetylene air combination is required.

Operating Flame photometer:

1) Switch ON the instrument, warm up to 30minutes

2) Insert proper filter

3) Start air compressor & adjust flow rate (0.48-0.6 kgcm^{2})

4) Place beaker containing distilled water for aspiration

5) Open gas cylinder & adjust gas / flow regulator, light the burner 5

6) Adjust the regulator to obtain a non-luminous flame with maximum blue cone

7) Aspirate the blank or distilled water

8) Adjust set zero knob of galvanometer to read zero

9) The most conc. standard is aspired first & reading is adjusted to 100 using sensitivity knob

10) Several standards of lower concentration are introduced & reading noted

11) Aspire the blank or distilled water, aspire the unknown solution and note down the reading.

** The conc. of unknown solution is calculated from the standard curve prepared with reading on x – axis & conc. on y – axis. Critically the curve should be straight line.

Steps to which off the Flame Photometer:

1. After aspiring all the samples turn OFF gas supply
2. Switch OFF galvanometer
3. Run distilled water & allow to flush out the atomizer & burner for sometimes
4. Remove the distilled water & allow only compressed air to flow
5. Switch OFF the compressor after 5 min to cool the instrument

Draw:

1. Photoelectric Colorimeter
2. Flame photometer

Exercise 2: Estimation of Soil Organic Carbon (Walkley and Black, 1934)

Principle:

Soil organic matter (SOM) is the seat of nitrogen in soil and its determination is often carried out as an index of nitrogen availability. Two major methods are generally followed: a) titration method (Walkley and Black, 1934) and b) colorimetry method (Datta et al., 1962). In both the methods, organic matter is oxidized with chromic acid (potassium dichromate + H_2SO_4). In the titration method, the unconsumed potassium dichromate is back-titrated against ferrous sulphate or ferrous ammonium sulphate (redox titration). Carbon in the sample is oxidized as follows:

$$2H_2Cr_2O_7 + 3C + 6H_2SO_4 = 3Cr_2(SO_4)_3 + 3CO_2 + 8H_2O$$

Thus, $2H_2Cr_2O_7$ or $2K_2Cr_2O_7 = 3C$

In the colorimetric method, the intensity of the green colour of chromic acid obtained due to its reduction is measured colorimetrically. This intensity is directly proportional to the amount of organic carbon present in the soil.

Reagents:

1. 1 (N) potassium dichromate: Dissolve 49.04g of $K_2Cr_2O_7$ in about 500 ml of distilled water and make the volume to one litre
2. Conc. Sulphuric acid
3. 0.5 (N) Ferrous Ammonium Sulphate- Dissolve 196g of ferrous ammonium sulphate in distilled water, add 20 ml of conc. H_2SO_4 and make the volume upto one litre.
4. Diphenylamine indicator- Dissolve 0.5g of the dye in a mixture of 20 ml of distilled water and 100 ml of conc H_2SO_4
5. Orthophosphoric acid (85%) or sodium fluoride

Procedure:

1. Weigh 1g of 0.2 mm soil sample into 500 ml dry conical flask of borosilicate glass
2. Add 10 ml of 1 (N) $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4
3. Swirl a little and keep on an asbestos sheet for 30 minutes
4. Add slowly 200 ml of distilled water and 10 ml of orthophosphoric acid
5. Add 1 ml of diphenylamine indicator
6. Add 0.5 (N) ferrous ammonium sulphate solution in 50 ml burette

7. Titrate the contents until green colour starts appearing
8. If the titre value is <6, repeat taking 0.2 to 0.5g of the sample

Calculation:

Organic carbon (%) in soil = 10 (B-S)/B X 0.003 X 100/wt. of the sample

Where, B and S stand for the titre value (ml) of blank and sample, respectively.

Result:

The organic carbon present in the soil is ___________%

Video Link:

https://www.youtube.com/watch?v=JCQxGqD--GE

Exercise 3: Estimation of Mineralizable Nitrogen in Soil (Subbiah and Asija, 1956)

Principle:

The easily mineralizable nitrogen is estimated using alkaline KMnO4, which oxidizes the organic matter present in the soil and hydrolyzes the liberated ammonia which is condensed and absorbed in boric acid, and titrated against standard acid. The method has been widely adopted to get a reliable index of nitrogen availability in soil due to its rapidity and reproducibility. The process of oxidative hydrolysis is, however, a progressive one and hence a uniform time and heating temperature should be allowed for best results. Use of glass beads checks bumping while liquid paraffin checks frothing during heating.

Apparatus:

a) Nitrogen distillation unit or Kelplus distillation unit.
b) Distillation tube, conical flasks, pipettes, burette etc.

Reagents:

1) 0.32% KMnO4: Dissolve 3.2 g of $KMnO_4$ in distilled water and make the volume to one litre.
2) 2.5% NaOH: Dissolve 25 g of sodium hydroxide pellets in water and make the volume to one litre.
3) 2% Boric acid: Dissolve 20 g of boric acid powder in warm water by stirring and dilute to one litre.
4) Mixed Indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 mL of ethyl alcohol. Add 20 mL of this mixed indicator to each litre of 2% boric acid solution.
5) 0.1M Potassium Hydrogen Phthalate: Dissolve 20.422 g of the salt in distilled water and dilute to one litre. This is a primary standard and does not require standardization.
6) 0.02M H_2SO_4: Prepare approximately 0.1M H_2SO_4 by adding 5.6 mL of conc. H_2SO_4 to about one litre of distilled water. From this, prepare 0.02M H_2SO_4 by diluting a suitable volume (20 mL made to 100 mL) with distilled water. Standardize it against 0.1M NaOH solution.

7) 0.1M NaOH: Dissolve 4g NaOH in 100 mL distilled water. Standardize against potassium hydrogen phthalate.

Procedure:

1) Measure 20 mL of 2% boric acid containing mixed indicator in a 250 mL conical flask and place it under the receiver tube. Carefully dip the receiver tube in the boric acid solution.
2) Take 2.5 g of soil sample in a 350mL distillation tube (run a blank without soil in a 350 mL distillation tube before started sample).
3) Moisten the soil with about 10 mL of distilled water, wash down the soil, if any, adhering to the neck of the flask.
4) Add 25 mL (Soil:0.32% $KMnO_4$::1:5) of 0.32% of $KMnO_4$ solution in the distillation tube manually or instrumentally.
5) Add a few glass beads or broken pieces of glass rod.
6) Add 2-3 mL of paraffin liquid, avoiding contact with upper part of the neck of the flask.
7) Add 25 mL of 2.5% NaOH solution and immediately attach to the rubber stopper fitted in the alkali trap.
8) Press the run switch and continue distillation until about 100 mL of distillate is collected.
9) First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
10) Titrate the distillate against 0.02M H_2SO_4 taken in burette until pink colour starts appearing.
11) Carefully remove the distillation tube and drain the contents in the sink.

**If brown colour not appearing in distillation tube at the time of distillation (after 6-7 min) then add 5-10 mL of 2.5% NaOH solution

Precautions:

a) Check the tap water and distilled water.
b) Dip the delivery tube end in the receiver containing standard boric acid solution before adding NaOH solution in the distillation flask.
c) Close the distillation tube to the distillation apparatus tightly then add NaOH to avoid the loss of ammonia.

d) During distillation, first remove the receiver flask and then distillation tube.

e) Collect about 100 mL of distillate in 10 minutes steady distillation.

Observation:

Sl No.	Initial burette reading (mL)	Final burette reading (mL)	mL of (0.02N H2SO4)

Calculation:

Mineralizable N (kg/ha) = Volume of acid used to neutralize ammonia in the sample = (A – B) mL

Where,

A = Volume of 0.02N H_2SO_4 used in titration of soil sample against ammonia absorbed in boric acid.

B = Volume of 0.02N sulphuric acid used in blank titration.

N= Normality of sulphuric acid

1 mL of 0.02N sulphuric acid = 0.56 mg N (1000 mL of 1N H_2SO_4 = 14 g Nitrogen).

****[Rating: Low = < 280 kg/ha, Medium= 280-560 kg/ha, High= > 560 kg/ha]**

Result:

The available nitrogen status of the soil is __________ kg /ha.

Video Link:

https://www.youtube.com/watch?v=l10NBPglZzI

Exercise 4: Estimation of Available Phosphorus in Acid Soil

Principle:

In soil, phosphorus exists in the form of various types of orthophosphates. A very small fraction of these is available to plants at a given time. Available phosphorus content of soil consists mainly of Ca-, Al-, and Fe-P. In the neutral and alkaline soils particularly, Ca-P is the dominant fraction. Organic -P fraction is also in considerable amount, but is usually not included in the determination of available phosphorus. A large number of extraction reagents from Dyer's 1% citric acid to some of the multi nutrient extractants, buffer solutions, acids and chelating agents have been suggested for available phosphorus from time to time. However, no single extractants appears to be suitable for all types of soils. Two types of extraction methods are more popularly adopted. Under acidic conditions, Bray's P-1 (or Bray No. 1), which involves soil extraction with a solution consisting of 0.03 (N) NH_4F and 0.025 (N) HCl is widely followed. The fluoride complexes Al and Fe in soil, thus releasing some bound P besides the easily acid soluble P (largely Ca-P). This extractant is suitable for soils containing less than 2% calcite or dolomite because in calcareous soils, carbonates quickly neutralize the acid, resulting in less extraction of P.

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

Apparatus and Instruments:

a) Spectrophotometer
b) Shaker
c) Pipettes
d) Beakers
e) Conical flasks
f) Volumetric flasks

Reagents:

1) Bray Extractant No 1 (0.03M NH_4F in 0.025M HCl): Dissolve 1.11 g of NH_4F (AR) in one litre of 0.025N HCl.

2) Dickman Bray's reagent (Molybdate reagent): Dissolve 15.0 g $(NH_4)_2MoO_4$ in 300 mL warm distilled water, cool and add the solution to 350 mL of 10 N HCl solution gradually with stirring. Dilute to one litre with distilled water.
3) Stannous chloride solution (40 % Stock Solution): Dissolve 10 g $SnCl_2.2H_2O$ in 25 mL of concentrated HCl. Add a piece of pure metallic tin and store the solution in a glass stoppered bottle.
4) Stannous chloride solution (Working Solution): Dilute 1 mL of the stock solution of stannous chloride to 66.0 mL with distilled after just before use. Prepare fresh dilute solution every working day.

Preparation of the Standard Curve:

Dissolve 0.439 g of pure dry KH_2PO_4 in about half a litre of distilled water. About 25 mL 7N H_2SO_4 is added and made up to one litre with distilled water. This solution contains 100 ppm stock solution of P (100 mg P /L). Preserve this as a stock standard solution of phosphate. From this, a 2 ppm P 12 solution is made. Take 0,1, 2, 3, 4, 5 and 6 mL of this 2ppm solution to 25 mL volumetric flasks. To these 5 mL of extracting reagent (Bray's) is added as described above by adding Bray's No. 1, 5 mL of Dickman Bray's reagent, 1 mL $SnCl_2$ and make the volume with distilled water and take reading as per sample readings.

Procedure:

a) Take 2.5 g of soil and 25 mL of the Bray's reagent (1:10 Soil: Solution) are shaken for 5 minutes in 250 mL conical flask and filtered.
b) Take 5 mL of the filtered soil extract with a bulb pipette in a 25 mL volumetric flask. To avoid interference of fluoride 7.5 mL of 0.8 M boric acid (50g H_3BO_3 per litre) can be added to 5 mL of the extract.
c) Add 5 mL of the molybdate reagent and add about 10 mL distilled water, shake and add 1 mL of the dilute $SnCl_2$ solution with a pipette. Make up the volume with distilled water and shake thoroughly.
d) Read the blue colour after 10 minutes on the spectrophotometer at 660 nm wavelength after setting the instrument to zero with the blank prepared similarly.

**Phosphorus is determined spectrophotometrically by Dickman and Bray's (Dickman and Bray, 1940) method.

Exercise 5: Estimation of Available Phosphorus in Alkaline soil

Principle:

The other and most widely used extractant is the 0.5 (M) $NaHCO_3$ solution at pH 8.5. The reagent is most suitable for neutral to alkaline soils and is designed to control the ionic activity of calcium through solubility product of $CaCO_3$, thus extracting the most reactive forms of P from Al-, Fe-, and Ca- phosphates. Phosphorus in the extract can be determined using suitable method of colour development and measuring the colour intensity at an appropriate wavelength.

Olsen's Method (Olsen et al., 1954)

Apparatus and Instruments:

a) Spectrophotometer
b) Shaker
c) Pipettes
d) Beakers
e) Conical flasks
f) Volumetric flasks

Reagents:

a) 0.5 M $NaHCO_3$: Dissolve 42 g Sodium bicarbonate in 1 litre of distilled water and adjust the pH to 8.5 by addition of dilute NaOH or HCl. Filter it, if necessary.
b) Activated carbon –Darco G 60 or P free charcoal.
c) Molybdate reagent: Same as for the Bray's Method No. 1 except that use 400mL of 10 N HCl instead of 350 mL/litre.
d) Stannous chloride solution: Same as in Bray's Method No. 1.

Preparation of the Standard Curve:

Dissolve 0.439 g of pure dry KH_2PO_4 in about half a litre of distilled water. About 25 mL 7 N H_2SO_4 is added and made up to 1 litre with distilled water. This solution contains 100 ppm stock solution of P (100 mg P /L). Preserve this as a stock standard solution of phosphate. From this, a 2 ppm P solution is made. Take 0,1, 2, 3, 4, 5 and 6 mL of this 2-ppm solution to 25 mL volumetric flasks. To these 5 mL of extracting reagent (Olsen's) is added as described above by adding, 5 mL of molybdate reagent (for Olsen's method), 1 mL $SnCl_2$ and make the volume with distilled water and take the colour reading on the spectrophotometer at 660 nm wavelength. Plot the absorbance reading against P mg/L and prepare the standard curve by adjoining points.

Procedure:

1) Weigh 2.5 g soil sample in a 250 mL conical flask.
2) Add 1-2 g of Darco G 60 or P free charcoal and 50 mL of the bicarbonate extractant (0.5M $NaHCO_3$, pH8.5).
3) Shake for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
4) Transfer 5 mL of filtrate in to 25 mL volumetric flask and gradually add 5 mL of ammonium molybdate containing 400 mL of 10 N HCl.
5) Stir slowly and carefully to drive out the CO_2 evolved.
6) After stop of bubbliness, add distilled water washing down the sides and bring the volume to 22 mL
7) Add 1 mL of freshly prepared diluted $SnCl_2$, shake a little and make up the volume.
8) Run a blank without soil in similar manner.
9) Read the blue colour after 10 minutes on the spectrophotometer at 660 nm wavelength (or red filter for colorimeter) after setting the instrument to zero with the blank prepared similarly.

Precautions:

a) Clean all glassware with distilled water.

b) Start filtration quickly after shaking

c) Before taking reading, warm up the instrument minimum 30 min.

d) Take reading after 10 min.

Observation:

Sl No.	Spectrophotometer reading (Abs)

Calculation:

Available P (kg/ha) = R × dilution factor × 2.24

Where,

R = ppm P in the sample (read from standard curve)

Dilution factor = 50/2.5 × 25/5 = 100 (Weight of the soil taken = 2.5 g,

Volume of the extract = 50 mL;

Volume of the aliquot taken for estimation = 5 mL and

Volume made for estimation (dilution = 5 times) = 25 mL)

Rating:

P kg/ha	Low	Medium	High
Bray's Method	< 30	30-60	>60
Olsen's Method	< 15	15-30	>30

Conversion factor: P × 2.29 = P2O5

Result:

The available phosphorus status of the soil is _______ kg /ha

Video link:

https://www.youtube.com/watch?v=y2FsPH5ZDXY

https://www.youtube.com/watch?v=R1lFrMjoraE

Exercise 6: Estimation of available Potassium in Soils (Jackson, 1973)

Principle:

Potassium in soil exists as water soluble, exchangeable, non-exchangeable (fixed) and lattice-K. The first two forms constitute only a small part, normally not more than 1% of the total content and are considered to be easily available to plants. On amount basis, the exchangeable form (K^+ ions adsorbed on exchange sites) far exceeds water-soluble fraction and the two are in equilibrium with each other. Only when these two forms are depleted, a part of the non-exchangeable K moves to the exchange sites and soil solution. This movement is a slow process and not sufficient to meet K requirement of plants. Therefore, most of the methods suggested are based on the determination of easily available fractions *ie.* water soluble and exchangeable K. attempts, however have been made to include a part of the non-exchangeable fraction in certain methods to increase the prediction value. Thus, methods for both available and non-exchangeable k have been in use. For all practical purpose, the extraction and estimation of exchangeable K along with water soluble K is good enough. The ammonium ions are very close in size to K^+ and replace the latter efficiently. During the estimation, accurate burns clearly and does not leave any residue on the burner of the flame photometer.

Instruments:

a) Flame Photometer
b) Glass electrode pH meter

Reagents:

1) 1(N) ammonium acetate solution: Dissolve 77.08 g of ammonium acetate ($NH_4C_2H_3O_2$) in about 800 mL of distilled water and adding to its 57 mL of glacial acetic acid and 68 mL of ammonium solution (sp.gr. 0.91) followed by dilution to one litre and adjusting pH 7.0 after cooling.
2) Standard potassium solution: Dissolve 1.908 g pure KCl (oven dried) in one litre of distilled water. This solution contains 1000 mg K/L. Dilute suitable volumes of this solution to get 100mL of working standards containing 0, 5, 10, 15, 20, 25 and 30 mg K/L. The working standards should be making up the volume with ammonium acetate solution.

Procedure:

a) Weigh 5 g of soil sample in 250 mL conical flask and add 25 mL of 1 N ammonium acetate solution and shake for 5minutes.
b) Filter through Whatman No. 1 filter paper.
c) Measure K concentration in the filtrate flame photometer.

Preparation of the Standard Curve:

Set up the flame photometer by atomizing 0 and 20 mg K/mL solutions alternatively to 0 and 100 reading. Atomize intermediate working standard solutions and record the readings. Plot these readings against the respective potassium contents and connect the points with a straight line to obtain a standard curve.

Precautions:

1. Start filtration quickly after shaking

2. Before taking reading, warm up the instrument minimum 30 min

3. Check the flame level

Observation:

Sl No.	**Flame photometer Reading (ppm)**

Calculation:

K (kg/ ha) = R × dilution factor × 2.24

Where,

Dilution factor = 25/5 (1:5:: Soil : Solution)

R = content of K (mg) in the sample, as read from the standard curve.

****[Rating (K kg/ha): Low = < 120 kg/ha, Medium= 120-280 kg/ha, High= > 280 kg/ha Conversion factor: %K × 1.2047 = %K2O]**

Result:

The available potassium status of the soil is ___________ kg /ha.

Compiled and prepared by Dr Madhurima Banik and Mr Sourav Mullick

Video link:

https://www.youtube.com/watch?v=IxK_I-Q2e8w

Exercise 7: Estimation of available Ca and Mg in Soils

Principle:

The method described here was developed by Lavkulich (1981) for standard analysis of a wide range of soil types. It involves fewer steps than some other similar methods such as that of Mc Keague (1978). Problems with this approach to measuring exchangeable cations and CEC have been discussed extensively in the literature (Chapman 1965; Bache 1976; Rhoades 1982; Thomas 1982) but we agree with the conclusion of Thomas (1982) that "there is no evidence at the present time that cations other than NH_4^+ give results that are less arbitrary than those obtained using NH_4^+." Errors due to the dissolution of CaCO3 and gypsum will result in an excess of Ca^{2+} being extracted by NH4+ and a decrease in the amount of NH_4^+ retained due to competition between Ca^{2+} and $NH4^+$ during equilibration in the saturating step. In soils containing these minerals, exchangeable Ca will be too high and total CEC too low. The former problem cannot easily be corrected (Thomas 1982); however, more accurate measurement of CEC in this type of soil can be obtained by using the method described by Rhoades (1982). Fixation of K^+ and $NH_4{}^+$ in phyllosilicates can result in either an over- or underestimation of exchangeable K^+ when $NH_4{}^+$ is used as an extractant depending on whether the $NH_4{}^+$ moves through the interlayer positions replacing the K^+ or whether it causes the collapse of the edges preventing further exchange. Compared to the other methods presented in this chapter, this method uses a larger sample size, which helps to decrease the sample to sample variability. Another advantage of this procedure is that there are no decantation steps that can cause the loss of sample, particularly in the case of organic soils. The method described below can be used to measure either exchangeable cations and CEC or just exchangeable cations. In the latter case, the sum of exchangeable cations (including Al) could be 26 used as an estimate of CEC. Due to the high pH of the extracting solution, the amount of Al measured will usually be lower than that displaced by $BaCl_2$ or KCl.

1. **Calcium by Versenate (EDTA) method:**

Apparatus:

a) Shaker
b) Porcelain dish
c) Beakers
d) Volumetric/conical flask

Reagents:

a) Ammonium chloride: Ammonium hydroxide buffer solution: Dissolve 67.5 g ammonium chloride in 570 ml of conc. ammonium hydroxide and make to 1 litre.
b) Standard 0.01N calcium solution: Take accurately 0.5 g of pure calcium carbonate and dissolve it in 10 ml of 3N HCl. Boil to expel CO_2 and then make the volume to 1 litre with distilled water.
c) EDTA solution (0.01N): Take 2.0 g of versenate, dissolve in distilled water and make the volume to 1 litre. Titrate it with 0.01N calcium solution and make necessary dilution so that its normality is exactly equal to 0.01N.
d) Muroxide indicator powder: Take 0.2 g of muroxide (also known as ammonium purpurate) and mix it with 40 g of powdered potassium sulphate. This indicator should not be stored in the form of solution, otherwise it gets oxidized.
e) Sodium diethyl dithiocarbamate crystals: It is used to remove the interference of other metalions.
f) Sodium hydroxide 4 (N): Prepare 16% soda solution by dissolving 160 g of pure sodium hydroxide in water and make the volume to 1 litre. This will give pH 12.

Procedure:

1) Take 5 g air dried soil sample in 150 ml conical flask and add 25 ml of neutral normal ammonium acetate. Shake on mechanical shaker for 5 minutes and filter through Whatman No.1 filter paper.
2) Take a suitable aliquot (5 or 10 ml) and add 2-3 crystals of carbamate and 5 ml of 16% NaOH solution.
3) Add 40-50 mg of the indicator powder. Titrate it with 0.01N EDTA solution till the colour gradually changes from orange red to reddish violet (purple). It is advised to add a drop of EDTA solution at an interval of 5 to 10 seconds, as the change of colour is not instantaneous.
4) The end point must be compared with a blank reading. If the solution is over titrated, it should be back titrated with standard calcium solution and exact volume used is thus found.
5) Note the volume of EDTA used for titration.

Observation:

Sl. No.	Initial burette reading (mL)	Final burette reading (mL)	Final volume (mL)

Calculation:

If N_1 is normality of Ca^{2+} and V_1 is volume of aliquot taken and N_2V_2 are the normality and volume of EDTA used, respectively, then

$N_1V_1 = N_2V_2$

Or, $N_1 = N_2V_2/V_1$ =Normality of EDTA X Volume of EDTA/ml of aliquot taken

Hence, N_1 (Normality) = equivalent of Ca^{2+} present in one litre of aliquot

Hence, Ca^{2+} (me/litre) = Normality of EDTA X Volume of EDTA X 1000/ ml of aliquot taken

When expressed in soil weight basis,

Ca^{2+} me/100g soil = 100/wt. of soil X extract volume/1000 X Ca as me/litre

Result:

The Ca content in soil is ______ me/100 g soil.

Video link:

https://www.youtube.com/watch?v=hTy9JBllUVg

2. Calcium plus Magnesium by Versenate (EDTA) method

Magnesium in solution can be titrated with 0.01N EDTA using Eriochrome black T dye as indicator at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer. At the end point, colour changes from wine red to blue or green. When calcium is also present in the solution this titration will estimate both calcium and magnesium. Beyond pH 10 magnesium is not bound strongly to Erichrome black-T indicator to give a distinct endpoint.

Apparatus:

a) Shaker
b) Porcelain dish
c) Beakers
d) Volumetric/conical flask

Reagents:

1) EDTA or Versenate solution (0.01N): Same as in calcium determination.
2) Ammonium chloride ammonium hydroxide buffer solution: Same as in calcium determination.
3) Eriochrome black T indicator: Take 100 ml of ethanol and dissolve 4.5 g of hydroxyl amine hydrochloride in it. Add 0.5 g of the indicator and prepare solution.
4) Hydroxylamine hydrochloride removes the interference of manganese by keeping it in lower valency state (Mn2+). Or mix thoroughly 0.5 g of the indicator with 50 g of ammonium chloride.
5) Sodium cyanide solution (2%) or sodium diethyl dithiocarbamate crystals: This is used to remove the interference of copper, cobalt and nickel.

Procedure:

a) Take 5 g air dried soil in 150 ml flask, add 25 ml of neutral normal ammonium acetate solution and shake on a mechanical shaker for 5 minutes and filter through Whatman No.1 filter paper.
b) Pipette out 5 ml of aliquot containing not more than 0.1 me of Ca plus Mg. If the solution has a higher concentration, it should be diluted.
c) Add 2 to 5 crystals of carbamate and 5 ml of ammonium chloride-ammonium hydroxide buffer solution. Add 3-4 drops of Eriochrome black-T indicator.

d) Titrate this solution with 0.01N versenate till the colour changes to bright blue or green and no tinge of wine-red colour remains.

Calculation:

If N_1 and V_1 are normally (concentration of Ca^{2+} and Mg^{2+}) and volume of aliquot taken and N_2V_2 are the normality and volume of EDTA used respectively, then

$N_1V_1 = N_2V_2$

Or, $N_1 = N_2V_2/V_1$ = Normality of EDTA X Volume of EDTA/ ml of aliquot taken

Here, N_1 (Normality) = equivalents of Ca^{2+} plus Mg^{2+} me/litre = Normality of EDTA X Volume of EDTA X 1000/ ml of aliquot taken

Milliequivalent (me) of Mg^{2+} = me (Ca^{2+} + M^{2+}) – me of Ca^{2+}

When expressed on soil weight basis

Ca^{2+} + Mg^{2+} me/100g soil = 100/wt. of soil X extract volume/ 1000 X Ca^{2+} + Mg^{2+} me/litre

Observation:

Sl No.	**Initial burette reading (mL)**	**Final burette reading (mL)**	**Final volume (mL)**

Result:

The Ca + Mg content in soil ________ me/100 g soil

Video link:

https://www.youtube.com/watch?v=yDGMgYwHoVA

Exercise 8: Estimation of Available Sulphur in Soils

Principle:

Besides some amount in the soil solution, available sulphur in mineral soils mainly adsorbed SO42- ions. Both CaCl2 and phosphate solutions (as monocalcium phosphate) are generally used for replacement of the adsorbed SO42-. Use of Ca salts have a distinct advantage over those Na or K, as Ca prevents deflocculation in heavy textured soils and leads to easy filteration. SO42- in the extract can be estimated turbidimetrically using a colorimeter/ spectrophotometer/ autoanalyzer. A major problem arises when the amount of extracted sulphur is too low to be measured precisely.

Apparatus and Instruments:

a) Spectrophotometer
b) Mechanical Shaker
c) Conical flasks
d) Volumetric flask

Reagents:

1) 0.15% $CaCl_2$: Take 1.5 g $CaCl_2$ in a volumetric flask and makeup volume 1000 mL mark with distilled water.
2) Sodium acetate acetic acid buffer (pH 4.8) [CH_3COONa-CH_3COOH]: 1000 mL volumetric flask add 100 g sodium acetate, add distilled water 500 mL to mixed, add 30 mL 99.5% acetic acid, shake to dissolved sodium acetate the makeup volume 1000 mL mark.
3) 0.25% Gum acacia solution: Dissolve 0.25g of chemically pure gum acacia powder in 100 mL of hot water and filter in hot condition through Whatman No.42 filter paper. Cool and and keep in refrigerator.
4) Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
5) 100 ppm S solution: Take 0.5434 g of K_2SO_4 and add 500mL distilled water to dissolved then make up to 1000 mL mark of volumetric flask

Procedure:

a) Weigh 5 g soil sample in a 250 mL conical flask.

b) Add 25 mL of 0.15%$CaCl_2$.
c) Shake with 230 rpm for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
d) Transfer 10 mL of filtrate in to 25 mL volumetric flask and gradually add 10 mL of sodium acetate acetic acid buffer.
e) Add 1 g $BaCl_2.2H_2O$ powder and shake well.
f) Then add 1mL gum acacia and make up the volume with distilled water.
g) Run a blank without soil in similar manner.
h) Measure the turbidity intensity at 440 nm (blue filter).
i) Run a blank side by side.

Preparation of standard curve:

Put 0, 1.25 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 mL of the working standard solution (10 mg S/litre) into a series of 25 mL volumetric flasks to obtain 0, 0.5, 1.0, 2.0,3.0, 4.0, 5.0 and 6.0 ppm. Develop turbidity as described above for sample aliquots. Read the turbidity intensity and prepare the curve by plotting readings against sulphur concentrations.

Precautions:

1) Before taking reading, warm up the instrument minimum 30min
2) Take reading within 30min

Observation:

Sl No.	**Spectrophotometer (Abs)**

Calculation:

Available S in soil (mg/kg) = R × 25/5 × 25/10 = R × 12.5

Compiled and prepared by Dr Madhurima Banik and Mr Sourav Mullick

Where,

R stands for the quantity of sulphur in mg as obtained on X-axis against an absorbance reading (Y-axis) on standard curve.

5 is the weight of the soil sample in g and 25 is the volume of the extractant in mL. 10 is the volume of filtrate solution in mL in which turbidity is developed and make up the volume to 25mL.

Result:

The available sulphur status of the soil is **_________** mg/kg or ppm.

Video link:

https://www.youtube.com/watch?v=8FsnaWA1Jp4

Exercise 9: Estimation of DTPA extractable Zinc in Soils

Principle:

Diethylene triamine penta acetic acid (DTPA), a chelating agent, combines with free metal ions in solution and forms soluble complexes. Due to the reduced ionic activity in solution desorption takes place, bringing in some more ions from solid phase. DTPA offers the most favourable combination of stability constants for the simultaneous complexing of Zn, Cu, Fe and Mn. Since, Fe and Zn deficiencies are frequently experienced in calcareous soils, the method is designed to avoid excessive dissolution of $CaCO_3$ with the release of occluded micronutrients, which are normally not available to plants. This is achieved by: a) the inclusion of soluble calcium and b) buffering the reagent the reagent at pH 7.3 with triethanolamine (TEA) which burns clearly during flame atomization. When the extractant is added to soil, additional Ca^{2+} and Mg^{2+} enter the solution. This is largely because the protonated TEA exchanges with the ions from the exchange sites and this leads to the increased ionic concentration of Ca^{2+} in the solution, which in turn helps in suppressing the dissolution of $CaCO_3$. DTPA extractant has the ability to chelate Zn, Cu, Fe and Mn in competition with Ca^{2+} and Mg^{2+}.

Apparatus:

a) Atomic Absorption Spectrophotometer
b) Mechanical shaker
c) Centrifuge,
d) Beaker
e) Pipette
f) Volumetric flask
g) Conical flask
h) Funnel

Reagents:

a) DTPA 0.005 M solution
b) TEA 0.1 M (AR or extra pure) solution
c) $CaCl_2.2H_2O$ (AR) 0.01M
d) Dilute HCl (1:1) AR diluted with double distilled water

The extracting reagent is prepared by taking 1.967 g of DTPA, 1.470 g, $CaCl_2.2H_2O$ and 13.3 mL of TEA in 100 mL of glass or double distilled water and dilute approximately to 900 mL. Adjust the pH of the solution to 7.3 by adding dilate HCl (1:1), while stirring make the volume of the extracting solution to one litre. The solution remains stable for several months.

Preparation of 100 ppm standard solutions:

Element	Atomic weight	Micro nutrient salt	Molecular weight	Quantity of salt in g for 1 litre solution
Zn	54.38	$ZnSO_4.7H_2O$	287.56	0.4398
Cu	63.54	$CuSO_4.5H_2O$	249.69	0.39259
Mn	57.94	$MnCl_2.4H_2O$	197.69	0.3602
Fe	55.85	$FeSO_4.7H_2O$	278.02	0.4977

From the above 100 ppm solution a 10ppm working standard solution is prepared for Mn, Cu and Zn by taking 10 mL of 100 ppm solution in a 100 mL volumetric flask and making the volume 100 mL with the help of glass distilled water. From the 10ppm solution take 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL in 50 mL volumetric flask and make up the volume. This will give 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ppm of respective micronutrient solution. For iron take direct 0, 1, 2, 3, 4, 5, 10 and 12 mL of 100 ppm solution in 50 mL volumetric flask and make up the volume with glass distilled water, this will give 0, 2, 4, 6, 8, 10, 16, 20 and 24 ppm iron solution.

Procedure:

a) Take 10 g of soil in a 250 mL conical flask and add 20 mL DTPA extracting solution toit and stopper it well.
b) Shake the conical flask for 120 minutes, using mechanical shaker or centrifuge it using centrifuge tube and filter the contents through Whatman No. 42 filter paper.
c) Prepare a blank by adding all the solution except soil by shaking and filtration.
d) Take reading directly by AAS, using acetylene gas and hollow cathode lamp of the element (nutrient) which is to be determined in AAS.
e) Feed the blank solution of 0 ppm and adjust the zero in AAS then feed 0, 1, 2 and 4ppm of standard solution of particular element.

f) Feed the sample extracts one by one into AAS and note the readings in ppm.

Calculation:

1. Weight of soil = 10 g

2. Volume of extractant = 20 mL

Dilution factor (d. f.) = Volume of extractant/ Weight of soil = 20/10 = 2

Ppm of micronutrient in soil = R (ppm of sample reading) X d. f.

= R X 2

Precautions:

a) Use only glass distilled water or double distilled water for the determination of micronutrients
b) Soil sample should be either ground in wooden pestle and mortar or stainless-steel grinder.
c) The glassware used should be of high quality i.e. either corning or borosil.
d) The glassware should be washed thoroughly by chromic acid ($K_2Cr_2O_7 + H_2SO_4$) then with distilled and finally with double distilled water before use. If possible, pipette etc. should be kept dipped into chromic acid.

Observation:

Results:

The soil contains available zinc ____ ppm (Soil having available zinc less than 0.6 ppm is rated as deficient in zinc.)

The soil contains available manganese _____ ppm (Soil having available manganese less than 2.0 ppm is rated as deficient in manganese.)

The soil contains available copper______ ppm (Soil having available copper less than 0.65 ppm is rated as deficient in copper.)

The soil contains available iron______ ppm (Soil having available iron less than 4.5 ppm is rated as deficient in iron.)

Video links:

https://www.youtube.com/watch?v=gzq8iPt6QOA

Exercise 10: Estimation of Total Nitrogen in Plants (Kjeldahl method)

Principle:

Nitrogen mostly present in soil in organic form. Relatively small amount of nitrogen usually occurs in ammonium and nitrate form, the available form. The Kjeldahl method of total N determination includes both organic and ammonium form and with modification nitrate form of N can also be included.

Plant samples for N determination are digested in sulphuric acid at a temperature below 360 and 410°C. Below 360°C, the digestion process is very slow or incomplete but above 410°C some of the ammonia may be lost. The rate of digestion is accelerated by using copper sulphate as a catalyst and anhydrous sodium sulphate or potassium sulphate to raise the boiling temperature of H_2SO_4. The temperature of digestion is carefully regulated for best and complete digestion, which normally takes less than 2 hours. On completion of digestion, the samples are cooled and diluted as concentrated alkali is to be added to H_2SO_4 digest for distillation. The distilled ammonia is quantitatively adsorbed in boric acid and titrated against standard acid.

Apparatus:

a) Kelplus digestion and distillation unit.
b) Distillation tube
c) Conical flasks
d) Burettes
e) Pipettes

Reagents:

1) Sulphuric acid – H_2SO_4 (93-98%)
2) 40% sodium hydroxide solution: Dissolve 350 g solid NaOH in water and dilute to one litre
3) 4% Boric acid: Dissolve 40 g of boric acid powder in warm water by stirring and dilute to one litre
4) Mixed Indicator: Dissolve 0.066 g of methyl red and 0.099 g of bromocresol green in 100 mL of ethyl alcohol. Add 20 mL of this mixed indicator to each litre of 4% boric acid solution

5) 0.2N H_2SO_4: Prepare approximately 0.2N acid solution and standardize against 0.1N sodium carbonate
6) Salicyclic acid or Devarda's alloy: Using for reducing NO_3 to NH_4^+, if present in the sample
7) Digestion mixture: 10 g Potassium sulphate (K_2SO_4) or anhydrous sodium sulphate (AR grade) + 1 g catalyst mixture (20 parts of Copper sulphate [$CuSO_4.H_2O$ (AR grade)] powder and 1part metallic selenium powder

Procedure:

a) Take 1 g soil or 0.5 g plant/seed sample in distillation tube.
b) Add 3-5 g digestion mixture and add 10 mL concentrated H_2SO_4.
c) Heat low temperature for 30 min. and then increased heat up to 410 °C and digest for 1-2 h or until solution is crystal clear green colour and then stop the digestion. If necessary, add small amount of paraffin or glass beads to reduce frothing.
d) Remove the flask from the heater and cool, add 50 mL water and transfer to distilling flask for distillation.
e) At the time of distillation, add 30 mL of 40% NaOH in the distilling flask in such a way that the contents do not mix.
f) The contents are distilled for 5 minutes by pressing run bottom of Kelplus distillation unit and the liberated ammonia collected in a conical flask (250 mL) containing 20 mL of 4% boric acid solution with mixed indicator.
g) First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
h) Titrate the distillate against 0.2N H_2SO_4 taken in burette until pink colour starts appearing.
i) Carefully remove the distillation tube and drain the contents in the sink

Precautions:

1) The material after digestion should not solidify.
2) No NH_4 should be lost during distillation.
3) If the indicator changes colour during distillation, determination must be repeated using either a smaller sample weight or a larger volume of standard acid.
4) Standard the 0.2N H_2SO_4 by using standard solution.
5) Check the tap water and distilled water.

6) Dip the delivery tube end in the receiver containing standard boric acid solution before adding NaOH solution in the distillation flask.
7) Close the distillation tube to the distillation apparatus tightly then add NaOH to avoid the loss of ammonia.
8) During distillation, first remove the receiver flask and then distillation tube.
9) Collect about 100 mL of distillate in 10 minutes steady distillation.

Observation:

Sl No.	Initial burette reading	Final burette reading	mL of (0.2N H_2SO_4)

Calculation:

N (%) = (A- B) X Normality of H2SO4 X 0.014 X 102/ Wt. of the sample

Volume of acid used to neutralize ammonia in the sample = (A – B) mL

Where,

A = Volume of 0.2N H2SO4 used in titration of soil sample against ammonia absorbed in boric acid.

B = Volume of 0.2N sulphuric acid used in blank titration.

Normality of sulphuric acid = 0.2

(1000 mL of 1N H2SO4 = 14 g Nitrogen).

Protein Content:

Crude protein was determined by multiplying percentage of nitrogen content in seeds of crops with a factor of 6.25 (Tai and Young 1974).

% Crude Protein = % N x 6.25

Result:

The total nitrogen content in seed/plant is _____________ %.

Video link:

https://www.youtube.com/watch?v=3uQOezl_Uos

Exercise 11: Estimation of Phosphorus in Plants

Sample digestion for plant analysis of P, K and S:

For the release of mineral elements from plant tissues, dry ashing and wet oxidation are the two widely adopted methods. Dry ashing is carried out usually at an ignition temperature of 550 to 600^{o}C followed by its extraction in dil. HCl or H_2SO_4 for determining various elements. Ashing at temperature exceeding 600^{o}C leads to considerable volatilization loss of P and K. to prevent this loss of P, dry ashing is carried out in presence of an alkali like Mg (NO_3) in alcohol. Volatilization loss of S and Cl also takes place during ignition, which can be prevented by adding Na_2CO_3. A part of P also gets occluded, causing lot of error. For these reasons and for being comparatively more time taking, dry ashing is only occasionally adopted. Wet oxidation employs oxidizing 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 in completing oxidation. $HClO_4$ on heating produces anhydrous $HClO_4$ which dissociates into nascent chlorine and oxygen, increasing the oxidation efficiently at high temperature. Direct contact with $HClO_4$ with plant samples might lead to explosion and fire, hence the pre-digestion of samples in HNO_3 is preferred. Digestion with HNO_3- $HClO_4$ instead of the tri-acid mixture is also adopted specially when S is also to be determined in the same digest.

Principle:

Phosphorus in the aliquot can be determined using the methods based on molybdophosphoric blue colour developed by reduction of the hetero- poly complex or by vanadomolybdophosphoric yellow colour method. The vanadomolybdophosphoric yellow colour method is suitable for P determination in plant extracts.

Apparatus:

a) Spectrophotometer
b) Volumetric flask
c) Pipette
d) Conical flasks
e) Beaker

Reagents:

1) Triacid mixture: Look over the procedure of sample digestion
2) Ammonium molybdate ammonium vanadate solution: Dissolve 22.5 g of $(NH_4)_6 MO_3O_7 .4H_2O$ in 400 mL of distilled water in a beaker. Take 1.25 g of ammonium vanadate in another beaker, add 300 mL distilled water and boil it. Add ammonium vanadate solution to the ammonium molybdate solution and cool the contents. Add 250 mL of concentrated HNO_3 and dilute it to 1 litre.
3) Phosphate standard solution: Take 0.22 g of AR grade KH_2PO_4 in a beaker and dissolve in distilled water, transfer the solution to a 1 litre volumetric flask and make up the volume. This solution contains 50 ppm of P.

Procedure:

a) After digestion (see digestion of Seed and Plant samples), take 10 mL filtered solution into a 25 mL volumetric flask. Add 10 mL ammonium molybdate-ammonium vanadate solution and make the volume to 25 mL with distilled water. Yellow colour will develop after 15-20minutes.
b) From the 50ppm standard solution of P pipette out 0, 2, 4, 6, 8 and 10 mL and transfer in 50 mL volumetric flaks then add 10 mL of ammonium molybdate ammonium vanadate solution and make the volume. This will contain 0, 2, 4, 6, 8 and 10 ppm P.
c) Measure the colour intensity of standard solutions at a wave length of 470 nm or by using blue filter in a Spectrophotometer. Prepare the standard curve by plotting concentration of P on X-axis and Spectrophotometer readings on Y-axis on a graph paper.
d) Take readings of seed/plant samples in the similar manner that of standard.

Precautions:

1) The temperature of the digestion mixture during digestion should not exceed 230^0C.
2) The reading of the P in solution should be taken after 30 minutes from the development of yellow colour.
3) Ammonium molybdate-ammonium vanadate solution should be stored in a coloured bottle to prevent oxidation.
4) A clear and white residue in flask should remain after digestion. In case of incomplete digestion, the material should be again digested after addition of 5 mL of triacid.

Observation:

Sl No.	Spectrophotometer Reading (Abs)

Calculation:

P (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

Dilution factor (d. f.) = Volume of extractant/ Weight of soil X Final volume of solution/ Volume of digest extract

= 100 X 25/ 0.5 X 10 = 500

Where,

Weight of plant sample = 0.5 g, Volume of digested extract prepared = 100 mL, Volume of digested extract taken = 10 mL, Volume of final coloured extract prepared = 25 mL

Results:

P content in sees/plant _______ mg/kg.

** **% P = P ppm / 10000**

Video link:

https://www.youtube.com/watch?v=y2FsPH5ZDXY

Exercise 12: Estimation of Potassium in Plants

Principle:

Potassium in the acid digest of plant samples can be determined using flame photometer. Depending on the concentration of K in the plant sample, the digest can be used either directly or after dilution foe flame photometric determination.

Apparatus:

a) Flame photometer
b) Volumetric flask
c) Pipette
d) Beaker
e) Conical flask
f) Funnel
g) Hot plate

Reagents:

1) Triacid digestion: see digestion of Seed and Plant samples.
2) Potassium standard solution: Dissolve 1.9103 g of AR grade KCl in distilled water. Transfer it to 1 litre volumetric flask and make the volume. This solution contains 1000 ppm of K. To prepare 100 ppm solution takes 10 mL of 100 ppm K solution in a 100 mL volumetric flask and make up its volume.

Procedure:

a) Digest the plant sample with triacid as per the method given in digestion of Seed and Plant samples and filtered the solution and make the volume in 100 mL volumetric flask.
b) Prepare 0, 2, 4, 6, 8 and 10 ppm K solution by taking 0, 2, 4, 6, 8 and 10 mL of 100 ppm K solution in 100 mL flask respectively and making up their volume.
c) Feed the standard in flame photometer and standardized the instrument on low and high level of K content and then take the readings in ppm of K content.
d) If the readings of sample are showed over calibration then dilute the extract 5 to 10 times or more as required.

Precautions:

1) Extract should be clear and it should be prepared from double distilled water otherwise it clogs the sucking capillary.
2) The air pressure should be maintained steadily at 0.48 kg/cm^2 to get uniform flame and proper atomization.
3) Do not feed K solutions having higher concentration than that prescribed for the instrument.
4) Be sure that the filter used in flame photometer is of potassium.
5) After taking 8-10 readings feed distilled water and then again take the 43 sample readings.
6) After completion of reading close the gas then after some time stop the air supply.

Observation:

Sl No.	Flame Photometer Reading (ppm)

Calculation:

K (mg/kg) content in seeds/plant = R × d.f

Where,

R = ppm reading of from standard curve

Dilution Factor (d. f.) = Volume of extractant/ Volume of digested extract

= 100/ 0.5 = 200

Where,

Weight of plant sample = 0.5 g,

Volume of digested extract prepared = 100 mL.

Results:

K content in seeds/plant is _______ ppm.

**** % K = K ppm / 10000**

Video Link:

https://www.youtube.com/watch?v=IxK_I-Q2e8w

Exercise 13: Estimation of Total Sulphur in Plants

Principle:

Since, dry ashing leads to volatilization loss of S present in the organic combination, and the wet oxidation based on tri- acid mixture includes H_2SO_4, both of these methods cannot be used for S determination in plant samples. Therefore, HNO_3- $HClO_4$ digest of plant is conveniently used. Turbidimetric method as described for soil sulphur or the barium chromate colorimetric method can be used for determination of S in the digest. Sulphur estimated by Turbidimetric method by using VIS-spectrophotometer at 490 nm (Chesnin and Yien 1950) and expressed the concentration in percentage.

Apparatus:

a) Spectrophotometer
b) Volumetric flask
c) Pipette
d) Conical flasks
e) Beaker

Reagents:

1) Triacid mixture: see digestion of Seed and Plant samples.
2) Sodium acetate acetic acid buffer (pH 4.8) [CH_3COONa-CH_3COOH]: 1000 mL volumetric flask add 100 g sodium acetate, add distilled water 500mL to mixed, add 30 mL 99.5% acetic acid, shake to dissolved sodium acetate the makeup volume 1000mL mark.
3) 0.25% Gum acacia solution: Dissolve 0.25g of chemically pure gum acacia powder in 100 mL of hot water and filter in hot condition through Whatman No.42 filter paper. Cool and keep in refrigerator.
4) Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
5) 100 ppm S solution: Take 0.5434 g of K_2SO_4 and add 500mL distilled water to dissolved then make up to 1000 mL mark of volumetric flask.

Procedure:

a) After digestion (see digestion of Seed and Plant samples), Transfer 10 mL of digest filtrate in to 25 mL volumetric flask and gradually add 10 mL of sodium acetate acetic acid buffer.
b) Add 1 g $BaCl_2.2H_2O$ powder and shake well.
c) Then add 1mL gum acacia and make up the volume with distilled water.
d) Run a blank without soil in similar manner.
e) Measure the turbidity intensity at 490 nm
f) Run a blank side by side.

Preparation of standard curve:

Put 0, 1.25 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 mL of the working standard solution (10 mg S/litre) into 47 a series of 25 mL volumetric flasks to obtain 0, 0.5, 1.0, 2.0,3.0, 4.0, 5.0 and 6.0 ppm. Develop turbidity as described above for sample aliquots. Read the turbidity intensity and prepare the curve by plotting readings against sulphur concentrations.

Precautions:

1) The temperature of the digestion mixture during digestion should not exceed 230^0C.
2) The reading of the S in solution should be taken within 30 minutes from the development of turbidity.
3) A clear and white residue in flask should remain after digestion. In case of incomplete digestion, the material should be again digested after addition of 5 mL of tri acid

Observation:

Sl No.	Spectrophotometer Reading (ppm)

Calculation:

S (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

Dilution Factor (d. f.) = Volume of extractant / Wt. of soil X Final Volume of solution/ Volume of digested extract

= 100 X 25/ 0.5 X 10 = 500

Where,

Weight of plant sample = 0.5 g, Volume of digested extract prepared = 100 mL, Volume of digested extract taken = 10 mL, Volume of final coloured extract prepared = 25 mL

Results:

S content in sees/plant _______ ppm.

**** % S = S ppm / 10000**

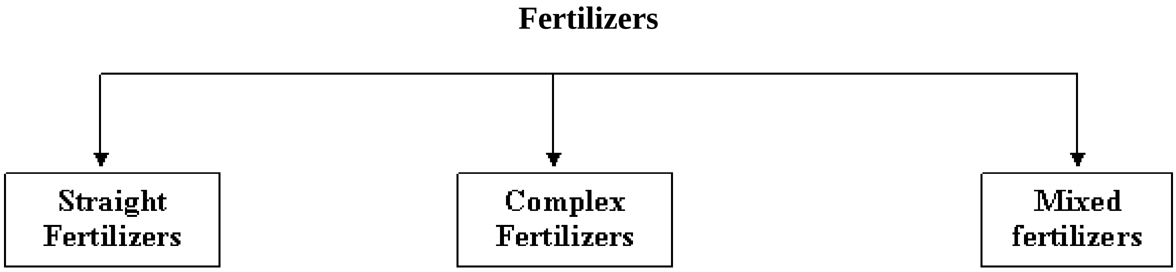
Video link:

https://www.youtube.com/watch?v=nDW3vW6PnR0

Exercise 14: Identification of Fertilizers

Fertilizer is any material of natural or synthetic origin added to the soil to supply one or more plant nutrients.

CLASSIFICATION OF FERTILISERS:



1. Straight fertilizers: Straight fertilizers are those which supply only one primary plant nutrient, namely nitrogen or phosphorus or potassium.
eg. Urea, ammonium sulphate, potassium chloride and potassium sulphate.

2. Complex fertilizers: Complex fertilizers contain two or three primary plant nutrients of which two primary nutrients are in chemical combination. These fertilizers are usually produced in granular form.
eg. Diammonium phosphate, nitrophosphates and ammonium phosphate.

3. **Mixed fertilizers**: are physical mixtures of straight fertilizers. They contain two or three primary plant nutrients. Mixed fertilizers are made by thoroughly mixing the ingredients either mechanically or manually.

A. Nitrogenous fertilizers:

1. Nitrogenous fertilizers take the foremost place among fertilizers since the deficiency of nitrogen in the soil is the foremost and crops respond to nitrogen better than to other nutrients.
2. More than 80 per cent of the fertilizers used in this country are made up of nitrogenous fertilizers, particularly urea.
3. It is extremely efficient in increasing the production of crops and the possibilities of its economic production are unlimited.

Urea [$CO(NH_2)_2$]

1. It is the most concentrated solid nitrogenous fertilizer, containing 46 per cent nitrogen.
2. It is a white crystalline substance readily soluble in water.

3. It absorbs moisture from the atmosphere and has to be kept in moisture proof containers. It is readily converted to ammoniacal and nitrate forms in the soil.
4. The nitrogen in urea is readily fixed in the soil in an ammoniacal form and is not lost in drainage.
5. Urea sprays are readily absorbed by plants.
6. It may be applied at sowing or as, a top-dressing.
7. It is suitable for most crops and can be applied to all soils.

B. Phosphatic fertilizers

1. Phosphatic fertilizers are chemical substances that contain the nutrient phosphorus in absorbable form (Phosphate anions) or that yield after conversion in the soil.

Super phosphate [$Ca (H_2PO_4)_2$]

1. This is the most important phosphatic fertilizer in use.
2. It contains 16 % P_2O_5 in available form.
3. It is a grey ash like powder with good keeping or storage qualities.
4. Phosphatic fertilizer hardly moves in the soil and hence they are placed in the, root zone.

C. Potassic fertilizers

1. Potassic fertilizers are chemical substances containing potassium in absorbed form (K^+).
2. There are two potassium fertilizers *viz*., muriate of potash (KCI) and sulphate of potash (K_2S0_4).
3. They are water soluble and so are readily available to plants.

Potassium chloride (KCI)

1. Potassium chloride or muriate of potash is a white or red, crystal containing 60.0 per cent K_2O.
2. It is completely soluble in water and therefore readily available to the crops.
3. It is not lost from the soil, as it is absorbed on the colloidal surfaces.
4. It can be applied at sowing or before or after sowing.
5. The chlorine content is about 47.0 per cent.
6. Its chlorine content is objectionable to some crops like tobacco, potato, etc where quality is the consideration.

Sulphate Fertilizers:

1. These are chemical substances containing the nutrient sulphur in the form of absorbable sulphate anions (SO_4^{2-}).

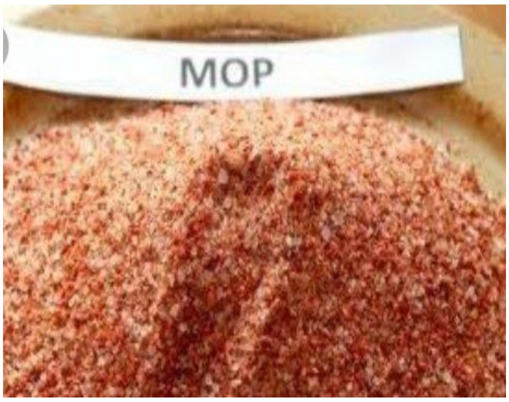
2. The sulphur requirements of plants are about two third of their phosphorus requirements.
3. Substantial sulphur supplies occur as minor constituents of various N, P and K fertilizers.
4. Fertilization with sulphur becomes necessary with increasing removal from the soil with rising agricultural production especially in plants with high sulphur requirements. e.g. mustard

Micronutrient Fertilizers:

1. The importance of fertilization of crops with micro-nutrients is increasing mainly because of greater removal from the soil, intensive liming of soil, intensive drainage of soil, higher use of nitrogenous, phosphatic and potassic fertilizers etc.
2. There are seven essential micronutrients required by plants.

These are iron, manganese, zinc, copper, chlorine, boron and molybdenum.

Urea **Single super phosphate**



Muriate of potash

Exercise 15: Utilization of Soil Testing Kit

ICAR - Indian Agricultural Research Institute (ICAR - IARI), PUSA- Delhi, is manufacturing Digital **S**oil **T**est and **F**ertilizer **R**ecommendation (STFR) Kit (Digital Soil Testing Mini Lab). This soil testing kit helps farmers in testing 14 parameters of soil & recommends crop-specific fertiliser dose for 100 crops within a short time and at a centre nearby, and thus helps them to achieve higher yields, increase their income and keeps soil fertile.

PUSA STFR METER TESTS FOLLOWING 14 PARAMETERS OF SOIL:

1. Available organic carbon (OC)
2. Available nitrogen (N)
3. Available phosphorous (P)
4. Available potassium (K)
5. Available zinc (Zn)
6. Available sulphur (S)
7. Available boron (B)
8. Available copper (Cu)
9. Available iron (Fe)
10. Available manganese (Mn)
11. Electrical conductivity
12. pH
13. Lime Requirement Test for Acid Soil
14. Gypsum Requirement Test for Alkaline Soil

TESTS 14 PARAMETERS OF SOIL

It tests 14 parameters of soil

ISSUES SOIL HEALTH CARD

It prints Soil Health Card for farmers.

In-built Battery Backup & Solar Charging

In-built battery backup in meter & solar panel for charging.

RECOMMENDS FERTILISER DOSE FOR 100 CROPS

It recommends crop-specific fertiliser dose for 100 crops.

SENDS SOIL REPORT ON SMS

Sends complete Soil Test Report to farmer on SMS.

KEEPS SOIL FERTILE

Helps in keeping soil fertile, increases yield & income of farmer.

The Kit Comprises Of

1. Digital STFR Meter
   With 6-hours battery back-up & solar charging.
2. Rotary Shaker
3. Thermal Printer
4. Reagents Kit
5. Accessories Kit
6. Glassware
7. Plasticware
8. Solar Panel
9. Pocket Weigh Balance
10. Computer Connectivity Functionality
11. Pen-Drive with Training Videos
12. Printed User Training Manual

Benefits of using PUSA STFR Meter Kit

1. Helps in determining the available nutrients in the soil
2. Helps in determining the correct dosage of fertilizers according to the crop
3. Saves money of farmers & nation by optimal usage of fertilizers
4. Optimal use of fertilizer also ensures good health of ground water
5. Prevents soil deterioration & maintains soil fertility
6. Increases crop yield & hence farmers have more income
7. Soil tests can be done within a short time frame
8. Testing can be done at a Centre near a Village or Panchayat
9. Accurate & reliable digital results
10. Easily portable to any region
11. Can be handled by an Under Graduate level person after training
12. Issues printed Soil Health Card & also sends Soil Test Report on SMS

Soil Testing Kit

Video link:

https://www.youtube.com/watch?v=2sHEIHOuk4M

Compiled and prepared by Dr Madhurima Banik and Mr Sourav Mullick

Exercise 16: Study of soil fertility map

There are different types of base materials used for mapping soils. They are mainly-

1. Cadastral map
2. Topographical and geographical map
3. Aerial photograph
4. Satellite imagery

Cadastral map

The cadastral maps are available on large scale mainly “16 to a mile or 8 ” to a mile. In hilly areas they are on 32” to a mile. The cadastral map shows field boundaries/parceling with numbers. The ownership and land use deteils can be obtained from Khasra and Khatoni records maintained by partwari.

Survey of India Maps:

The Survey of India (SOI) is the national survey and mapping organisation of our country. The SOI publishes topographical and geographical maps on different scale.

Definition of Topographical and Geographical Maps:

A topographical map is on a sufficiently large scale to enable the individual features shown on the map to be identified on the ground by their shape and position. On the other hand a Geographical Map is on such a small scale that strict representation of individual features for identification on the ground is not possible. A geographical map is intended to give a picture of the country as a whole.

Maps on scale 1 inch to 4 miles or 1:250,000 and larger are classified as topographical maps while the maps on scales smaller than 1:250,000 are termed as geographical maps.

Details regarding Topographical Maps

Topographical Maps:

The layout and numbering of all topographical maps (1”,1/2”, ¼”,1:50,000 and 1:250,000) are based on 1:1,000,000 (1:M) India and adjacent countries series maps.

Each 1:M map is divided into sixteen 1 inch to 4 miles (1/4inch) or 1:250,000 sheet, each ¼ inch or 1:250,000 sheet is divided into four 1 inch to 2 miles (1/2 inch sheet and each ½ sheet is further divided into four 1 inch to 1 mile (1 inch) or 1:50,000 sheets.

Gradient = (Vertical distance/ Horizontal distance)

Vertical distance is the rise or drop between two points
Working out of the gradient
Height of A= 19m
Height of B =139m

Gradient = (Vertical distance/ Horizontal distance)
= (120/960)
= 1/8 or 1 in 8

Landforms:

Each landform has a fairly distinctive contour pattern. Contour patterns help us to identify and to explain how they were created.

The human landscape in maps:

SOI maps provide a wealth of information about the human landscape. They show the place, where people live (settlements), the roads and railways along which people and good travel (communications), how the land is used (land use) and some of man's activities (economic and non-economic activities).

Survey of India Maps.

Maps published by Survey of India are available for sale with authorized Maps Sale Agents in important cities and towns and can also be obtained from:

1. Officer-in-Charge, Map Records and Issue Office, Survey of India. Dehra Dun-248001 (Main supplier of Maps).
2. Head Clerk, Map Sales Office, Survey of India, Janpath Barraks, "A"Janpath,

New Delhi-110001.

3. Director, Southern Circle, Survey of Indian, 8, Richmond Road, Banglore, 560 027.
4. Director, Eastern Circle, Sruvey of Undia, 13, Wood Street, Calcultta-700 016.
5. Incharge, Map Sales Office, Survey of India, 3-6-561/1, Himayatnagar, Hyderabad-500 029.
6. Director, South Eastern Circle, Survey of India, Lewis Road, Bhubaneshwar-751 014.

The recent maps are printed in about seven colours viz. Black for outline and typescript Blue for water features Red for roads and sites Yellow for cultivation Green for wooded area Brown for contours, cliffs etc. Grey for hill shading.

In addition, administrative boundaries are printed with ribands in various colours. Hills are shown by contours and more important ranges are emphasized by hill shading.

Scale: Is relationship between distance on the map and distance on the ground. Every unit of distance measured on the map represents a fixed number of units on the ground. There are three ways of showing scale on a map.

A **statement of scale** uses words to indicate the scale of the map. One centimeter to half a kilometer means that one centimeter measured on the map represents half a kilometer measured on the land. The units of measurement are clearly stated in this kind of scale.

A **Linear scale** is the easiest kind to use. It is a straight line devided up into sections which are numbered according to the real distances on the land. Map distance can be red off a linear scale immediately.

The **ratio or representative fraction** is the third way of showing the scale of map. Here, the units must always be the same 1:50,000 (1/50,000) meant that 1 unit on the map represents 50,000 units on land. If the units are centimeters then a statement of the scale can be worked out like this.

1 cm represents 50,000 cm
1 cm represents 500 m
1 cm represents 05 km

Some of the older SOI maps have a scale of 1:63, 360. In this scale the units are inches so that one inch on the map represents 63,360 inches on the land. These symbols are called conventional signs and are kind of shorthand allowing cartographers to show may features without cluttering on the map.

Relief:

Relief is shown of SOI maps **contour lines.** A contour is line that joins places that are the same height above sea level. On SOI maps contours are drawn as brown lines. The pattern and spacing of contours lines show the shape or relief of the land. Spot heights are also used to show the height of a place above sea level.

Land Use

This refers to the main use to which land is put.

Communications

This section includes roads, railways and paths

Water features: All water areas like the sea, lakes, rivers and canals are shaded or drawn in blue.

MAP READING

Map reading is a skill which brings a map to life. This skill can best be achieved by trying to imagine the map as a real landscape and not just a printed sheet of paper. Map reading may also identify physical and human aspect of landscape. The physical aspects includes the natural features of the landscape which are formed by physical forces such as earth movements, rives, ice and the sea. Human aspects include all those feature of the landscape that have been or modified by man. So a mountain, river and cliff are examples of physical features. A town, orchard and reservoir are human features. Sometimes the physical and human aspects are closely related.

The Physical Landscape in Maps

The height and shape of the land is called relief. It is shown on maps by spot heights and contour lines. Sometimes the relief forms particular shapes known as landforms which can be detected form contour patterns.

Cross-Section

On should be able to draw cross-section from the contours on SOI map. First, decide on the line of section. It should cross the features you want to show. Next, mark off and number contours that cross the line of section. Use the straight edge of a sheet of paper for this. Now transfer the marks and contour numbers to the correct position in the section box. The heights can be found on the left hand side on the box. Finally, join the marks together with a smooth curved line. Never use a ruler. Label both ends of the section with a letter or name from the map to show where the section was taken. The cross section is then complete.

Gradient means the slope or angle of the land. Suppose a hill has a gradient of 1 in 9. That means for every 9 meters we go along the road, we will also go down 1 meter. The gradient can be mapped in degrees with the help of protector. There is a formula for working out gradients.

Soil fertility Map

Soil testing provides information regarding nutrient availability in soils which forms the basis for the fertilizer recommendations for maximizing crop yields. The texture, structure, colour etc. are important soil physical parameters. Similarly, soil reaction (pH), organic matter, macro and micronutrients etc. are also important soil chemical parameters. Soil properties vary spatially from a field to a larger regional scale and it is affected by soil forming factors which can be termed as intensive factors and extrinsic factors such as soil management practices, fertility status, crop rotation etc thus having a pictoral view of the estimated parameters helps use to interprete the the particular region to greater extent.

Reference link

http://www.iiss.nic.in/mapd_7.htm