LABORATORY MANUAL

Manures, Fertilizers and Soil Fertility Management

Course Code: 13A.312

Credit: 3(2+1)

Semester: 5th

B.Sc. (Hons.) Agriculture
Department of Agriculture

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Experiment No. 1 Introduction of analytical instruments

Object: Basic principles of the instruments

Analytical instruments are devices which are used to measure the physical or chemical properties of assayed substance.

Basic concepts in Instrumental analysis

Colorimetry:

In colorimetry, color reactions using appropriate reagents are made use of to measure concentration of a substance. Higher the concentration of the substance being determined, greater is the intensity of the color. The intensity of color is measured using an instrument called photoelectric colorimeter.

Colorimeter deals with the determination of concentration of the substance in solution by measurement of the relative absorption transmittance of light with respect to a known concentration of the substance.

About the instrument:

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 makes use of the characteristic radiation given out by different elements when their atoms are excited in a flame. When a fine atomized spray of a solution of a compound containing the element is introduced into the flame atoms of elements absorbs thermal energy from the flame & get excited. As a result extra nuclear electrons are raised to higher energy level, fall back to original lower energy level energy initially absorbed given off in the form of radiation of discrete wavelength. The amount of radiation given out in this manner will be proportional to the conc. of atoms in the flame. When all other things will be equal the concentration of atoms in the flame will depend upon the conc. of elements in the solution. In flame Photometry the characteristic radiation is isolated using a filter & intensity of isolated radiation is measured by a suitable mechanism such as photoelectric cell &galvanometer.

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

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. At the end of determination the following steps are taken to switch OFF flame potentiometer.

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

Estimation Of Soil Organic Carbon

Objectives:

To determine the available organic carbon in the soil.

Principle:

In the detection of soil Organic Carbon a known weight of soil is heated with an excess volume of standard $K_2Cr_2O_7$ in the presence of Con. H_2SO_4. The soil is slowly digested at the low temperature by the heat of dilution of H_2SO_4 and the organic carbon in the soil is thus oxidized to CO_2. The highest temperature attained by the heat of dilution reaction produced with the addition of Con. H_2SO_4 is approximately 12^oC which is sufficient to oxidize the active forms of the soil organic carbon but not the more inert forms of carbon that may be present. The excess of $K_2Cr_2O_7$ not reduced by organic matter is titrated against a standard solution of Ferrous Ammonium Sulphate in the presence of Phosphoric acid and Diphenylamine as indicator. While the actual measurement of oxidisable Organic Carbon, the data are normally converted to percentage organic matter using a constant factor, assuming that organic matter contains 58% Organic Carbon. However, as this proportion is not in fact constant, we prefer to report as oxidisable Organic Carbon, or multiplied by 1.334 as Organic Carbon.

Apparatus:

Conical flask, Pipette, Burette.

Procedure:

- Take 1 g of soil in a 500 mL conical flask.
- Add 10 mL of 1N $K_2Cr_2O_7$ solution and shake to mix it.
- Then add 20 mL Con. H_2SO_4 and swirl the flask 2 or 3 times.
- Allow the flask to stand for 30 minutes on an asbestos sheet for the reaction to complete.
- Pour 200 mL of water to the flask to dilute the suspension. Filter if it is expected that the end point of the titration is not to be clear.

- Add 10 mL of 85% H_3PO_4 and 1 mL of Diphenylamine indicator and back titrate the solution with 0.5 N Ferrous Ammonium Sulphate, till the colour flashes from violet through blue to bright green. H_3PO_4 gives sharper endpoint, by making the colour change, distinct through a flocculating effect.
- Note the volume of Ferrous Ammonium Sulphate.
- Carryout blank titration (without soil) in a similar manner.

i. Calculation:

% of Organic Carbon in Soil (R) is,

$$R = \frac{(V_1 - V_2) \times N \times 0.003 \times 100}{W} \times C$$

Where,

W - Weight of Sample

V_1 - Blank Titre value

V_2 - Titre value of the Sample

N - Normality of $K_2Cr_2O_7$ (Here it is 1N)

C - Correction Factor (1.334, 1.724)

Experiment No. 3 Determination of mineralizable Nitrogen (Subbia and Asija, 1956)

Principle:

In case of soils, mineralizable N is estimated as an index of available nitrogen content and not the total nitrogen content. The easily mineralizable nitrogen is estimated using alkaline $KMnO_4$, which oxidizes and hydrolyses the organic matter present in the soil. The liberated ammonia is condensed and absorbed in boric acid, which is 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. In the process of oxidative hydrolysis, a uniform time and heating temperature should be allowed for bestresults.

Apparatus:

• Nitrogen distillation unit or Kelplus distillation unit.

• Distillation tube, conical flasks, pipettes, burette etc.

Reagents:

• **0.32% $KMnO_4$:** Dissolve 3.2 g of $KMnO_4$ in distilled water and make the volume to one litre.

• **2.5% NaOH:** Dissolve 25 g of sodium hydroxide pellets in water and make the volume to one litre.

• **2% Boric acid:** Dissolve 20 g of boric acid powder in warm water by stirring and dilute to one liter.

• **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.

• **0.1*M* 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.

• **0.02*M* H_2SO_4:** Prepare approximately 0.1*M* H_2SO_4 by adding 5.6 mL of conc. H_2SO_4 to about one litre of distilled water. From this, prepare 0.02*M* H_2SO_4 by diluting a suitable volume (20 mL made to 100 mL) with distilled water. Standardize it against 0.1*M* NaOH solution.

• **0.1*M* NaOH:** Dissolve 4g NaOH in 100 mL distilled water. Standardize against potassium hydrogenphthalate.

Procedure:

- 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.
- 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).
- Moisten the soil with about 10 mL of distilled water, wash down the soil, if any, adhering to the neck of the flask.
- Add 25 mL (Soil:0.32% $KMnO_4$ :: 1:5) of 0.32% of $KMnO_4$ solution in the distillation tube manually or instrumentally.
- Add a few glass beads or broken pieces of glass rod.
- Add 2-3 mL of paraffin liquid, avoiding contact with upper part of the neck of the flask.
- Add 25 mL of 2.5% NaOH solution and immediately attach to the rubber stopper fitted in the alkali trap.
- Press the run switch and continue distillation until about 100 mL of distillate is collected.
- First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
- Titrate the distillate against 0.02*M* H_2SO_4 taken in burette until pink colour starts appearing.
- 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:

1. Check the tap water and distilled water.
2. Dip the delivery tube end in the receiver containing standard boricacid solution before adding NaOH solution in the distillation flask.
3. Close the distillation tube to the distillation apparatus tightly then add NaOHto avoid the loss of ammonia.
4. During distillation, first remove the receiver flask and then distillation tube.
5. Collect about 100 mL of distillate in 10 minutes steady distillation.

Observation:

Sl.	Initial burette reading (mL)	Final burette reading (mL)	mL of (0.02*N* H_2SO_4)

Calculation:

$$\text{Mineralizable N (kg/ha)} = \frac{(A\text{-}B) \times N \times 0.014 \times 2.24 \times 10^6}{\text{Wt. of soil}}$$

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

Where,

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

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

N= Normality of sulphuric acid

1 mL of 0.02*N* sulphuric acid = 0.56 mg N (1000 mL of 1*N* 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.

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Experiment No. 3 Determination of Available Phosphorus in soil

Principle:

Soil is extracted with suitable extractant according to the pH of the soil. The soil is treated with an acid-molybdate solution to form phospho-molybdate complex. This complex can be reduced either by H_2S / Ascorbic acid / $SnCl_2$ solution to give a molybdenum blue colour depends upon the amount of phosphorus present in the system. In this particular system (blue colour) there is the formation of heteropoly complex in which there is coordination of molybdate ion with phosphorus as the central coordinating atom.

$$H_3PO_4 + 12\ H_2MoO_4 \rightarrow H_3P(Mo_3O_{10})_4 + 12\ H_2O$$

Heteropoly compound has Mo^{6+} which reduced to Mo^{3+} and or Mo^{3+} with the help of reducing agent H_2S or $SnCl_2$ or Ascorbicacid.

Unpaired or unshared electrons of molbdenum gave resonance line of blue colour and have maximum absorbance of incident light at 660 nm.

Phosphorus in soil occurs as orthophosphate in several forms and combinations and only a fraction of the total amount present may be available to plants which are of direct relevance in assessing the phosphorus fertility level. A wide variety of chemical soil tests for available P has been proposed which extract variable quantities of phosphorus. These include water, carbonated water, solution of neutral salts viz. KCl and $CaCl_2$, strong mineral acids of different concentrations (2.5% HCl, 0.2*N* HNO_3, 0.2*N* H_2SO_4), weak organic acids like citric (1%), lactic and acetic (2.5%), buffered dilute acid solutions both organic and inorganic such as Truog's reagent (0.002*N* sulphuric acid and ammonium sulphate pH 3.0), Egner's lactate extractant (ammonium lactate-lactic acid pH 3.8), Morgan's solution 9sodium acetate-acetic acid pH 4.8) and alkaline extractants such as NaOH (0.1N), K_2CO_3 (1 and 2%) and $NaHCO_3$ (0.5M). Chelating agents (like EDTA or DTPA) and specific chemicals for anionic exchange has been suggested for extracting certain forms adsorbed and chemically bound phosphate in the soilsystem.

Two methods are most commonly used for determination of available phosphorus in soils: Bray’s Method No.1 for acidic soils and Olsen’s Method for neutral and alkaline soils.

1. Bray's method No. 1 (Bray and Kurtz, 1945) for acid soils

The estimation of specific categories of soil P considered to be of significance to crop growth has lead to the introduction of neutral NH_4F (ammonium fluoride) solution as an extracting for adsorbed phosphate and NH_4F-HCl combination for adsorbed plus acid soluble form. This method is primarily meant for soils which are moderately to strong acid. The Bray's No. 1 reagent consists $0.03N$ NH_4F in 0.025 N HCl.

Apparatus and Instruments:

- Spectrophotometer
- Shaker
- Pipettes
- Beakers
- Conical flasks
- Volumetric flasks etc.

Reagents:

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

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.

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.

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 7*N* 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

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 (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:

- 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.
- 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.
- 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.
- 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.

Olsen's method (Olsen *et al*., 1954) for neutral and alkaline soils

A 0.5*M* $NaHCO_3$ solution adjusted to pH 8.5 has been designed to control the ionic activity of Ca through the solubility product of CaCO3 in case of neutral and calcareous soils. By this process, the most reactive form of P is extracted from the phosphates of iron, aluminium and calcium present in the soil.

Apparatus:

Same as for Bray's Method No. 1.

Reagents:

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.

Activated carbon –Darco G 60 or P free charcoal.

Molybdate reagent: Same as for the Bray's Method No. 1 except that use 400mL of 10*N* HCl instead of 350 mL/litre.

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:

- Weigh 2.5 g soil sample in a 250 mL conical flask.
- Add 1-2 g of Darco G 60 or P free charcoal and 50 mL of the bicarbonate extractant (0.5*M* $NaHCO_3$, pH8.5).
- Shake for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
- 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.
- Stir slowly and carefully to drive out the CO_2evolved.
- After stop of bubbliness, add distilled water washing down the sides and bring the volume to 22mL.

- Add 1 mL of freshly prepared diluted $SnCl_2$, shake a little and make up the volume.
- Run a blank without soil in similar manner.
- Read the blue color 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:

1. Clean all glassware with distilled water.
2. Start filtration quickly after shaking
3. Before taking reading, warm up the instrument minimum 30min.
4. Take reading after 10min.

Observation:

Sl	Specrtophotometer 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 = P_2O_5

Result: The available phosphorus status of the soil is_____kg /ha.

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Experiment No. 5 Determination of available Potassium (Jackson, 1973)

Principle:

The term available potassium represents both exchangeable and water soluble forms of the nutrient present in soil. The readily exchangeable plus water soluble forms of K is determined by the neutral normal ammonium acetate extract of soil. The ammonium ion provides a sharp and rapid separation from exchange complex while other cations bring about a gradual replacement of lesser or greater amount of potassium which generally increases with the period of contact. The estimation of available K in the extract is carried out with the help of flame photometer.

Instruments:

- Flame Photometer
- Glass electrode pH meter

Reagents:

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.

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 make up the volume with ammonium acetate solution.

Procedure:

- 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.
- Filter through Whatman No. 1 filter paper.
- Measure K concentration in the filtrate flamephotometer.

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 30min.
3. Check the flame level.

Observation:

Sl	Flamephotometer 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 = %K_2O]

Result: The available potassium status of thesoilis_____kg /ha.

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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 McKeague (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 $CaCO_3$ and gypsum will result in an excess of Ca^2+ being extracted by NH_4+ and a decrease in the amount of NH_4+ retained due to competition between Ca^2+ and NH_4+ 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

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.

Calcium by Versenate (EDTA) method

Apparatus:

Shaker, Porcelain dish, Beakers, Volumetric/conical flask.

Reagents:

Ammonium chloride:

Ammonium hydroxide buffer solution: Dissolve 67.5 g ammonium chloride in 570 ml of conc. ammonium hydroxide and make to 1 litre.

Standard 0.01N calcium solution:

Take accurately 0.5 g of pure calcium carbonate and dissolve it in 10 ml of 3*N* HCl. Boil to expel CO_2 and then make the volume to 1 litre with distilled water.

EDTA solution (0.01*N*):

Take 2.0 g of versenate, dissolve in distilled water and make the volume to 1 litre. Titrate it with 0.01*N* calcium solution and make necessary dilution so that its normality is exactly equal to 0.01*N*.

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 getsoxidized.

Sodium diethyl dithiocarbamate crystals: It is used to remove the interference of other metalions.

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:

- 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 filterpaper.
- Take a suitable aliquot (5 or 10 ml) and add 2-3 crystals of carbamate and 5 ml of 16% NaOH solution.
- Add 40-50 mg of the indicator powder. Titrate it with 0.01*N* 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.
- 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 thusfound.
- Note the volume of EDTA used for titration.

Observation:

Sl.	Initial burette reading (mL)	Final burette reading (mL)	Final volume (mL)

Calculation

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

$$N_1V_1 = N_2V_2$$

$$\text{Or } N_1 = \frac{N_2V_2}{V_1} = \frac{\text{Normality of EDTA x Vol. of EDTA}}{\text{ml of aliquot taken}}$$

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

$$\text{Hence, } Ca^{2+} \text{ me/litre} = \frac{\text{Noramlity of EDTA x Vol. of EDTA x 1000}}{\text{ml of aliquot taken}}$$

When expressed on soil weight basis,

$$Ca^{2+} \text{ me/100 g soil} = \frac{100}{\text{wt. of soil}} \times \frac{\text{extract volume}}{1000} \times \text{Ca as me/litre}$$

Result: The Ca content in soil is_____me/100 g soil.

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Calcium plus Magnesium by Versenate (EDTA) method

Magnesium in solution can be titrated with 0.01*N* 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:

Shaker, Porcelain dish, Beakers, Volumetric/conical flask.

Reagents:

EDTA or Versenate solution (0.01*N*): Same as in calcium determination. **Ammonium chloride-ammonium hydroxide buffer solution:** Same as in calcium determination.

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. Hydroxylamine hydrochloride removes the interference of manganese by keeping it in lower valency state (Mn^{2+}). Or mix thoroughly 0.5 g of the indicator with 50 g of ammonium chloride.

Sodium cyanide solution (2%) or sodium diethyl dithiocarbamate crystals: This is used to remove the interference of copper, cobalt and nickel.

Procedure:

- 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.
- Pipette out 5 ml of aliquot containing not more than 0.1 meq of Ca plus Mg. If the solution has a higher concentration, it should be diluted.
- 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.
- Titrate this solution with 0.01*N* 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 normality (concentration of Ca^{2+}+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 $N_1 =$

$$\frac{N_2V_2}{V_1} = \frac{\text{Normality of EDTA x Vol. of EDTA}}{\text{ml of aliquot taken}}$$

Here N_1 (Normality) = equivalents of Ca^{2+} plus Mg^{2+} present in one litre of aliquot.

$$\text{Hence, } Ca^{2+} \text{plus } Mg^{2+} \text{ me/litre} = \frac{\text{Normality of EDTA x Vol. of EDTA x 1000}}{\text{ml of aliquot taken}}$$

Meliequivalent (me) of Mg^{++} = me (Ca^{++} + Mg^{++}) – me of Ca^{++}

When expressed on soil weight basis.

$$Ca^{++} + Mg^{++} \text{ me/100 g soil} = \frac{100}{\text{wt. of soil}} \times \frac{\text{extract volume}}{1000} \times Ca^{++} + Mg \text{ me/litre}$$

Observation:

Sl.	Initial burette reading (mL)	Final burette reading (mL)	Final volume (mL)

Result: The Ca + Mg content in soil _____me/100 g soil

Signature of Faculty In-charge

Experiment No. 6 Estimation of Soil Extractable Sulphur/Available S in soils

Principle:

Available sulphur in mineral soils occurs mainly as adsorbed SO_4 ions. Phosphate ions (as monacalcium phosphate) are generally preferred for replacement of the adsorbed SO_4 ions. The extraction is also carried out using $CaCl_2$ solution. However, the former is considered to be better for more efficient replacement of SO_4 ions. Use of Ca salts has a distinct advantage over those of Na or K as Ca prevents deflocculation in heavy textured soils and leads to easy filtration. SO_4 in the extract can be estimated turbidimetrically using a spectrophotometer. A major problem arises when the amount of extracted sulphur is too low to be measured. To overcome this problem, seed solution of known S concentration is added to the extract to raise the concentration to easily detectable level. Barium sulphate precipitation method is described here.

Apparatus and Instruments:

- Spectrophotometer
- MechanicalShaker
- Conicalflasks
- Volumetric flasksetc.

Reagents:

0.15% $CaCl_2$: Take 1.5 g $CaCl_2$ in a volumetric flask and makeup volume 1000mL mark with distilled water.

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 1000mLmark.

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.

Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.

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:

- Weigh 5 g soil sample in a 250 mL conical flask.
- Add 25 mL of 0.15%$CaCl_2$.
- Shake with 230 rpm for 30 minutes on the mechanical shaker and filter through Whatman No. 42 filter paper.
- Transfer 10 mL of filtrate in to 25 mL volumetric flask and gradually add 10 mL of sodium acetate acetic acid buffer.
- Add 1 g $BaCl_2.2H_2O$ powder and shake well.
- Then add 1mL gum acacia and make up the volume with distilled water.
- Run a blank without soil in similar manner.
- Measure the turbidity intensity at 440 nm (blue filter).
- 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	Specrtophotometer reading (Abs)

Calculation:

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

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.

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Experiment No. 7 Estimation of DTPA Extractable Zn in soils

Object: Estimation of available (DTPA extractable) iron, manganese copper and zinc in soil (Lindsay and Norwell, 1978)

Principle:

Diethylene tetramine penta acetic acid (DTPA) being a chelating agent is used in the determination of available Fe, Mn, Cu and Zn. When the soil is shaken with a solution of DTPA, it combines with metal ions in the solution and form soluble complexes of Fe^{++}, Mn^{++}, Cu^{++} and Zn^{++}. The $CaCl_2$ and tri-ethanolamine (TEA) solution slightly raise and buffer the soil pH and mitigates the effect of Ca^{++} and Mg^{++}. From DTPA extract Fe^{++}, Mn^{++}, Cu^{++} and Zn^{++} are determined with the help of Atomic Absorption Spectrophotometer by using their respective hollow cathodes.

Apparatus:

Atomic Absorption Spectrophotometer, shaking machine, Centrifuge, beaker, Pipette, volumetric flask, conical flask, funnel etc.

Reagents:

1. DTPA 0.005 M solution
2. TEA 0.1 M (AR or extra pure) solution
3. $CaCl_2.2H_2O$ (AR) 0.01M
4. 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	$ZnSO_4.7H2O$	287.56	0.4398
Cu	63.54	$CuSO_4.5H2O$	249.69	0.39259
Mn	57.94	$MnCl_2.4H2O$	197.69	0.3602
Fe	55.85	$FeSO_4.7H2O$	278.02	0.4977

From the above 100 ppm solution a 10 ppm 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 10 ppm 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 an d 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:

1. Take 10 g of soil in a 250 mL conical flask and add 20 mL DTPA extracting solution toit and stopper it well.
2. 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.
3. Prepare a blank by adding all the solution except soil by shaking and filtration.
4. Take reading directly by AAS, using acetylene gas and hollow cathode lamp of the element (nutrient) which is to be determined in AAS.
5. 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.
6. 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 = 20mL

$$\text{Dilution factor (d.f.)} = \frac{\text{Volume of extractant}}{\text{Weight of soil}} = \frac{20}{10} = 2$$

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

= R × 2

Precautions:

1. Use only glass distilled water or double distilled water for the determination of micronutrients
2. Soil sample should be either ground in wooden pastle and mortar or stainless steel grinder.
3. The glassware used should be of high quality i.e. either corning or borosil.
4. 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.)

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Experiment No. 8 Basics of Plant analysis and estimation of N in plant samples

Objective: Determination of Total Nitrogen in Plant (Kjeldahl Method)

Principle:

The total Nitrogen in soil includes the organic N compounds like proteins, amino acids and other derivatives and all forms of inorganic N, like NH4 –N, NO_3 -N and also NH2 –N. For the determination of total nitrogen in soil, specific method is to be adopted for getting the total nitrogen value depending on the form of N present in a particular soil sample. The organic N components can be converted into inorganic ammoniacal salt by digestion with sulphuric acid, for reducing nitrates into ammoniacal form, use of salicylic acid or Devarda's alloy is made in the modified Kjeldahl method. At the end of digestion, all organic and inorganic salts are converted into ammonium form which is distilled and the liberated ammonia is condensed and absorbed in boric acid, which is titrated against standard acid.

The precision of the method depends upon complete conversion of organic N into NH4 - N, the digestion temperature and time, solid:acid ratio and the type of catalyst used have an important bearing on the method. The ideal temperature for digestion is $320^o – 370^o$ C. At lower temperature, the digestion may not be complete, while above 410^o C, the loss of NH3 may occur. The salt : acid (weight : volume) ratio should not be less than 1:1 at the end of digestion. Commonly used catalysts to hasten the digestion process are CuSO4 or Hg. Potassium sulphate is added to raise the boiling point of the acid so that loss of acid by volatilization is prevented.

Apparatus:

- Kelplus digestion and distillation unit.
- Distillation tube
- Conical flasks
- Burettes
- Pipettes etc.

Reagents:

Sulphuric acid – H_2SO_4 (93-98%)

40% sodium hydroxide solution: Dissolve 350 g solid NaOH in water and dilute to one litre.

4% Boric acid: Dissolve 40 g of boric acid powder in warm water by stirring and dilute to one litre.

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.

0.2N H_2SO_4: Prepare approximately 0.2*N* acid solution and standardize against 0.1*N* sodium carbonate

Salicyclic acid or Devarda's alloy: Using for reducing NO_3 to NH_4^+, if present in the sample.

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 1 part metallic selenium powder.

Or

10 g Potassium sulphate (K_2SO_4) or anhydrous sodium sulphate (AR grade) + 1 g of Copper sulphate[$CuSO_4.H_2O$ (AR grade)] powder.

Procedure:

- Take 1 g soil or 0.5 g plant/seed sample in distillation tube.
- Add 3-5 g digestion mixture and add 10 mL concentratedH_2SO_4.
- 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.
- Remove the flask from the heater and cool, add 50 mL water and transfer to distilling flask for distillation.
- 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.
- 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.
- First remove the conical flask containing distillate and then remove the distillation tube to avoid back suction.
- Titrate the distillate against 0.2*N* H_2SO_4 taken in burette until pink colour starts appearing.
- 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 3 min) then add 5-10 mL of 40% NaOH solution

Precautions:

1. The material after digestion should not solidify.
2. No NH4 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 0.2*N* 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	Initial burette reading	Final burette reading	mL of (0.2*N* H_2SO_4)

Calculation:

$$N\ (\%) = \frac{(A\text{-}B) \times \text{Normality of } H_2SO_4 \times 0.014 \times 10^2}{\text{Wt. of sample}}$$

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

Where,

A = Volume of 0.2*N* H_2SO_4 used in titration of soil sample against

ammonia absorbed in boric acid.

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

Normality of sulphuric acid = 0.2

(1000 mL of 1*N* H_2SO_4 = 14 g Nitrogen).

Note:

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 insoil/seed/plantis_____%.

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Experiment No. 9 Estimation of Phosphorus in plant samples

Objective: Determination of total phosphorus in seeds/plant:

Phosphorus content in digested seeds/plant was determined by Vanado-molybdophosphoric yellow colour method (Jackson 1973) by using VIS-spectrophotometer at 470 nm and expressed the concentration in percentage.

Apparatus:

Spectrophotometer, volumetric flask, pipette, conical flasks, beaker etc..

Reagents:

Triacid mixture: see digestion of Seed and Plant samples.

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.

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:

- 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.
- From the 50 ppm 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.
- 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.
- Take readings of seed/plant samples in the similar manner that of standard.

Note:

Digestion of Seed and Plant samples:

The oven dried plant samples were ground in a willey mill and the ground material was collected in a polythene bag, later 0.5 g of sample was used for each chemical analysis

Powdered seeds (0.5 g) were digested separately with 2 mL of conc. HNO_3 in a 100 mL conical flask with attaching funnel. The material was digested on a low temperature for half an hour and then 2 mL of 60% perchloric acid was added and heated on a low flame for 30 minutes. After that the temperature was increased and boils for one hour. Cooled and 5 mL of 5 *N* HCl was added to remove the entire nitrate present. Again digest the material by heating until fumes of perchloric acid disappear or residue becomes totally white. After cooling it was diluted with 20mL distilled water and filtered by Whatman no. 42 filter paper in a 100 mL volumetric flask and washed the residue with distilled water until the volume becomes 100 mL. A known quantity of liquid was used for further analysis (for P, K, S and Zn).

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	Specrtophotometer reading (Abs)

Calculation:

P (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

$$\text{Dilution factor (d.f.)} = \frac{\text{Volume of extractant}}{\text{Weightof soil}} \times \frac{\text{Final volume of solution}}{\text{Volume of digest edextract}} = \frac{100 \times 25}{0.5 \times 10} = 500$$

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.

Note: % P = P ppm / 10000

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Experiment No. 10 Estimation of Potassium in plant samples

Objective: Determination of total potassium in seeds/plant:

Potassium content in digested seeds/plant was estimated by Flame photometer (Jackson 1973) and the concentration was expressed in percentage.

Apparatus:

Flame photometer, volumetric flask, pipette, beaker, conical flask, funnel, hot plate etc.

Reagents:

Triacid digestion: see digestion of Seed and Plant samples.

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:

- 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.
- 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.
- 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.
- 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/cm2 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

sample readings.

6. After completion of reading close the gas then after some time stop the air supply.

Observation:

Sl	Flame photometer reading (ppm)

Calculation:

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

Where, R = ppm reading of from standard curve

$$\text{Dilution factor(d.f.)} = \frac{\text{Volume of extractant}}{\text{Volume of digested extract}} = \frac{100}{0.5} = 200$$

Where,

Weight of plant sample = 0.5 g, Volume of digested extract prepared = 100 mL.

Results: K content in seeds/plantis_____ppm.

Note: % K = K ppm / 10000

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Experiment No. 11 Estimation of Sulphur in plant samples

Objective: Determination of total Sulphur in seeds/plant:

Seeds/plant were digested using the method described in 3.8.2 and sulphur estimated by Turbidimetric method by using VIS-spectrophotometer at 490 nm (Chesnin and Yien 1950) and expressed the concentration in percentage.

Apparatus:

Spectrophotometer, volumetric flask, pipette, conical flasks, beaker etc..

Reagents:

Triacid mixture: see digestion of Seed and Plant samples.
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.

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.

Barium chloride crystal AR grade ($BaCl_2.2H_2O$): Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.

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:

- 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.
- Add 1 g $BaCl_2.2H_2O$ powder and shake well.
- Then add 1mL gum acacia and make up the volume with distilled water.
- Run a blank without soil in similar manner.
- Measure the turbidity intensity at 490nm
- 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. 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	Specrtophotometer reading (Abs)

Calculation:

S (mg/kg) in plant sample = R × d.f.

Where,

R = ppm reading of plant from standard curve

$$\text{Dilution factor (d.f.)} = \frac{\text{Volume of extractant}}{\text{Weight of soil}} \times \frac{\text{Final volume of solution}}{\text{Volume of digested extract}} = \frac{100 \times 25}{0.5 \times 10} = 500$$

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.

Note: % S = S ppm / 10000

Note:

Uptake of nutrients (kg/ha)

Total uptake of sulphur was calculated for each treatment separately using the following formula.

$$\text{Nutrient uptake by seed/plant (kg/ha)} = \frac{\text{\% nutrient concentration} \times \text{yield (kg/ha)}}{100}$$

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