DR. D.Y. PATIL EDUCATIONAL ACADEMY, MUMBAI'S
PADMABHUSHAN VASANT DADA PATIL
COLLEGE OF AGRICULTURE,

AMBI-TALEGAON DABHADE, TAL.-MAVAL, DIST.-PUNE.
(AFFILIATED TO MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI)

DIVISION OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

PRACTICAL MANUAL

COURSE NO : SSAC 353

CREDIT : 3 (2+1)

COURSE TITLE: Manures, Fertilizers and Soil Fertility Management

NAME : _____

REG. NO. _____ SEMESTER : V (NEW)

BATCH._____ ACADEDMIC YEAR 2019-20

INDEX

Course No. : **SSAC-353** Credits : **2 + 1= 3**

Course Title : **Manures, Fertilizers and Soil Fertility Management**

Sr. No.	Title of experiments	Page No.	Date	Remarks
1	Principle and application of Spectro-photometry/Colorimetry.		**/ /2019**	
2	Principle and application of Flame photometry and Atomic Absorption Spectrophotometer.(AAS)		**/ /2019**	
3	Determination of moisture from organic manure and its preparation for nutrient analysis.		**/ /2019**	
4	Determination of organic carbon from organic manure (compost / FYM / oil cake) by **Ignition method**.		**/ /2019**	
5	Estimation of **available Nitrogen** in soil by **Alkaline permanganate method**. (Subbiah and Asija, 1956).		**/ /2019**	
6	Estimation of **available Phosphorous** Content in soil by using **spectrophotometer**. (**Olsen's method**)		**/ /2019**	
7	Determination of **available Potassium** in soil (**Ammonium Acetate Extractable) using Flame Photometer**.		**/ /2019**	
8	Determination of **Exchangeable Ca & Mg** in soil by **EDTA Method.**		**/ /2019**	
9	Estimation of **available Sulphur** in soil (**Turbidity Method).**		**/ /2019**	
10	Estimation of **DTPA extractable micronutrients** from soil using **AAS.**		**/ /2019**	
11	Determination of **Total Nitrogen (Protein) in plants** by **Micro Kjeldahl Method.**		**/ /2019**	
12	Estimation of **Total Phosphorous from plant** sample (**Vando Molybadte Method),** Total		**/ /2019**	

	Potassium, Secondary and micronutrients like Fe, Zn, Mn and Cu from plant sample.			
13	Fertilizer Adulteration test / Identification of Adulteration in fertilizer / Detection of adulteration in fertilizers (**Rapid test**)		**/ /2019**	
14	Determination of nitrate nitrogen content of potassium nitrate by **Kjeldahl's method.**		**/ /2019**	
15	Determination of water soluble phosphorus in superphosphate **(Pumberton method)**		**/ /2019**	
16	Determination of acid soluble phosphorus from rock phosphate **(Pumberton method)**		**/ /2019**	
17	Determination of total potassium content of muriate of potash (**flame photometer**).		**/ /2019**	
18	Determination of Zinc content from micronutrient fertilizer (**EDTA Method**).		**/ /2019**	

CERTIFICATE

This is certify that, Mr./Miss _____

Registration No._____ Semester _____ has completed the practical works in SSAC-353 (Manures, Fertilizers and Soil Fertility Management) during the academic session _____.
His/Her performance duly the semester was satisfactory.

Place :R.A.C AKLUJ

Date : / /2019. Course Teacher

Basic terms in Soil Chemistry ***Date :- / /2019***

1) Mass:-it is the definite property which can be used as measure of quantity.

2) Weight:-it is the force from the interaction of the gravitational field on the substance.

3) Atom:-Smallest particle of element which retain its property and take part in chemical reaction.

4) Atomic weight :-

Atomic weight of an element is the **relative weight of the atom** on the basis of oxygen as 16.

Molecule:-Smallest part of the substance which retain the property of the substance and exist in the free state.

5) Molecular weight :-

The **sum of the atomic weights** of all the atoms in a molecule is its molecular weight.

e.g. Molecular weight of H_2SO_4

H = 2 x 1 = 2

S = 1 x 32 = 32

O = 4 x 16 = 64

= 98

6) Equivalent weight :-

A equivalent weight of a substance is that weight equivalent in reacting power to an atom of hydrogen.

OR

Equivalent weight of a substance is the number of gram of the substance required to react with, replace or furnish one of H^+ or OH^-.

e.g. Equivalent weight of H_2SO_4 is 49 since, H_2SO_4 contains two replicable hydrogen's, eq. wt = 98/2 = 49.

7) Titration :-

The process of adding solution of known strength to another solution that the reaction is just completed is known as titration.

8) Titre :-

A titre is defined as the weight of solute contained in a ml. of solution or the equivalent weight of any substance which will react with or be equivalent to 1 ml. of the solution.

9) End point :-

The point at which the reaction is complete is termed the end point (the equivalence point or stoichometric point). The end point at which the titration is just completed can be found out by some change in coloration or a coloured precipitate developed during the raction either by one of the reagent added or by the addition of an reagent known as the indicator.

1. **Indicator :-**

Indicator indicates the end point generally by a change of colour of the solution. Indicator is that substance which indicates the physico-chemical reaction of a chemical.

pH range	Indicator	Acid colour	Basic colour
3.1 to 4.4	Methyl orange	Pink	Yellow
4.2 to 6.2	Methy red	Red	Yellow
6 to 7.6	Bromothymol blue	Yellow	Blue
8.2 to 10.0	Phenolphthalein		Pink

10) Solution :-

It is the resulting mixture of solute and solvent.

11) Solute :- The substance to the dissolved in solvents.

12) Solvent :- The substance in which the solute is dissolved.

Standard solution :-

It is a solution which contains known weight of a reagent in a definite volume of the solution.

a) Normal solution :-

A normal solution is that contains one equivalent weight expressed in grams (one gram equivalent weight) of dissolved substance per liter of solution, or one gram – milliequivalanent.

b) Normality :-

It is the number of gram molecular weight of the reagent contained in 1.0 lit. of solution.

e.g. 1.0 lit of solution containing 53 g (eq.wt.) of Na_2CO_3 has normality of 1 N.

c) Molarity :-

It is the number of gram molecular weight of the reagent contained in 1.0 lit. of solution.

d) Molality :-

It is the number of gram molecular weight of the reagent contained in 1000 g of the solvent (w/2). Eq. 1000 g solvent containing 40 g of HaOH has got molaity of 1 M.

e) PPM solution:-Gram of solute per million milliliters of solution. OR milligram of solute per Lit of solution.(1ppm=1mg/lit)

f) Per cent solution :- Amount of solute in gram or in ml dissolved in the solvent and volume made to 100 ml or 100 g is called per cent solution.

g) Mole :- It is defined as the molecular weight of a compound in grams. The basic S1 unit of quantitatively the mole, which gives the amount of substance present in flask or test tubes.

Exercise No.-1 ***Date :- / /2019***

Principle and application of Spectro-photometry/Colorimetry

Principle :-

Colorimetry is the determination of the concentration of a substance by measuring the relative absorption, or transmission of light with respect to a known concentration. Colorimetric analysis is based on the measurement of intensity of radiant energy after it passes through a sample solution. A monochromatic light beam of known intensity is passed through the test solution and the intensity of the transmitted beam is determined with the help of a photo-electric cell. Thus, colorimetry can be considered as the absorption spectrophotometer in the visible range. It is based on Beer's law, which states that the intensity of a monochromatic light beam decreases exponentially as the concentration of absorbing substance increases arithmetically as expressed below.

$\log 1_0/l_1 = kC$

where 1_0 and l_1 are the intensities of the incident radiation and transmitted radiation, respectively, C denotes the concentration in solution and k is a constant.

The above relationship holds good only when the light is of the wavelength at which its absorption is maximum by the sample. Therefore, monochromation of the incident light through a suitable filter or grating is an essential pre-requisite for colorimetry or absorption sprectrophotometry (Willard et. Al.,1965). The Beer-Lambert's law relates the concentration is the logarithm of the ratio of the intensities of incident and transmitted radiations. Hence, the absolute intensities sites need not be determined.

The colorimeter tube acts as a cylindrical lens, converging the light to a sharp line on the photocell. If the tube is not exactly circular in cross section, its focal length changes at different angles. This leads to a change in the intensity of the light falling on the photo-cell. A perfectly circulate tube when filled with distilled water will not show any deflection of the galvanometer needle at any angle of tube rotation.

Type of colorimeters

Absorption photometers may be classified as visual comparators, filter, photometers and spectrophotometers. On the basis of their construction, these could be either single beam or double beam type. Also the instruments may be of direct reading type or based on balanced circuit. Special features of some instruments are the double monochromator and dual wave-length monochromators, plus automatic recording (Willard et. Al., 1965). While selecting an instrument one should consider the initial and maintenance cost, flexibility of operation and adaptability to varying situation.

Visual Comparators

The simplest colour comparators used side by side viewing of light coming from a common source through a pair of matching, flat bottom tubes, one containing the test solution and the other the standard. On matching the two, the ratio of the vertical heights of the solution would be inversely related in the ratio of their intensity.

Filter Photometer

In a single beam direct reading photo-electric colorimeter the optical path is from the light source through the filter and sample holder to the detector. The light passes through the solution and then strikes the surface of the barrier layer cell, the output of which is measured as deflection of the galvanometer. EEL colorimeter, Unicam SP 300, Hilger Biochem and Evelyn photoelectric colorimeter. Leitz photometer and Lumetton colorimeter model 450 are the examples of this type. Besides these, some indigenously made instruments e.g. AIMIL, ELICO, Systronics are also available under this category.

Double beam colorimeters employ barrier layer matching photo-cells and the incident filtered light is divided into two parallel beams one passing through the solution in the tube/curette before falling on the photo-cell and the other passing directly on to the reference photo-cell through an adjustable slit. Fisher electro photometer Klett-Summersion photo-electric colorimeter and Lumetron photo-electric colorimeter model 402 belong to the category. A colorimeter essentially has i) a light source, usually is tungsten lamp. ii) a monochromatic (filter prism or grating) as dispersion device.

iii) absorption cell and iv) a photometric system of measuring the light intensity as electric current.

Spectrophotometer

There is no difference between the basis principles of ordinary filter colorimeter and spectrophotometer. In the spectrophotometers a more refined monochromatic is employed and the measurement may be made slightly beyond the visible region of the electromagnetic spectrum. The wavelength ranges from 375 to 650 nm and can be extended to 950 nm by adding a red filter and exchanging the photo-tubes. The effective band width is 20 nm.

Care and Maintenance- For a better service and maintenance of the instrument follow the important tips given below :-

1. Do not disturb the galvanometer assembly.

2. Protect the instrument from mechanical shocks. Keep it on a vibration free surface.

3. Consult a competent engineer, if the instrument has developed some fault.

4. If the projection lamp fails to light and if there is a dangling wire it is a sign of damaged bulb. Replace it with the same wattage bulb. If the bulb filament is intact set righ the loose connections, if any.

5. If the photo-cells filed in the instrument lose their sensitivity on prolonged use or for any other reason, clean them or get them replaced. Never open them.

6. For replacement of photo-cell contact any competent firm undertaking the jobs of repairs/sercie.

7. Use an automatic voltage stabilizer in the AC supply line.

8. Always place the colorimeter tube (absorption cell) gently in order to avoid scratches.

9. Ensure that the outer surface of the tube/curette is absolutely clean and no liquid is sticking to it before placing in the socket.

Reference- Practical manual on Soil water and plant analysis by Dhyan Singh et. al.

Exercise No.-3 *Date :- / /2019*

Principle and application of Flame photometry and Atomic Absorption Spectrophotometer(AAS)

Flame photometer :-

Atomic emission spectrophotometer using flame photometers has been extensively used for the estimation of the **alkali metals (Na & K)** and **alkaline earth metals (Ca & Mg)** in soil, plant and water samples. The precision and accuracy for Na and K determination using flame photometer are better than those for Ca and Mg. The use of improved burner systems, high dispersion monochromators and nitrous oxide-acetylene flame has markedly improved the performance of this technique by improving precision and detection limit capabilities for may other elements while at the same time minimizing certain types of interferences.

The development of AAS and ICP-AES instruments, despite their higher cost have completely replaced flame photometers in many laboratories in the advanced countries. But flame photometry has advantages of comparatively much less initial and maintenance/running costs because of its simple optical system and the operation procedures are well suited to the laboratories that can not afford costly equipment.

Principle

When a solution of a salt is sprayed into a flame the salt breaks into the component atoms due to high temperature. The energy provided by the flame excites the orbital electrons to higher energy levels. When these electrons return to their ground state, they emit a characteristic radiation. Flame emission spectrophotometers are designed to measure the intensity of the characteristic light (resonance line) emitted from the excited atoms or ions of its element to be determined as opposed TO AAS which involves measurement of the absorption of radiant energy by excited atoms (Rich 1965). Each excited individual atom emits one quantum of radiation when it returns to the ground state. Therefore, the increase in the intensity of radiation emitted from steady and non-luminous flame will be proportional to the number of stomps of the particular element in the flame i.e. its concentration. This concentration is directly related to the content of the element in the test solution. Like most of the analytical techniques, this method is also not absolute but comparative and concentration of the analyte in the sample is to be computed from the calibration curve.

The instrumental set-up consists of three parts.

1) Atomization assembly comprising a nebulizer (atomizer) to produce a fine mist from the solution and a burner which vaporizes the analyte (in atomized form).

2) Monochromation system (filter, prism) that separates out the analytical wavelength from the other radiations.

3) Photometric system for measuring the intensity of the emitted radiation.

Types of Flame Photometers

Flame photometers are two types – single beam and double beam.

Single beam type :- A single beam equipment commonly referred to as direct reading type comprises only one set of optics. Light emitted from the conc of the flame just above the inner cone is collected by a reflector and focused by a lens of heat resistant glass through interchangeable optical filters (interference type filter) on to a single photo-detector. Alternatively, light from the burner passes into a monochromator and radiation leaving the exit slit is focused on to the photo-detector unit.

Double beam type :- In the double beam type instrument, a second light path is provided for the radiation emitted by the internal standard element (e.g. lithium) that is added in the fixed amount to each test solution as well as calibration standard. The wavelengths of internal standard and analytical materials are isolated by means of special filters in a dual optical system and each beam is focused on separate photoelectric cell.

The various types of flame photometer available commercially are classified into four main categories viz., (i) direct – reading, absorption filters, barrier-layer cell (Cole man, Evans-Electroselenium, Eel-Corning models 2 and 3, Schukhnecht, systronics, EUCO); (ii) Internal standard, interference filters, barrier-layer cell (Analytical Instruments, Baird model KY-2, Electrosynthese, lankc, lange model 4, Noreico Patwin, Barclay); (iii) prism monochromator, direct reading or recording, photomultiplier tube (Beackman models B-DB and DU, Unicam SP 900, Hilger UV Spek, Perkin-lElmer model 52C); and (iv) grating monochromator, recording photomultiplier tube Jerrell-Ash, Ebert; Hiltachi, Perkin-Elmer model 139).

Operation of Flame Photometer

While the ordinary type of manual flame photometers can be operated “with great ease, modern flame photometers are operated with the help of computer software and necessary operational parameters and can be set through keyboard commands. The actual steps involved may vary with the type of the instrument. Therefore, the users are advised to consult the manual provided by the manufacturer. However, the common steps required are given below.

1. Select the desired optical filter (K, Na or Ca).
2. Switch on the instrument.
3. Switch on the compressor to get air supply.
4. Adjust the air control knob to give a pressure as prescribed in the operation manual.
5. Leave the instrument for about five minutes to bet stabilized.

6. Turn on the gas supply and light the flame.

7. Adjust the gas control until formation of separate stable blue colour cones in the flame without sound.

8. Set the galvanometer to zero by means of the control knob against a reagent blank solution (medium).

9. Set the galvanometer to 100 with the highest concentration of standard solution.

10. Repeat the last two steps till the above setting is obtained without further adjustment.

11. Proceed with the determination of the remaining standards and samples.

12. For switching of the instrument close the gas supply first, run distilled water for about 2 minutes more and then switch off the compressor. Turn-off all the knobs and switches.

Precautions

1. Keep the standards and samples free from any suspended material to prevent clogging of the nebulizer and the capillary.

2. Do not feed highly concentrated acid and salt solution.

3. Maintain a constant supply of air-gas mixture to the burner.

4. Do not leave the flame unattended.

5. Periodically replace the pressure tubing of the fuel gas.

Reference

Practical manual on Soil, water and plant analysis by Dhyan Sing et. al.

Atomic Absorption Spectrophotometer :-

Principle :-

In the analysis employing Atomic Absorption spectrophotometer (AAS), the sample in the from of a homogeneous liquid is aspirated into a flame where “free” atoms of the element to be analysed are created. A light source (hollow cathode lamp) is used to excite the free atoms formed in the falme by the absorption of the electromagnetic radiation. The decrease in energy (absorption) is then measured which follows the Lambet-Beer law, i.e. the absorbance is proportional to the number of free atoms in the ground state (Baker and Suhr 1982).

Most of the leading instrument manufacturers have launched tandem models of AAS with interchangeable flame and graphite furnace versions. The graphite furnance has added

advantage of analyzing larger number of elements including mercury, arsenic and selenium with greater precision upto parts per billion (ppb) level. With the help of on-line dilution and calibration in the fully PC-based models, the sample throughout has increased tremendously, while minimizing the human-error at various steps.

Preparation of standards and sample solutions

Prepare stock standards in concentration of **1000 mg L^{-1}** from pure metal wire, granules, foil, metal oxides or other suitable compounds of the elements s given in appendix X is not enclosed. Make the sample solutions free from interfering elements and suspended solids that may cause clogging of the nebulizer. Adjust both standards and unknowns to a concentration range, which a compatible with the analytical range of the instrument.

Operational Steps of AAS

1. Check for the proper fittings of all the tubings, connections, required type of burner (air-acetylene or nitrous oxide-acetylene), hollow cathodes lamps etc.
2. Fill the liquid trap with the solvent (medium) used for the analysts.
3. Align the hollow cathode lamp of the lament to be analyzed with the optical path of the instrument by rotating the lamp turret.
4. Switch on the instrument and allow at least 30 minutes for warm up.
5. Switch on the deuterium lamp for background correction which is generally required when the wavelength of the resonance line of the element is less than 250 nm.
6. Use lamp current recommended by the lamp manufacturer.
7. Select the desirable wavelength and the band pass width or slit width or slit width.
8. Optimise burner position by using vertical, horizontal and rotational adjustment knobs util the burner slot is aligned with the beam and just below the position from where it start blocking the light path.
9. Switch on the compressor to get air supply in case of air-acetylene flame. If N_2O-acetylene flame is used, turn on the N_2O supply cylinder. Select air with the support selector Knob. Adjust the support flow (air) reading between 6 and 9 flow units.
10. If nitrous oxide-acetylene flame is used, first ignite an air-acetylene flame and then change over to a nitrous oxide-acetylene flame.
11. Turn on the main gas supply from the cylinder, followed by fuel-control knob of the instrument and light the flame immediately.

12. Adjust the fuel control (acetylene) and support control (air or nitrous oxide) knobs to produce required kind of flame of air-acetylene or nitrous oxide-acetylene flame.

13. Set the instrument to zero against a reagent "blank" solution.

14. Feed a standard (or sample) and optimize fuel, oxidant and sample flow rates by adjusting fuel knob, fuel support knob and nebulizer so that a maximum signal (absorbance) is achieved.

15. Prepare calibration curve by recording absorbance of series of working standards. The calibration must be done fresh for each set of samples.

16. In case the instrument shows a sign of high or over or error, dilute the samples depending on the absorbance of the sample and feed the sample again.

17. If the instrument has been used in higher concentration range, run distilled water until the reading returns to zero, before closing down.

Steps for Switching-off AAS

1. Turn-off the gas supply from the cylinder first.

2. Wait for extinction of the flame and then turn off the fuel control knob.

3. Turn-off the air compressor and fuel support knob.

4. The shut-down sequence for a nitrous oxide-acetylene flame involves first changing over to an air-acetylene flame and then extinguishing it.

5. Switch-off the instrument.

Precautions

1. Acetylene cylinder should always be used in a vertical position to prevent liquid acetone entering the gas line.

2. Acetylene cylinders should not be run at a pressure lower than 500 kpa (70 psi). Never operate Acetylene lines above **100 kpa (15psi).** At a higher pressure acetylene can spontaneously decompose or explode.

3. Never run the nitrous oxide-acetylene flame without red filter visible or with less than 5 flow units of acetylene.

4. Do not leave uncovered containers of the volatile organic solvents near the uncovered flame.

5. Do not look at flame without the aid of safety glasses or the flame shield.

6. Do not leave the flame completely unattended.

7. Do not ignite the flame if the air flow is below 6 flow units.

8. Do not adjust the air (or N_2O) and gas supply to alter the sensitivity of the instrument after the calibration of the instrument.

Reference Practical manual on Soil water and plant analysis by Dhyan Singh et. al.

Exercise No.-3 *Date :- / /2019*

Determination of moisture from organic manure

Principle :-

The fertilizer release free water and absorbed / adsorbed moisture on heating at varying temperatures. Some fertilizers do not give volatile substances at drying temperature. However, some fertilizers yield volatile substances other than water on drying in oven or at $105^{O}C$. Based on the nature of manures and fertilizers, different methods for moisture determination i.e. oven dry method, vacuum desiccator method and Karl Fisher titration method are used. Moisture in liquid fertilizers is determined by evaporation method.

Solid fertilizer :- 1, 2, 3,

Liquid fertilizer :- evaporation method

1) Oven dry method :-

This method is used for stable substances which do not give volatile substances at $105^{O}C$ like manures, mixed fertilizers, fertilizers other than urea and ammonical fertilizers like DAP, CAN and nitrophosphates etc. are not analyzed by this method.

Procedure :-

Take about 5 gm of sample in a pre-weighed porcelain or silica or glass dish. Keep the dish in hot air oven maintained at $100 \pm 2^{O}C$, for about 4 hours. Cool in desicator and weigh accurately. For sodium nitrate, ammonium sulphate and potassium salts, heat upto $130 \pm 2^{O}C$ .Calculate moisture as percentage loss in weight and report on fresh weight basis.

Observations

1) Weight of empty dish	=	X	=	gm
2) Weight of dish + sample	=	Y	=	gm
3) Weight of dish + sample after drying	=	Z	=	gm

Calculation :-

$$\text{Per cent moisture in sample by Weight} = \frac{Y - Z}{Y - X} \times 100$$

Where,

X = weight of empty dish Y = weight of dish + sample Z = weight of dish and sample after drying.

2) Vacuum desiccator method :-

This method is used for substances that yield volatile substances other than water at drying temperature (examples ammonium chloride, ammonium nitrate, diammonium phosphate, urea, calcium ammo. Nitrate, potassium nitrate).

Procedure :-

Weigh accurately 5.0 gm sample in a pre weighed dish place the dish in vacuum oven at $30^{O}C$ or vacuum desiccator over previously boiled concentrated H_2SO_4 for 24 hrs. Now weigh again.

Calculation :-

$$\text{Percent moisture in sample by weight} = \frac{Y - Z}{Y - X} \times 100$$

Where,

X = Weight of empty dish Y= Weight of dish and sample

Z = Weight of dish and sample after drying in vacuum desiccator or vacuum oven.

Reference :-

1. P.K. Gupta (2004) Soil, Plant Water and Fertilizer Analysis. pp. 376-377
2. Shivanand Tolanur (2004). Practical Soil science & Agril. Chemistry. pp. 142-143.
3. A.K. Gupta (2007). Practical manual of Agricultural Chemistry. pp. 90-91.

Exercise No.-4 *Date :- / /2019*

Determination of organic matter from compost / FYM / oil cake (*Ignition method*)

Principle :-

The organic matter content of the sample is ignited (Burnt) in muffle furnace at $550^{O}C$. From loss in weight the per cent organic matter is calculated and from this per cent organic matter value per cent organic carbon is calculated.

Procedure :-

1. Weigh 2-3 gm sample in silica crucible
2. Keep it on burner (low flame) until sample is charred.
3. Transfer the silica crucible into muffle furnace and heat at $550^{O}C$ until greyish white ash is obtained.
4. Remove and cool it in a desiceator and weigh.
5. The residue represents the ash i.e. mineral matter and the loss in weight represents the moisture and organic matter.
6. Calculate the per cent organic carbon from per cent organic matter.

Observations :-

1. Wt. Of silica crucible = W_1
2. Wt. Of silica crucible + sample = W_2
3. Wt of sample = (W_2-W_1)
4. Wt. Of silica dish + ash (after igrotion) = W_3
5. Wt of ash = W_3-W_1

Calculation :-

$$\% \text{ ash on original basis} = \frac{\text{Wt. Of ash}}{\textit{Wt. Of sample}} \times 100$$

$$Z = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

% organic matter = 100 – (z + % moisture) …….oven dry basis.

% organic carbon = organic matter x 0.58* ……oven dry basis.

* organic matter is assumed to contain 58 per cent organic carbon.

Reference :-

1. Chopra and Kanwar (1980). Analytical Agricultural chemistry pp. 156.

Exercise No.-5 *Date :- / /2019*

Estimation of available Nitrogen in soil by *Alkaline Permanganate Method.(Subbiah and Asija,1956)*

Principle

The organic matter in the soil is oxidized by $KMnO_4$ in presence of NaOH. The ammonia released during oxidation is absorbed in boric acid to convert the ammonia to ammonium borate. The ammonium borate formed is titrated with std. H_2SO_4. From the volume of std. H_2SO_4 required for reaction with ammonium borate the nitrogen is calculated.

Reagents

i) Potassium permanganate $KMnO_4$ (0.32%) : Dissolve 3.2 g of potassium permanganate in 1 litre of distilled water with the intermittent shaking till it is completely dissolved. Store it in amber coloured bottle in dark.

ii) Sodium hydroxide, NaOH (2.5%) :- Dissolve 25 g of pure sodium hydroxide pellets in one litre of distilled water.

iii) Boric acid H_3BO_3 (2%) :- Dissolve 20 g Boric acid of A.R. Grade in 800 ml distilled water by heating the content, cool it and dilute to 1000 ml. volume.

iv) **Mixed indicator** (**Bromocresol green + Methyl red)** :- Weigh out separately 99 mg of bromocresol green and 66 mg of well powdered methyl red and dissolve them together in 100 ml ethyl alcohol.

v) Working boric acid solution :- Add 20 ml of mixed indicator to one litre of 2% boric acid solution and adjust the pH to 5.0 after shaking or add 0.1 N NaOH continuously until the solution assumes reddish purple tinge/wine red colour appears.

vi) Std. Sulphuric acid H_2SO_4 (0.02 N) :- Standardise the H_2SO_4 soultion using std. NaOH. NaOH should be standardized against 0.02 N $H_2C_2O_4$ or 0.02 N potassuium phthalate.

vii) Liquid paraffin.

viii) Glass beads.

Procedure

1. Weight of 20 g soil, transfer it into distillation flask (1000 ml round bottom flask).
2. Add to it 20 ml. distilled water with the help of jet in such a way that the particles of soil do not remain stuck to the sides of the flask.

3. Add 2 to 3 glass beads to **prevent bumping** and 1 ml of liquid paraffin to **prevent frothing**.

4. Add 100 ml of potassium permanganate (0.32%) and 100 ml of sodium hydroxide solution (2.5%) to the flask.

5. Stopper the flask immediately and start distillation. The tip of condenser should dip in the 20 ml of boric acid solution (with mixed indicator) in the beaker, on heating ammonia will be liberated which will be absorbed in boric acid. The original wire red/pink red colour turns to green with absorption of ammonia.

6. Collect nearly 100 ml of the distillate in about 30 minutes and titrate with 0.02 N H_2SO_4 to the original pink red/wine red colour and record the burette reading.

7. Run a blank without soil and use the reading.

8. Express results on over dry basis.

Reactions

1. $2KMnO_4$ Na0H $K_2O + 2MnO_2 + 3$ O (nescent oxygen)

   The oxygen evolved from the $KMnO_4$ xidizes, organic matter.

2. Organic matter + 3 O Release $R - NH_2$ (amines)

3. $R\ NH_2 + H_2O$ Hydrdysis $R - OH + NH_3$

4. $NH_3 + H_2O$ Release NH_4OH

5. $H_3BO_3 + 3\ NH_4OH = (NH_4)_3 + Bo_3 + 3\ H_2O$ ammonium borate

   Green colour during ammonia absorption.

6. Ammonium borate formed is further titrated with standard H_2SO_4

   $2(NH_4)_3\ BO_3 + 3\ H_2SO_4 = 3\ (NH_4)_2\ SO_4 + 2H_3\ BO_3$

   Wine red colour end point.

Observation

1. Weight of the soil sample taken :- 20 g

2. Volume of std. H_2SO_4 required for sample X :- ------ml

3. Volume of std. H_2SO_4 required for blank Y :- ------ml

4. Normality of H_2SO_4 :- 0.02N

Calculations

$$\text{Available nitrogen (kg/ha)} = (X - Y) \times N \text{ of } H_2SO_4 \times 0.014 \times \frac{2.24 \times 10^6}{\text{Wt. of soil sample taken (g)}}$$

1 ml. 1 NH_2SO_4 = 0.014 g nitrogen.

From the reactions above the relationship is

$3H_2SO_4$	=	$2(NH_4)_3BO_3$	=	$6\ NH_3$
$3H_2SO_4$	=	$6\ NH_3$	=	$6NH_3$
H_2SO_4	=	$2NH_3$	=	$2\ N$ or N_2
1 Mole of H_2SO_4	=	2 moles of NH_3	=	1 mole of N_2
98 g H_2SO_4	=	2 x 14	=	28g N
49 g H_2SO_4 (eq. Wt. of H_2SO_4)		=	14 g N.	
1000 ml 1 N H2SO4			=	14 g N
1 ml 1 N H2SO4			=	0.014 g N

Rating for soil available nitrogen

Based on the observed value, the soil can be rated in the following categories :-

Very low	:- **Less than 140 kg N/ha**
Low	:- **141-280 kg/ha**
Medium	:- **281-420 kg/ha**
Moderately high	:- **421-560 kg/ha**
High	:- **561-700 kg/ha**
Very high	:- **Greater than 700 kg/ha**

Questions

1) What do you mean by available N in soil?

2) Explain the principle involved in the estimation of available nitrogen from soil?

3) Explain the role of $KMnO_4$, NaOH, boric acid and mixed indicator in the estimation.

4) What precautions are to be taken while carrying out distillation?

5) What is the weight of 1 hecatre soil in kg.

Reference books

1. Subbaih, B.V. and G.L. Asija (1956). A rapid procedure for the estimation of available nitrogen in soil. Curr. Sci. 25 : 259-260.
2. Black C.A. 1965. Methods of soil analysis. Part 2 Amer. Soc. Agron, inc. Soil Sci. Sco. Amer. Madizon Wisconsin, USA.
3. Saharwat K.L. and J.R.Burford (1980) : Soil Science 133 : 53-57.

Exercise No.-6 ***Date :- / /2019***

Estimation of available Phosphorous Content in soil by using *spectrophotometer (Olsen's method)*

Phosphorus in soils ranges from **0.01 to 0.3 per cent** and occurs in several forms and combinations. The apetite group of primary minerals is the original source of about 95 per cent or more of the soil phosphours. The different phosphate compounds in soils can be generally classed as fluoro carbonate and hydroxyl phosphate of Fe, Al, Ti, Mn, Ca and Mg of which the Fe, Al and Ca – phosphates are the most important ones quantitatively. The total amount of phosphorus present in soil is not available to the plants only small fraction of it may be available which is of direct relevance in assessing the phosphorus fertility levels.

Several chemical tests for available P has been proposed by various workers which extracts variable quantities of phosphorus. Most commonly used methods for determination of plant available phosphorus in soil are

1. The Olsen's method used for neutral – alkaline soils (Olsen et. Al. 1954)
2. The Bray and Kurtz method used for acid soils (Bray and Kurtz, 1945)

Olsen's method or sodium bicarbonate extractable – P

Sodium bicarbonate ($NaHCO_3$) of pH 8.5 is used as an extractant in this method.

Principle

Phosphorus is extracted from the soil with **0.5 M NaHCO3** at nearly constant **pH 8.5**. In calcareous, alkaline or neutral soil containing calcium phosphate, this extractant decreases the concentration of calcium in solution by causing precipitation of calcium as calcium carbonate, as a result, the reactive (high specific surface) from of phosphorus is extracted from the phosphate of iron, aluminum and calcium present in soil.

Phosphate in the extract is measured by the reaction of phosphate with ammonium molybadte in an acid medium to form molybdophosphorc acid. The molybdophosphoric is then reduced to a blue colured complex (reduced **phosphomolybdenum blue**) through reaction with ascorbic acid. Absorbance readings are taken at **882 nm using spectorophotometer**.

$$H_2PO_4 + 12H_2M_oO_4 \qquad H_2P(M_{o3}O_{10})_4 + 12H_2O$$

Reagents

1. Sodium bicarbonate ($NaHCO_3$) 0.5 M extracting solution : Dissovle 42 g of $NaHCO_3$ in 1000 ml of distilled water. Mixed thoroughly. Adjust the pH of the solution to 8.5 with 1 M NaOH.

2. Darco – G 60 or equivalent grade phosphorus free charcoal : Phosphorus fee Darco – G – 60 charcoal is available in the market which can be used directly after testing.

3. Standard solution (Stock solution) : Analytical grade KH_2PO_4 is dried in an oven at 60^0C for one hour and after cooling in desiccator, weight 0.4387 g and dissolve in about 500 ml distilled water. Add 25 mL of 7 N H_2SO_4 to it and volume is made upto 1 litre with distilled water. Add 5 drops of tolune to diminish microbial activity. This gives 100 ppm stock solution of P (100 ug mL^{-1}).

4. Dilute 'P' solution (working solution) : From the above stock solution a 2 ug P mL^{-1} working solution is made. Pipette out 20 mL of stock solution of P and volume is made upto 1 litre with distilled water. This solution contains 2 ug P mL^{-1}.

5. Solution – 4

    a. Dissolve 6 g ammonium molybdate [(NH_4) 6 $MO_7O_{24}.4H_2O$] in 125-150 ml warm distilled water.

    b. Dissolve 0.145 antimony potassium tartarate in 50 ml distilled water and make 500 ml volume.

    c. Prepare 500 ml of 5 N H_2SO_4. Add slowly 70.5 ml conc. H_2SO_4 in distilled water and make 500 ml volume.

    Add cooled ammonium molybadate (solution 'a') to 500 ml 5 N H2SO4 solution, followed by cooled antimony potassium tartatarate (solution – b). Volume is made up to 1 litre. Thus, a + b +c forms solutin – A. this is also called acid molybdate stock solution.

6. Solution – B : Dissolve 1.056 g ascorbic acid (LR) in 200 mL of solution – A. this solution is prepared fresh as and when required just prior to colour development.

7. Solution C : 0.25% P – nitrophenol $NO_2C_6H_4OH$ (250 mg/100 mL)

8. Solution – D : 5 N H_2SO_4.

Procedure

I) Extraction of P

1.Take 2.5 g of soil in 250 ml conical flask.

2.Add one teaspoon of carbon black or equivalent grade of activated charcoal (Free of P).

3.Add 50 ml of the 0.5 M NaHCO3 (pH 8.5

4.shake the flask for 30 minutes on platform shaker.

5.Filter the suspension immediately through dry filter paper (whatman No.1) in to clean and dry beakers.

6.Shake the flask immediately before pouring the suspension into funnel. Add more carbon black to obtain clear filtrate.

7) A blank is run without soil.

II)Colour development

1. Pipette out 5 ml $NaHCO_3$ extract into 25 ml volumetric flask.
2. Add 2 drops of **P-nitrophenol** indicator, **acidified with 5 N H2SO4 drop** with intermittent shaking till yellow colour disappears at pH 5.0.
3. Dilute the contents to about 20 ml with distilled water and then add 4 L solution – B, L ascorbic acid plus solution – A. Make volume to 25 ml with distilled water and mix the content.
4. Run a blank without soil.
5. Make upt the volume and **measure the intensity of blue colour at 882 nm** after 30 minutes.

The colour is more stable than with stannous chloride reduction method.

III) Preparation of standard curve

1. Prepare a series of standards by taking 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of 2 μg mL^{-1} 'P' solution in 25 ml volumetric flasks. These solutions contains 0.08, 0.16, 0.24, 0.32, 0.40, 0.48, 0.56, 0.64, 0.72 and 0.80 μg P mL^{-1} respectively.
2. Add 5 mL of 0.5 M NaHCO3 solution ot each flask and adjust the pH to 5 with 5 N H2SO4 by using P – nitrophenol.
3. Develop the colour and record the per cent transmittance, (T) reading as above, convert per cent transmittance in to absorbance. (A = 2 – lgo T).

4. Construct a graph by plotting reading on 'Y' axis and concentration of P in μgmL^{-1} on 'X' axis.

Calculation :-

$$\text{Soil available Phosphorus (kg ha}^{-1}) = \frac{R}{10^6} \times \text{Vol. of extractent added} \times \frac{\text{coloured sol}^n \text{ made}}{\text{Vol. of filtrate taken}} \times \frac{2.24 \times 10^6}{\text{Weight of soil}}$$

$$\text{Soil available Phosphorus } (kg\ ha^{-1}) = \frac{R}{10^6} \times 50 \times \frac{25}{5} \times \frac{2.24 \times 10^6}{2.5}$$

Soil available Phosphorus $(kg\ ha^{-1}) = R \times 1.12$

Where R = μg P/mL from standard curve.

Rating

Sr. No.	Category	Soil available nitrogen ($kg\ ha^{-1}$)
1	Very low	**0 – 7**
2	Low	**7 – 14**
3	Moderate	**14 – 21**
4	Moderately high	**21 – 28**
5	High	**28 – 35**
6	Very High	**More than 35**

II)Bray's method

In is method the soil phosphorus is extracted with the dilute acid fluoride solution. Easily acid soluble P from phosphates bound to Al, Fe and Ca is extracted from the soil. From the extract phosphorous is then determined colorimetrically using phosphomolybdenum blue using ascorbic acid as a reducing agent and antimony gives a stable compound of MO-P-Sb.

Reagents

Extracting solution (0.03 N NH_4F in 0.25 N HCl solution) : Dissolve 2.137 g NH_4F in 2.5 litres of 0.024 N HCl, Store in polyethylene or glass bottle. Other reagents and preparation of standard curve are as per the Olsen's method.

Procedure

Weigh 5 g soil in 150 ml concial flask. Add 50 ml of extracting solution (1:10 soil to

solution ratio). Shake te suspension for 5 minutes and filter through Whatman No.42. To avoid the interference of fluoride 7.5 ml of 0.8 M boric acid (50 g boric acid/litre) should be added to 5 ml of the extract if necessary Pipette out 5 ml of extract in a 25 ml volumetric flask. Add 10-15 ml distilled water followed by 4 ml of Reagent B and make upto the volume. After 10 minutes read the intensity of blue colour as described in Olsen's method.

$$\text{Soil available Phosphorus } (kg\ ha^{-1}) = \frac{R}{10^6} \times \text{Vol. of extractent added} \times \frac{\text{coloured sol}^n \text{ made}}{\text{Vol. of filtrate taken}} \times \frac{2.24 \times 10^6}{\text{Weight of soil}}$$

$$\text{Soil available Phosphorus } (kg\ ha^{-1}) = \frac{R}{10^6} \times 50 \times \frac{25}{5} \times \frac{2.24 \times 10^6}{2.5}$$

$$\text{Soil available Phosphorus } (kg\ ha^{-1}) = R \times 1.12$$

Where R = μg P/mL from standard curve.

Questions

1. Explain the principle involved in the estimation of available phosphours.
2. What is Olsen's reagent? Explain its extraction behaviour.
3. Explain the role of following reagents.
    1) Activated charcoal (Darce G-60)
    2) Ascorbic acid
    3) 5 N H_2SO_4
    4) Para-nitro phenol
4. How will you prepare a standard curve for P determination? Explain in Brief.
5. What do you mean by available P in soil?

Reference Books

Ghosh et. al. (1983) Soil and Water method – A laboratory Manual pp.16-21.

Olsen, S.R., Coles C.V., Watanabe F.S. and Dean, L.A. (1954). Estimation of available phosphours in soil by extraction with sodium bicarbonate circ. U.S. Dept. Agric. : 939.

Watanabe, F.S. and Olsen, S.R. (1965). Test for ascorbic acid method for determining phosphorus in water and sodium bicarbonate extract of soil Proc. Soil. Sci. Am. 29 : 677-678.

Exercise No.-7 *Date :- / /2019*

Determination of available Potassium in soil by Ammonium Acetate Extractable method using Flame Photometer

Principle

The term available potassium incorporates both exchangeable and water soluble forms of the nutrient present in the soil. The readily exchangeable plus water soluble K is determined in the netural normal ammonium acetate extract of soil. The NH_4 ion provides a sharp and rapid separation from exchange complex. The concentration of K ions in the solution is then determined by using flame photometer.

Reagents and material required

1. Netural normal ammonium acetate extracting solution. : Dissolve 77.1 g reagent grade ammonium acetate in 900 ml. distilled water, adjust pH to 7.0 with 3 N acetic acid or 3N ammonium hydroxide. Dilute to 1 litre.
2. Standard Potassium stock solution (1000 ppm) : Dissolve 1.908 g oven dry AR grade crystals of

   KCl in distilled water make up volume to 1 liter. This solution contains 1000 mg. K/L. prepare 10 ppm K standard by dissolving 10 ml of 1000 ppm stock solution to one litre with distilled water.

Procedure

1. Weigh 5 g. soil and transfer it to 150 ml conical flask.
2. Add to it 25 ml extracting solution and shake it for exactly 5 minutes on a mechanical shaker.
3. Filter the contents immediately through ordinary filter paper and collect the filtrate in a beaker

   (Reject First few ml of filtrate).
4. Atomize the above extract on flame photometer and record the readings. Dilute if necessary.

Preparation of std. Curve for K

1. Pipette out 0, 1, 2, 4, 6, 8 and 10 ml. of 100 ppm solution into 100 ml volumetric flask and bring the volume to mark with extracting solution (Neutral N ammonium acetate) the solution contains 0, 1, 2, 4, 6, 8 and 10μ g K/ml.

2. Use potassium filter in a flame photometer, adjust the gas and air pressure.
3. Adjust the flame photometer at zero for the blank at zero ppm K and by 100 for 10 ppm K.
4. Construct the std. Curve by plotting the reading (on Y-axis) against the different concentrations (like 0,1,2,4,6,8 and 10 µg K/ml. (X-axis). Find out factor from straight ling graph.

N.B: Avoid fluctuation in gas and air pressure for steady reading of the meter.

Observations

Record the flame photometer readings for the standard curve and for the soil sample.

Calculations

$$\text{Soil available Potassium (kg ha}^{-1}\text{)} = \frac{R}{10^6} \times \text{Vol. of extractent added} \times \frac{2.24 \times 10^6}{\text{Weight of soil}} \times \text{Dilution Factor}$$

$$\text{Soil available Potassium (kg ha}^{-1}\text{)} = \frac{R}{10^6} \times 25 \times \frac{2.24 \times 10^6}{5} \times \text{Dilution Factor}$$

Soil available Potassium (kg ha^{-1}) = R X 11.2 X Dilution Factor

Where R = PPM of K in the extract (obtained from standard curve.)

Categories the soil using flowing rating chart

Very low	:-	**Less than 100 kg/ha**
Low	:-	**101-150 kg/ha**
Medium	:-	**151-200 kg/ha**
Moderately high	:-	**201-250 kg/ha**
High	:-	**251-300 kg/ha**
Very high	:-	**Greater than 300 kg/ha**

Questions

1. What do you understand by a term available potassium?
2. Explain the principle involved in the extraction of available K?
3. Why neutral normal ammonium acetate is used for extraction of K?
4. What care should be taken while recording the reading on flame photometer?
5. Explain the principle of flame photometer?

Reference Books

Page, Millier and Keeney (1989) : Methods of Soil Analysis Part II. Pp.228-238.

Ghosh et. al. (1983) : Soil and Water Testing Methods : Laboratory Manual. pp.21-22.

Exercise No.-8 *Date :- / /2019*

Determination of Exchangeable Ca & Mg in soil by *EDTA Method*

Principle:-

Exchangeable Ca and Mg can be determined in ammonium acetate extracts of soils by titration with EDTA (versenate). However, ammonium acetate and dispersed organic matter must be almost entirely removed from the soil extract prior to titration with EDTA (Versenate).

Evaporation of an aliquot of the soil extract to dryness flowed by treatment with aqua-regia (3 parts conc. HCl + 1 part conc HNO_3) and a second evaporation to dryness usually sufficies for the removal of ammonium acetate and organic matter. Very dark coloured soil extracts may require additional treatment with aqua regia. After treatment dissolve the residue in a quantity of distilled water equal to the original volume of the aliquot taken for treatment.

Equipments and Reagents

1. Neutral normal ammonium acetate (pH.7) – Dissolve 77.08 g of ammonium acetate into 900 mL distilled water mix thoroughly and adjust pH 7.0 with dilute ammonium hydroxide solution or dilute acetic acid solution as required and make up the volume with distilled water.

2. Concentrated nitric acid.

3. Concetrated hydrochloric acid.

4. Ammonium chloride-ammonium hydroxide buffer solution : Dissolve 67.75 g of ammonium chloride in 570 ml. of conc. ammonium hydroxide and make to 1 litre.

5. 4 N sodium hydroxide : Dissolve 160 g of NaOH in 1 Litre of water.

6. Standard calcium chloride solution (0.01 N) ; Dissolve 0.5 g of pure calcium carbonate in 10 ml of approximately 3 N (1 + 3) HC1 and dilute to a volume of exactly 1 litre.

7. Eri-chrome black T indicator : Dissolve 0.5 g of Eri-chrome black T and 4.5 g of hydroxylamine hydrochloride in 100 ml. of 95% ethanol.

8. Calcon indicator : Dissolve 20 mg. Of calcon in 50 mL of methanol prepare freshly before use.

9. Ethylene diamine tetra acetic acid (Versenate) solution (0.01 N) : Dissolve 2.00 g of disodium dihydrogen ethylene diamine tetra acetic acid and 0.05 g of magnesium chloride hexahydrate in water and dilute to a volume of 1 litre. Standardize the solution against standard $CaCl_2$ using the titration procedure given below. The solution should be standardized using each of the indicators separately for Ca and Ca + Mg.

Method

1. Place 5 g. air dried soil in 150 ml. Erlenmeyer flask and add 25 ml. neutral normal ammonium acetate.

2. Shake on mechanical shaker for 5 minutes and immediately filter through whatman paper No.1. First few ml. of filtrate may discarded. Ca & Mg concentration in the extract is determined after pre-treatment.

Pre-treatment of Soil Extract

1. Transfer ammonium acetate extract to a 250 ml beaker and evaporate to dryness on a hot plate or steam bath.

2. Wash down the walls of the beaker with a small quantity of water and again evaporate to dryness.

3. Add 1 ml of nitric acid and 3 ml of hydrochloric acid and again evaporate.

4. Dissolve the residue after evaporation in 20 ml of 0.1 N acetic acid.

5. Filter through Whatman filter paper (low ash content filter paper) into a 50 ml volumetric flask using water to wash the beaker and filter paper. Dilute upto volume.

Calcium

1. Pipette a 5 to 25 ml aliquot (obtained in after step 5) into a porcelain casserole. Dilute to a volume of approximately 25 ml. with distilled water.

2. Add 0.25 ml (5 drops) of 4 N sodium hydroxide and approximately 5-10 drops **calcon indicator**

   prepared freshly before used.

3. Titrate with 0.01 N EDTA using 10 ml micro burette. The colour changes from **wine red to blue.**

4. Record the volume of EDTA used.

Calcium + Magnesium

1. Pipette a 5 to 25 ml aliquot into (obtained in after step 5) a 125 ml. Erlenmeyer flask. Dilute to

   volume of approximately 25 ml. with distilled water.

2. Add 0.5 ml (10 drops) of ammonium chloride-ammonium hydroxide buffer and 3 or 4 drops of

   Erichrome blak T indicator.

3 .Titrate with 0.01 N EDTA using 10 ml micrbourette. The colour change is from **wine red to blue.** No tinge of the wine red colour should remain at the end point.

4. Record the volume of EDTA used.

Observations

1. Weight of soil sample
   :- 5 g.
2. Volume of netural N ammonium acetate extractant added :- 20 mL
3. Volume of aliquot taken of Ca estimation :- 10 mL.
4. Volume of 0.01 EDTA used in Ca titration R_1:- ml.
5. Volume of aliquot taken for Ca + Mg estimation :- 10 ml.
6. Volume of 0.01 EDTA used in Ca + Mg titration R:- ml.

Calculations :-

$$\text{Ca or Ca + Mg (me/litre)} = \frac{R \times \text{Normality of EDTA} \times 1000}{\text{Volume of aliquot taken}}$$

Where R or R_1 : Volume of standard EDTA used in titration (Ca or Ca + Mg).

$$\text{Ca or Ca + Mg (me/100 of soil)} = \text{Ca or Ca + Mg me/litre} \times \frac{\text{Volume of extraction Added}}{1000} \times \frac{100}{\text{Wt. of soil}}$$

Mg me/100 g soil = (Ca + Mg me/100 g soil – Ca me/100 g. soil)

Here volume of ammonium acetate extract taken (obtained after centrifugation or filtration) is 100 ml.

Exchangeable Ca or Ca + Mg = (Ammo acetate extractable Ca or Ca + Mg in me/100 g) - (Water soluble Ca or Ca + Mg in me/ 100 g)

Water soluble Ca or Ca + Mg in me/100 g = (Ca or Ca + Mg + saturation extract in me/l) X (Saturation percentage)/1000

Exercise No.-9 ***Date :- / /2019***

Estimation of available Sulphur in soil by *Turbidity Method*

Principle

In the method sulphate sulphur is extracted with Morgan's solution i.e. sodium acetate-acetic acid mixture from the extract the sulphate sulphur ion determined by precipitating as barium sulphate by adding barium chloride. Fin colloidal suspension of barium sulphate is stabilized by gum acacia. The resulting turbidity is measured by colorimetrically using blue filter.

Reagents

1. **Morgan's reagent (Extracting solution) :-** Dissolve 100 g sodium acetate in about 500 ml. water add 30 ml of 99.5% acetic acid and make the solution to a volume of one litre.
2. **Gum acacia (0.25%) :-** Dissolve 2.5 g gum acacia in a litre of distilled water. Keep over night and filter.
3. **Barium chloride powder :-** Ground $Bacl_2$ powder in an agate mortar until they pas through a 30 mesh sieve.
4. **Standard sulphate solution (100 pp) :-** Dissolve 0.543 g potassium sulphate in the extracting solution and dilute to one litre. This solution contains 100 mg S/mL.

Procedure

Weigh out 10 g air dried soil and transfer to a 250 ml conical flask. Add 50 ml Morgan's extracting solution and shake for one and half hour. Filter the contents through Whatman No.42 filter paper or centrifuge to remove the solid particles from the suspension. Transfer a 10 to 20

ml. aliquot of the extract to a 100 ml volumetric flask. Add 1.0 g of 30 to 60 mesh barium chloride crystals and shake for one minute then add 1.0 ml gum acacia solution. Make the flask contents up to volume and shake for one minute Again mix the contents thoroughly and immediately measure the degree of turbidity at 340 nm on spectrophotometer. Simultaneously prepare the standard curve using a series of standards as described as below.

Preparation of standard curve

From 100 ppm solution of sulphate Stake 0, 5, 10, 15 and 20 ml into 100 ml volumetric flask (containing 0, 5, 10, 15 and 20 mg S/ml). Add 1.0 g of 30-60 mesh barium chloride crystals and shake for one minutes. Then add 1 ml gum acacia solution : Make the flask contain upto volume and shake for 1 minutes again mix the content thoroughly and immediately measure the degree of turbidity at 340 nm on spectrophotometer.

Observation

Sr. No.	Particulars	Quantity
1	Weight of soil sample	5 g.
2	Volume of extract and added	50 ml.
3	Volume of aliquot taken	10 ml.
4	Vol. of turbid solution	100 ml.
5	Colorimeter reading ®	Mg S/ml.

Calculations

$$SO_4 \text{ (ppm)} = R \times \frac{\text{Vol. of extractant added}}{\text{Vol. of aliquot taken}} \times \frac{\text{Vol. of turbuid solution}}{\text{Weight of soil sample}}$$

$$= R \times \frac{50}{10} \times \frac{100}{5}$$

$$= R \times 50$$

Where R = µg S/mL in aliquot read from standard curve.

Exercise No.-10 ***Date :- / /2019***

Estimation of DTPA extractable micronutrients from soil using *AAS*

Method-DTPA Method.

Instrument-AAS.

Micronutrients are as important to plant nutrition as major and secondary nutrients though plants

require them in small quantities. A lack of any one of the micronutrients in the soil can limit plant

growth, even when all other essential nutrients are present in adequate amounts. The need for

micronutrients has been known for many years. But their commercial use as fertilizer is a recent

practice because micronutrient deficiencies became more and more common in recent past

particularly in the irrigated area of high cropping intensity. The reasons for deficiencies of these

nutrients are increasing crop yield, past fertilizer practices, and lack of addition of organic manures.

Suitable test methods for the diagnosis and assessment of micronutrient deficiencies are needed for delineation of soil fertility to formulate recommendations for fertilizers under varying soil and crop situations for sustainable agriculture.

The method commonly used for determining the available micronutrient cations in soil sample is given by Lindsay and Norvell (1978). The method consists of use of **DTPA** (Diethylene triamine penta acetic acid) as an extracting which has been widely acdepted for the simultaneous extraction of metal micronutrient cations viz; zine (Zn), copper (Cu), iron (Fe) and managanese (Mn) in neutral and alkaline soils. The content of these cations in the extract is determined on an Atomic Absorption spectrophotometer (**AAS**).

Principle

DTPA as a Chelating agent combines with free metal ions in the solution to from soluble complexes, stability contantas fro the simultaneous complexing of Zn, Cu, Mn and Fe shows DTPA as a most suitable extractant. Excessive dissolution of CaCO3 which may release occulded micronutrients that are not available for plants particularly in calcareous soils may mislead the results. The avoid this, the extractant is buffered in a slighhlty alkaline pH range and included souble Ca++.

Triethanolamine (**TEA**) is used as buffer because it burns cleanly during atomization. At the selected pH of 7.3, three fourth of TEA is protonated (HTEA+) which exchanges with Ca++ and some Mg++ from the soil exchange sites. This increases the concentration of calcium ions (Ca++) by two to three fold and helps in suppressing the dissolution of CaCO3 in calcareous soils. The DTPA has a capacity to complex each of the micronutrient cations as 10 times of its atomic weight. The capacity ranges from 550-650 mg kg^{-1} depending on the micronutrient cation.

Apparatus

1. Analytical balance.
2. Narrow mouth polyethylene bottles with stopper 100 mL capacity
3. Pipette : 20 ml capacity.
4. Reciprocating electric shaker.
5. Whatmen No. 42 filter papers
6. Polyethylene glass funnels.
7. Polyethylene vials 50 mL capacity.
8. Atomic Absorption Spectrophotmeter (AAS)
9. Hollow Cathode lamps of Zn, CU, Fe and Mn.

Reagents

1. Micronutrient standard stock solutions

i. Fe 100 PPM(ug mL^{-1}) :-dissolve 7.021 g Fe (HN_4) or (4.964 gm – $FeSO_4$) SO_4O in 700 mL water, add 15 mlL of conc H_2SO_4 and volume made to 1000 mL with distilled water.

ii. Zn 1000 ug mL^{-1}, dissolve 4.398 g $ZnSO_4$, $7H_2O$ volume made 1000 ml distilled water.

iii. Cu 1000 ug mL^{-1}, dissolve 3.929 g $CuSO_4$, $5H_2O$ and volume made 1000 ml distilled water.

iv. Mn 100 ug mL^{-1}, dissolve 3.077 g MnSO4, $2H_2O$ and volume made 1000 ml distilled water.

Prepare secondary or working standards from the above stock solutions for atomic absorption spectrophotometer.

2.Extracting solution :-

It constist of 0.005 M DTPA, 0.01 M $Cacl_2.2H_2O$, 0.01 M Triethanolamine (TEA). Dissolve 14.92 g TEA, 1.967 g diethylene tramine penta acetic acid, and 1.47 g $Cacl_2.2H_2O$ in 900 mL distilled water. Adjust the pH to 7.3$\pm$ 0.05 with 1 : 1 HCl or NaOH and dilute to one litre.

Procedure

Extraction procedure

i. Ten g of air-dried soil sample is placed in 125 mL conical flask.

ii. Add 20 mL of the DTPA xtracting solution.

iii. Covered with plastic sheet and secured tight on a horizontal shaker. Shake for 2 hours.

iv. Suspension is then filtered by gravity through what man No.42 filter paper.

v. Filtrate is then analyzed for Zn, Mn, Fe and Cu using atomic absorption spectrophotometer and by using appropriate standards of each element.

Observations and Calculations

Most of the modern AAS are cailibrated to display the concentration of a micronutrient in ppm directly in the soil extract. In such cases t6he concentration of the given micronutrient in the soil sample is calculated by multiplying the displayed reading by the dilution factor which is 2 in this case.

If the AAS displays the reading in absorbance then a standard curve has to be prepared for the known standards on a graph paper (Fig.) and the absorbance reading should be converted in to concentration (ug mL^{-1}) from the standard curve. The amount of the given micronutrient is then calculated as below.

Weight of soil taken :- 10 g

Volume of DTPA extracting added :- 20 mL

Dilution :- 2 times

Absorbance shown by the AAS :- A

Concentration of the micronutrient as

Read from the standard curve against A :- C ug mL^{-1}.

Content of the micronutrient cation in the soil sample :- C X 2 mg kg^{-1} or ppm

ug mL^{-1} = mg kg^{-1} = ppm

Precautions

1. Ensure that the deionized or double glass distilled water used is free of the micronutrients cations.
2. The apparatus (glass/polyethylene/polypropyle) to be used fro the analysis must be thoroughly washed with acidified water and then with deionzed water.
3. Shaking time, DTPA concentration, pH and temperature during shaking influence the amount of Zn, Fe, Cu and Mn extracted by DTPA. The mostsuitable pH of extracting solution is 7.3, shaking time 2 hours and temperature during shaking 25 $\pm$ 1^0C. So do not forget to adjust the DTPA extracting solution to 7.3, shaking must be carried out at 25^0C and the suspension must be filtered immediately after the shaking time of 2 hours.
4. Before feeding the extracts, it should be ensured that they are not turbied otherwise, they may block the capillary of the AAs.

DTPA Micronutrients

Sr. No.	Soil micronutrient	Rating (ug g^{-1})		
		Low	Medium	High
1	Fe	0 – 2.0	2.1 - 4.0	> 4.0
2	Mn	0 – 1.0	-	> 2.0
3	Cu	0 – 0.2	-	> 0.2
4	Zn	0 – 0.5	0.6 – 1.0	> 1.0

Exercise No.-11 *Date :- / /2019*

Determination of Total Nitrogen (Protein) in plants by *Micro Kjeldahl Method*

Principle

The organic nitrogen from the proteins and other nitrogenous compounds is converted to inorganic nitrogen (ammonium sulphate) by complete oxidation of sample with conc. H_2SO_4. The digest is trated with excess of 40% NaOH to liberate ammonia form ammonium sulphate. The ammonia is collected in boric acid and titrated with standard H_2SO_4. **The protein contains about 16% nitrogen. Therefore, the protein content calculated by multiplying 6.25 to per cent nitrogen**.

Reactions :- Digestion

Distillation ---------- $R\text{-}NH_2 + H_2SO_4$ oxidation $(NH_4)_2SO_4$

1. $(NH_4)_2SO_4 + 2\ NaOH$ ---------- $Na_2SO_4 + 2\ NH_3 + 2H_2O + 2H_2O$

2. $H_2O + NH_3$ ---------- NH_4OH

3. $3NH_4OH + H_3BO_3$ ---------- $(NH_4)_3\ BO_3 + 3H_2O$

Ammonium borate formed is further titrated with standard H_2SO_4

4. $2(NH_4)_3BO + 3H_2SO_4 = 3(NH_4)_2SO_4 + 2H_3BO_3$

Reagents

1. 36 N, N-free H_2SO_4, Sp. Gr. 1.84

2. 30% Hydrogen peroxide

3. NaOH (40%) : Dissolve 40 g NaOH + 60 mL distilled water make 100 ml (prepare fresh).

4. Boric acid : Dissolve 2 gm boric acid in water to make 100 ml.

5. Bormocresol green 99 mg + methyl red 66 mg in 100 mL ethanol.

6. Standard H_2SO_4 (0.02N).

Materials

1. Microkjeldahl digestion unit.

2. Microkjeldahl distillation unit.

3. Digestion flask.

Procedure

Wet digestion :- (H_2SO_4 & 30% H_2O_2)

Weight 0.2 g dried 20 mesh plant tissues in digestion tube.

Add 5 ml conc. H_2SO_4 and let it stand for 30 minutes.

Add 5 ml 30% H_2O_2

Place the funnel in digestion tube and tube into a port in digestion block set at 250^0C temp.

Heat for 30 minutes. Remove tube and let it cool. Add 1 mL. 30% H_2O_2 until the digest is clear upon cooling when clear upon cooling dilute to 20 mL with pure water. Transfer the content in 100 mL volumetric flask and make up the volume with distilled water.

The digest is ready for elemental assay.

Distillation

1. Pipette out 10 ml boric acid solution into 100 ml beaker containing mixed indicator, place the beaker below the condenser so that tip of condenser dips in the solution.
2. Pipette out 10 ml aliquot of digested acid extract into distillation apparatus, funnel is washed with 2-3 ml of distilled water.
3. Add 10 ml of 40% NaOH solution and carry out distillation. When all ammonia is evolved stop distillation (Collect about 60-70 ml of distillate).
4. Titrate the distillate with std. H_2SO_4 till the colour changes from green to red.
5. Blank should also be run and titration should be carried out to the same end point as that of sample.

Observations

1. Wt. of sample: w1:- gm
2. Volume of acid digest made : 100 ml.
3. Volume taken for distillation : 10 ml.
4. Vol. std. H2SO4 required for sample titration. :- X
5. Vol. std. H2SO4 required for blank.:- Y

For the reaction.

$3H_2SO_4 = 2(NH_4)_3 BO_3 = 6 NH_3$

$3H_2SO_4 = 6 NH_3$

$H_2SO_4 = 2NH_3 = 2 N = N_2$

1 mole H_2SO_4 = 2mole NH_3 = 1 mole N_2

98 g H_2SO_4 = 2 x 14 g N

49 g H_2SO_4 = (Eq wt. of H_2SO_4) = 14 g N

1000 mL 1 N H_2SO_4 = 14 g N

1 ml 1 H_2SO_4 = 0.014 g N

Calculations

$$\% N = (X - Y) \times \text{Normality of } H_2SO_4 \times 0.014 \times \frac{\text{vol. digest acid extract}}{\text{Vol. Aliquot taken}} \times \frac{100}{\text{wt of sample}}$$

Per cent protein = % N X 6.25

Questions

1. Give the principle of protein estimation by Kjeldahl method.
2. Complete the following reaction $(NH_4)_3 BO_3 + 3 H_2SO_4$
3. Why do you multiply nitrogen content by 6.25
4. Give the constituents and role of each chemical used in digestion mixture.
5. Give the reaction during distillation process of nitrogen, why the tip of the condenser should dip into the boric acid solution.

Exercise No.-12 *Date :- / /2019*

Estimation of Total Phosphorous from plant sample by Vando Molybadte Method, Total Potassium, Secondary and micronutrients like Fe, Zn, Mn and Cu from plant sample

Principle

P in the triadic extract of plant is complexed by adding vanadomolyb date solution in HNO_3 medium. The intensity of yellow colour is measured on **spetronic – 20 at 470 nm** or photoelectric colormeter at 470 nm wavelength (blue filter). The nature of the yellow chromogen of the vanadomolybdophosphoric system is not known, but the colour is attributed to substitution of oxyvanadium and oxymolbdenum radicles for the O of PO_4 to the give a heteropoly compound which is chromogenic.

Vanadate, molybadte and orthophosphates react together to give a yellow colour complex in nitric acid medium. The optimum concentration of nitric acid for development of colour is 0.5 N. Hence five ml of 5 N nitric acid per 50 ml of final volume is sufficient to give optimum acidity.

The colour develops in about 30 minutes and is stable for 2-8 weeks at high P concentrations, but at low P concentrations of 5 ppm, it is table for only 2 weeks.

Reagents

Reagents required for digestion of plant sample for P, K determination by Tri-acid method.

1 .Conc.HNO_3 2. Conc. H_2SO_4 3. Conc. $HclO_4$ (perchloric acid)

Procedure for preparation of tri-acid extract for P and K.

1. Weight 0.2 g finely ground plant sample in a conical flask.
2. Add 5 ml of conc. HNO_3 and 2 ml mixture of equal volume of H_2SO_4 and $HclO_4$.
3. Digest initially with gentle heat and then with high temp. on hot plate till contents are colourless. If digestion mixture becomes dry, add conc. HNO_3 occasionally till sample is digested completely.
4. Cool, dilute in 100 ml volumetric flask by filtering and give washing till the filtrate runs free of acid.
5. Filter and used the filtrate for P and K determinations.

Observations

1. Wt. of plant sample taken 0.2 g

2. Volume of acid extract made 100 mL

Reagents

1. Ammonium molybdate-ammonium vanadate in nitric acid : Dissolve 22.5 g of $(NH_4)_6 Mo_7 O_{24}. 4H_2O$ in 400 ml of water. Dissolve 1.25 g of ammonium vanadate in 300 ml of boiling water. Add the ammonium vanadate solution to the ammonium molybdate solution and cool to room temperature. Add 250 ml of concentrated nitric acid and make of the volume to 1 liter.

2. Standard phosphorus solution : Weight 0.2195 g of potassium dihydrogen phosphate (AR grade KH_2PO_4 dried in an oven at 400C and cooled in a desiccator) and dissolve in 400 ml of distilled water in a 100 ml volumetric flask. Then 25 ml of 7 N H2SO4 (approx) is added and the solution made to 1000 ml volume and mixed. This solution contains 50 ppm or 5o micrograms phosphorus per milliter.

Procedure for colour development

1. Pipette out 10 ml of the plant acid extract into a 50 ml volumetric flask.

2. Add 10 ml of the ammonium molybdate – ammonium vandadte reagent.

3. Dilute to 50 ml with distilled water and mix well.

4. The colour develops rapidly but is usually read after 30 min.

5. A blank must be prepared and read with the samples because the blank vanadate colour is itself is noticeable even when phosphorus impurities have been carefully excluded. The colour is rad on the colorimeter/spectronic 20, at 470 nm. Wave length.

Preparation of std curve

1. Pipette our 0, 1, 2, 4, 6, 8, 10 ml of (50 ppm) standard phosphorus solution separately into 50 ml volumetric flask.

2. Add 10 ml of vanado-molyybdate reagent to each volumetric flask make upt the volume with distilled water.

3. Read the colour intensity with the help of colorimeter / spectronic – 20 at 470 nm wave length after 30 minutes. It gives 0, 1, 2, 4, 6, 8 and 10 ppm concentration. (0-10 μg P/mL)

4. Draw a curve by ploting absorbance on Y-axis and P concentration µg P/mL on X-axis. This should be straight line.

Observations

i. Wt. of plant sample taken for Triacid extract :- 0.2 g

ii. Volume of acid extract made :- 100 mL

iii. Record colorimeter reading for std. Curve and draw a std. Curve. (microgram p/ml on X – axis and readings on Y – axis).

iv. Record colorimeter reading for plant sample and read P conc. From std. Curve.

1. Observations for standard curve.

Draw a standard curve by ploting absorbance versus (against) concentration of phosphours.

mL of 50 ppm P solution	Final Vol. of colour solution mL.	Conc. Of P (µg/mL)	% T	Absorbance
0	50	0		
1	50	1		
2	50	2		
4	50	4		
6	50	6		
8	50	8		
10	50	10		

Absorbance (A) = 2-10 g T

Calculations

Phosphorus content in plant

$$\%\ P = \frac{R}{10^6} \times \text{final vol. of coloured solution} \times \frac{\text{Vol of acid digest extract}}{\text{Vol. of acid digest extract taken}} \times \frac{100}{\text{Wt. of plant sample}}$$

$$\%\ P = \frac{R}{10^6} \times 50 \times \frac{100}{10} \times \frac{100}{0.2}$$

$$= R \times 0.25$$

Where R = µg/mL from standard curve.

Questions

1. What is the principle employed in the determination of P?
2. How will you prepare 1000 ml of 100 ppm P solution by using KH_2PO_4?

Reference

Jackson M.L. (1973) Soil Chemical Analysis, pp 151-152, Chapman H.D. and P.F. Pratt (1961). Methods of analysis for soils, plants and water. Pp-169-170.

Estimation of total Potassium from plant sample by *Flame Photometer*

Principle

The plant extract is atomized in the flame where the atoms of the element of K are excited and emit radiations of characteristics wavelength. The radiation emitted by K atoms is passed through K filter which falls on a photocell emitting electrons i.e. electric current which is measured on galvanometer of flame photometer. The electric current generated is proportional to the concentration of K in the extract.

Reagent

Std. Potassium stock solution (1000 ppm) : Dissolve 1.908 g oven dry A.R. grade crystals of KCl in distilled water and volume made up to one titre. This solution contains 1000 mg K/L. prepare 100 mg K/L solution by diluting the 100 mg K/L solution 10 times.

1. **Preparation of std. Curve for K.**

   Pipette out 0, 1, 2, 4, 6, 8, 10 ml of 100 ppm K solution in 100 ml volumetric flask. Make up the volume with distilled water and shake well. This gives 0, 1, 2, 4, 6, 8 and 10 mg K/L respectively. Read the intensity of K on flame photometer, using K filter. Draw a curve by plotting flame photometer reading on y-axis and K mg/L on x-axis.

Procedure

Read the plant extract (diacid / triacid digested) directly on flame photometer or after appropriate dilution so that final concentration lies between 0 to 10 mg K/L. Run a blank i.e. without sample (ppm K=μgK/mL)

Observations

Record the flame photometer readings for the standard curve and for the plant sample,

Sr. No.	Vol. of 100 mg K/L solution	Final vol. of solution	Concentration of K g/mL	Flame photometer reading
1	0	100	0	
2	1	100	1	
3	2	100	2	
4	4	100	4	
5	6	100	6	
6	8	100	8	
7	10	100	10	

Calculations

$$\% K = \frac{R}{10^6} \times \text{Vol. of acid digest} \times \frac{100}{\text{wt. of plant sample}} \times \text{Dilutionfactor,if any}$$

Where – R = g K/ml from standard curve.

Questions

1. Write the principle of flame photometry?
2. Calculate the quantity of KCl required for reparation of 500 ml of 100 ppm K solution.
3. How potash is extracted from plant?

Determination of Secondary nutrients from Plant sample-

Determination of Fe, Zn, Mn & Cu from Plant analysis by **AAS-**

Zinc, manganese, copper and iron are estimated in plant digest obtained from dry ashing or from wet digestion by (HNO_3 and $HclO_4$) Triacid digestion (HNO_3 : H_2SO_4 $HclO_4$ 9:4:1) and digestion of plant samples with H_2SO_4 and H_2O_2 is avoided for this estimation because H_2SO_4 used in digestion can contribute some micronutrients and heavy metals.

Principle

The principle that atoms of metallic elements (Fe, Mn, Cu, Zn etc.) which normaly remain in ground state under flame conditions absorbs energy when subjected to radiations of specific wavelength absorb energy when subjected to radiations of specific wavelength. The absorption of radiation is proportiona to the concentration of atoms of that element. The AAS has a distinct advantage over flame emission spectroscopy because the absorption of radiation by the atoms is independent of the wavelength of radiations and temperature of the atoms. It also has greater sensitivity and accuracy.

Apparatus / Equipment

1. Analytical balance.
2. Measuring cylinder 25 mL capacity.
3. Hot plate (s) with temperature control system.
4. Concical flasks, 100 / 150 mL capacity.
5. Polyethylene / glass funnels.
6. Polyethylene bottles 60 / 100 mL capacity.
7. Measuring flasks 50 / 100 mL capacity.
8. Piptte graduated 1 ml.
9. Atomic absorption spectrophotometer.
10. Hollow cathode lamps of Zn, Cu, Fe and Mn.
11. Whatman No.1 filter papers.

Reagents

1. Digest obtained from dry asking or from HNO_3 – $HClO_4$ digestion.

2. **Stock Standard solutions :-** The standard solution of different micronutrient captions should be prepared as detailed under soil analysis.

3. **Working standard solutions :-** They should be prepared as described under soil analysis with the exception that dilute HNO_3 (0.25 M) should be used in place of DTPA for making final volume in each case.

4. **0.25 M HNO_3 :-** Dilute 16 ml reagent grade concentrated HNO3 to 1 Litre with deionized or double distilled water.

Method

Digestion of plant material

Wet Diacid Digestion by **Nitric Acid and perchloric acid** (for nutrients other than N) Plant material is digested either in HNO_3 – $HClO_4$ mixture or ashed and dissolved in acid (for detail see on page nos. 261, 265). Also carry blank digestion using all steps, excluding the plant material to avoid any impurity in the reagents being used.

1. Acid mixture – dilute two parts HNO_3 with one part $HCLO_4$ or nine part HNO_3 with four parts $HCLO_4$.

2. Weight 0.2 to 0.5 gm plant material into 50 ml. digestion tube.

3. Add 5 ml of the acid mixture.

4. Place small Dyren funnel into tube and put-tube into block digester.

5. Heat-sample at 60^0C for 15 min or until reaction is complete.

6. Interacts heat to 1200C and digestion for 75 min or until sample clears.

7. Remove tube from block digester when sample is clear cool and add sufficient distilled water to bring solution up to 100 ml (if block digester is not available digestion may be carried out in 100 ml volumetric flask in a digestion chamber as detail given below).

Analysis of Plant Digest

Zn, Cu, Fe and Mn concentration in plant digest can be determined by atomic absorption spectrophotometer as follows :-

1. Read operator's manual prior to start instrument.

2. Set zero of the instrument with blank (blank digest).

3. Feed standards of element to be determined to the AAS to standardize the instrument for element in sample. Feed plant digest and record the absorbance / concentration of the element in question.

4. Repeat the above steps for every element.

5. In case the instrument shows a sign of over for some element in a particular sample then make further dilution of the sample (say 2-5 times) and feed again to record absorance or concentration.

Calculation

$$\text{Zn, Cu, Mn or Fe (µg/mL)} = R \times \frac{\text{Final volume acid extract}}{\text{Wt of plant tissue (g)}} \times \text{Dilution factor}$$

Where R = Read from AAS – in µg/mL

Exercise No.-13 *Date :- / /2019*

Fertilizer Adulteration test / Identification of Adulteration in fertilizer /

Detection of adulteration in fertilizers (Rapid test)

The commonly used fertilizers are normally adultered by either cheap fertilizer or non fertilizer substances such as sand, ash, gypsum, lime etc.

The following commonly used adulterants are used in fertilizers.

	Name of fertilizers	**Adulterants**
1.	Urea	Common salt, silica, sands
2.	Diammonium phosphate (DAP)	Granular single super phosphate
3.	Single super phosphate (SSP)	Sand, ash, granular gypsum.
4.	Calcium ammonium nitrate (CAN)	Clay gypsum
5.	Muriate of potash (MOP)	Sand common salt.
6.	NPK	Single super phosphate (granular)
7.	Zinc sulphate	Magnesium sulphate.
8.	Copper sulphate	Sand, common salt.
9.	Ferrous sulphate	Sand, common salt.

Reagents :-

1. NaOH (Conc.) : 40 % in water, NaOH dilute – 1 %.
2. Acetic acid : glacial
3. $AgNO_3$ – Dissolve 1 gm in 100 ml distilled water.
4. Cobalt nitrate : Dissolve 5 gm cobalt nitrate in 5 ml distilled water. Add 25 gm $NaNO_2$ and 2.5 ml glacial acetic acid. Mix and dilute to 100 ml with distill water
5. $FeCl_3$ solution : Dissolve 7 gm $FeCl_2$ and 12 gm ammonium acetate in 1 litres of distill water.
6. Formaldehyde : (37-40 %) Add 1 ml method red indicator in 100 ml formaldehyde.
7. Ammonium acetate
8. Calcium oxide (CaO)
9. Conc. And diluted acids (5 N) H_2SO_4, HNO_3, HCl

10. K_3 [Fe $(CN)_6$] – dissolve 5 gm potassium fero cyanide in 100 ml water.

Detection of adulterants

1) Urea :-

a) Dissolve 1 gm sample in 5 ml water in a test tube. Add 5-6 drops of $AgNO_3$. If white ppt does not appear, the sample is not adulterated with salt.

b) Filter the solution, if no residue on filter paper. It is not adulterated with sand / silica.

c) Heat dry urea in a test tube, if melts completely the sample is pure, if not and solid residue remains it contains adulterated with sand / silica.

2) Diammonium phosphate (DAP) :-

a) Dissolve 1 gm powdered sample in 5 ml distilled water. Add 1 ml conc. HNO_3 and mix. It dissolve completely, it is pure DAP. If it contains undissolved material, it is adulterated.

b) To 1 gm sample add 2-3 drops of NaOH or 1 gm CaO, smell of ammonia indicates the presence of nitrogen. If not, the sample is not DAP.

c) Dissolve 1 gm of sample in warm water and filter. To the filtrate add 1 ml $AgNO_3$. If yellow ppt. It contains phosphate. If not, it is not DAP but SSP.

3) Muriate of potash (MoP) :-

Take about 1 gm sample and dissolve in 10 ml dist. Water. If sample does not dissolve completely and undissolved material settles, it is adulterated. Place powder in blue flame, which burns with yellow flame, if adulterated.

4) NPK fertilizers :-

a) Dissolve 1 gm of sample in 5 ml distilled water in a test tube. Add 1 ml conc. NaOH and heat place the moist (water) red litmus paper to the mouth of test tube. Paper turns blue indicates the presence of nitrogen. If paper remains unchanged, the sample does not contain nitrogen and highly adulterated.

b) Dissolve 1 gm sample in 5 ml of water and filter. Add 0.5 ml of $FeCl_3$, if yellow ppt. Forms which is soluble in conc. HNO_3 it indicates the presence of phosphorus

c) To the 5 ml solution of sample add 2 ml formaldehyde. The colour of solution turns red. Add NaOH drop wise till yellow colour. Add 1 ml cobalt nitrate reagent. Yellow ppt indicates the presence of potassium (Potash).

5) Phosphours

Dissolve 1 gm of sample in 5 ml of water. Add 1 ml dilute NaOH and 1 ml of $AgNO_3$. Yellow colour ppt indicates phosphate. If yellow ppt is not formed it is not phosphatic fertilizer.

6) Zinc sulphate :

Dissolve 1 gm sample in 5 ml water and filter. Add 8-10 drops of NaOH to the filtrate, if white ppt appears and dissolve in 10-12 drops of conc. NaOH the sample is pure. If ppt does not dissolve in conc. NaOH, it is adultered.

7) $CuSO_4$:

Dissolve 1 gm of sample in 5 ml water. The solution must be transparent blue colour. Add potassium fernocynide to the solution, if brown ppt copper is present.

8) $FeSO_4$:

To the aqueous solution add 1 ml $K_3[Fe(CN)_6]$, appearance of blue coloured ppt confirms iron.

Reference :

1. A.K. Gupta (2007) Practical manual of Agril. Chemistry, pp. 108-109.

Exercise No.-14 *Date :- / /2019*

Determination of nitrate nitrogen content of potassium nitrate by *kjeldahl method*

Principle :- Nitrate -N is reduced to ammonia by Devarda's alloy in strongly alkali medium. The ammonia liberated is absorbed in known excess std. H_2SO_4. Excess std. H_2SO_4 is titrated with std NaOH. From the volume of std. H_2SO_4 neutralised, nitrogen is calculated.

Reagent :-

1. Std H_2SO_4 0.1 N
2. Std. NaOH 001 N
3. Methyl red indicator (0.2% solution in 60 % ethyl
4. 40% NaOH or MgO powder (free from $MgCO_3$)
5. Devards's alloy (50% Cu, 45% A1 and 5% Zn) finally ground – 100 mesh.

Procedure :-

1. Weigh one gram potassium nitrate and dissolve in distilled water and volume is made up to 250 mI.
2. Pipette out of 25 ml solution in a distillation flasK and dilute by adding distilled water.
3. Add 5-10 ml 40% NaOH or 2-3 g MgO powder to the distillation flask.
4. Add 2.0 gram of finely powdered Devarda's alloy to distillation flask.
5. Distill as in exercise NO. 4 & 5.
6. Run the blank simultaneously.

Reactions :-

During distillation :- Reduction of NO_3 to NH_3 by Devarda's alloy :

$3\ K\ NO_3 + 8\ Al + 5\ NaOH + 2\ H_2O = 3NH_3 + 8K\ (AlO_2)$

$K\ NO_3 + 4\ Zn + 7\ NaOH = NH_3 + 4K\ (Zn\ O_2) + H_2O$

In Condenser :-

$NH_3+N_2O = NH_4OH$ (Liquer ammonia)

$NH_4OH + H_2SO_4 = (NH_4)_2\ SO_4 + H_2O$ OR

$2NH_4OH + H_2SO_4 = (NH_4)_2\ SO_4 + H_2O$

During Titration:-

$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$

Observations:-

1. Weight of KNO_3	-	W_1 g
2. Vol. of KNO_3 solution made	-	250 ml
3. Vol. of KNO_3 solution taken	-	25 ml
4. Vol. of std. H_2SO_4 taken for sample	-	x ml
5. Vol. of std. NaOH required for sample	-	y ml
6. Vol. of std H_2SO_4 taken for blank	-	A ml
7. Vol. of std NaOH required for blank	-	B ml
8. Normality of std. H_2SO_4	-	0.1 N
9. Normality of std. NaOH	-	0.1 N

Calculations :-

1 ml of 1 N H_2SO_4 = 0.014 g N

Volume of std. H_2SO_4 required for neutralisation ammonia.

C ml = [(X x N) – (Y x N)] – [(A x N) – (N x N)] = 'C' ml

$$\% N = C \times 0.014 \times \frac{\text{Volume made}}{\text{Volume taken}} \times \frac{100}{\text{Wt. Of sample}}$$

Questions :-

1. Explain the principle involved in the estimation of nitrate nitrogen from potassium nitrate.
2. What is the composition and role of Deverda's alloy in NO_3 determination?
3. What reactions are involved in reduction of nitrate by Devarda's,alloy?

Reference: - Motiramani and Wankhede Second Edition, Laboratory Manual in Agril. Chemistry, pp. 84-85

Exercise No.-15 *Date :- / /2019*

Determination of water soluble phosphorus in superphosphate by *Pumberton method*

Principle :-

The phosphate is precipitated as ammonium phosphomolybdate by adding ammonium molybdate. The precipitate is dissolved in a known excess of standard alkali. The excess of alkali is titrated against std. acid. From the amount of std. alkali required to dissolve the precipitate, the percent p is calculated.

Reagents :-

1. Ammonium molybdate solution :- Dissolve 200 g finely ground ammonium molybdate (A.R. grade) in distilled water. If necessary heat it and add ammo hydroxide solution till the solution becomes clear and make up the volume to one litre. Now pour 9 ml of above solution into 11 ml of conc. nitric acid and shake the solution.
2. Dillute ammonium hydroxide- 67.6ml of Lit = 1 N 6.76 ml
3. Conc. nitric acid
4. Std. NaOH (O.1 N) = 4 gm
5. Std. H_2SO_4 (0.1 N) = 2.8 ml
6. Ammonium nitrate (solid)
7. KNO_3 (3%) = 3 gm in 100 ml
8. Indicator-phenolphthalein (1 % in ethanol)

Procedure :-

a) Preparation of water extract :-

1 . Weigh accurately 1.0 g of superphosphate in beaker & add hot water, stir well.

2. Filter through whatman No. 40 filter paper

3. Wash the residue with successive portion of hot distilled water and collect the filtrate in 250 ml volumetric flask.

4. Continue the washing with water till the filtrate runs free from acid (test with blue litmus paper)

5. Make up the volume upto the mark 250 ml.

b) Precipitation :-

1. Pipette out 10 ml of the extract in 400 ml beaker.

2. Make the aliquot alkaline by adding amm. hydroxide and then acidic by adding conc. nitric acid (Use litmus).

3. Add 3-10 g ammonium nitrate and place the beaker on thermostat 60-65^{O}C. On the same thermostat place ammonium molybdate reagent to attain the same temperature.

4. After 15 minutes add about 20 ml of ammonium molybdate reagent and stir slowly with the help of policeman. Canary yellow precipitte appears.

5. Again place both the beakers on their respective places. Intermitenetly stir the precipitate centrifugally with policeman taking care not to grind the precipation at the interval of five minutes, continue this process for half an hour.

6. Allow the beaker to remain again for 15 minutes and test with the ammo molybdate reagent, if precipitate appears again add more amount of the reagent.

c) Filtration :-

Carefully transfer the supernatant liquid on Whatman No. 40 filter paper and continue the filtration adding small quantity of 3% KNO_3 at each time allowing the liquid to drain completely before adding next portion of KNO_3. As for as possible the precipitate should not be disturbed. Wash the precipitate till it is free from acid, (Test with phenolphthalein by taking one or two drops of alkali in a test tube dilute to 10 ml with distilled water, collect the filtrate in test tube, if pink colour persisted as such, it means precipitate is free from acid).

d) Dissolution of precipitate and titration :-

1. Carefully transfer the filter paper into the beaker in which precipitation was carried out. Then wash the sides of the beaker and funnel with distilled water.

2. Add about 50 to 100 ml of distilled water and macerate.

3. Add two or three drops of phenolphthalein indicator.

4. Add known excess standard NaOH in the beaker until the precipitate is completely dissolved as indicated by pink colour. Note the volume of alkali added.

5. Titrate the excess of alkali against std. H_2SO_4 till the content becomes colourless. Note the volume of standard acid required for titration.

Reactions :-

During Precipitation :-

1. . $Ca(H_2PO_4)_2 + 24(NH_4)2MO_4 + 44HNO_3 = 2(NH_4)_3\ PO_4.12MnO_3$ Cannary yellow precipitate of Ammo. Phosphomolybdate $42NH_4NO_3 + Ca(NO_3)_2 + 24H_2O$.

During Dissolution of precipitate :-

2. $(NH_4)_3PO_4$ $12MoO_3$ + 23NaOH = 11 Na2MnO_4 + $(NH_4)_2MnO_4$ + NaNH4HPO_4 + 11 H_2O

During Titration :-

3. H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O

Observations :-

1 . Weight of sample - W_1 g

2. Vol. of water extract made - ml

3. Vol. of water extract taken - ml

4. Vol. of std NaOH added to dissolve the precipitate - x ml

5. Normality of std. NaOH - 0.1 N

6. Vol. of std. H_2SO_4 required for titration - y ml

7. Normality of std. H_2SO_4 - 0.1 N

Calculations :-

Derivation of Factor :-

1 ml of 1 N NaOH = 0.0031 g P_2O_5

23 NaOH = $(NH_4)_3PO_4$. $12MnO_3$

46 NaOH = 2$(NH_4)_3PO_4$.$12MnO_3$

46 NaOH = 2P = P_2O_5

46 x 40 g NaOH = 2 x 31 P or 142 g P_2O_5

1000 ml 1 N NaOH = 1.347 g P or 3.086 g P_2O_5

1 ml 1 N NaOH = 0.0013 g P or 0.0031 g P_2O_5

$$\% P_2O_5 = (\text{ml of NaoH x N}) - (\text{ml } H_2SO_4 \text{ x N}) \times 0..0031 \times \frac{\text{Vol. made}}{\text{Vol. taken}} \times \frac{100}{\text{Wt. of sample}}$$

% P = % P_2O_5 X 0.44

$$\%P_2O_5 = (\text{ml of NaOH X N}) - (\text{ml } H_2SO_4 \text{ x N}) \times \frac{\text{Vol. made}}{\text{Vol. taken}} \times 0.0031 \times \frac{100}{\text{Wt. of sample}}$$

Questions :-

1. Write the form of phosphorus present in single supper phosphate.

2. Explain the role of following reagents

a) ammonium molybdate b) std. NaOH and std.H_2S0_4

c) ammo hydroxides d) KNO_3

3. Why the temperature of the precipitation medium should be 60- 70°C.

4. Explain the principle involved in the estimation of phosphorus by Pemberton method.

5. How will you judge whether the precipitation is complete?

6. Explain how 1 ml of 1 N NaOH = 0.0031 g P_2O_5

Reference book :-

Chopra S.L. and J.S. Kanwar (1991) Analytical Agril.Chemistry pp. 122-124.

Exercise No.-16 *Date :- / /2019*

Determination of acid soluble phosphorus from rock phosphate by *Pumberton method*

Principle:- Phosphorus from rock phosphate is extracted by treating with cone. HNO_3 and conc. HCl. The principle of p estimation from acid extract is the same as given in the estimation of water soluble phosphorus in single superphosphate (Experiment NO. 9).

Reagents :- 1) Conc. HNO_3 2) Conc. HCI 3) 0.1 N $AgNO_3$ - 16.98 gm

All other same as expt. for the determination of WSP from SSP.

Procedure :-

a) Preparation of acid extract :-

1. Weigh 1.0 g rock phosphate sample and dissolve in 30 ml conc. HNO_3 and 3.5 ml of conc. HCl boil until organic matter is destroyed. Cool the solution.
2. Filter through Whatman No. 40 filter paper. Collect the filtrate in 250 ml volumetric flask.
3. Wash the residue with hot water till filtrate is free of chlorides (Test with Silvernitrate).
4. Make up the volume.

b) Precipitation :-

1. Pipette out 5-10 ml of acid extract and proceed as per procedure described in Experiment No.11.

Questions :-

1. Write the total per cent P_2O_5 content of rock phosphate.
2. Write the suitability of rock phosphate for its use in crop production under different soils.
3. Write other examples of water insoluble phosphate fertilizers.

Reference book :-

Chopra and Kanwar (1980) Analytical Agril. Chemistry, pp. 136

A.O.A.C. (1975), pp. 11.

Exercise No.-17 *Date :- / /2019*

Determination of total potassium content of muriate of potash by *flame photometer*

Principle :- The water extract of K fertilizer is atomised in the flame where the atoms of the elements are excited emitting radiations of characteristic wavelength. The radiation emitted by K atoms is passed through K filter (768 nm), which falls on photocell, emitting electrons i.e. electric current which is measured on galvanometer of flame photometer. The electric current generated is proportional to the concentration, of K in the extract.

Reagents :-

Standard stock solution (1000 ppm K)

Dissolve 1.2931 g KNO_3 in distilled water and make up to 500 mI.

Working solution (100 ppm K) = 10 ml of stock solution is diluted 100 mI.

Standard curve:

1. Pipette out 0, 10, 20, 40, 60, 80, 100 ml of working solution in 100 ml volumetric flasks separately and make the volume. This will be 0, 10, 20, 40, 60, 80 and 100 ppm K-solutions, respectively.

Preparation of water extract of muriate of potash :-

1. Weigh 0.5 g muriate of potash and dissolve in distilled water and make up the volume to 250 ml (Filter if necessary) Dilute the extract if necessary.
2. Feed the extract to the flame photometer after adjusting the scale of flame photometer with standards.

Observations :-

1. wt. of sample
2. Vol. of extract made
3. Flame photometer reading

   Record the standard curve readings and plot a standard curve.

Calculations :-

$$\% K = \frac{\text{Graph Reading (in ppm)}}{} \times \text{made} \times \frac{\text{Volume}}{1000} \times \frac{1}{1000} \times \frac{1}{\text{Wt. Of sample}} \times 100$$

$\% K_2O = \% K \times 1.20$

NOTE:- If dilution of water extract is made, introduce the dilution factor in calculations.

Questions:-

1. Write the principle of flamephotornetry.

2. How will you prepare 100 ppm K solution of K_2SO_4?

3. Write the wave length of radiation emitted by K at excited state.

4. What colour of flame appear when K is burnt in flame ?

Reference book :-

Chopra S.L. and J.S.Kanwar (1991) Analytical Agril. Chemistry pp. 130-133.

Exercise No.-18 *Date :- / /2019*

Determination of Zinc content from micronutrient fertilizer by *EDTA Method*

Principle :-

Ethylenediamine tetra acetic acid (EDTA) forms complexes with divalent cations, like Ca^{+2}, Mg^{+2}, Zn^{+2}. The metal replaces hydrogen atoms of (-COOH) groups and is also linked by coordinate bonds to nitrogen atoms of EDTA. This principle is used in determination of cations such as Ca^{+2}, Mg^{+2}, Zn^{+2}, Cu^{+2} etc. by titration. The indicator eriochrome Black T is used to detect the end point.

Reagents :-

1) EDTA (0.01 N) : Dissolve accurately weighed 2.00 gm sodium salt of EDTA in 100 ml distilled water and finally dilute to 1 litre in volumetric flask.
2) Standard zinc solution : weigh 1.250 gm of zinc metal and dissolve in 20 ml 1:1 warm HCl. Dilute and make final volume 500 ml.
3) Ammonium hydroxide - 20 %.
4) Ammonium chloride
5) Sodium cyanide
6) Formaldehyde solution : Dilute / volume of formaldehyde 36 % (M/V) with 80 volumes of water.
7) Friochrome black – T mix 1 gm of EBT with 100 gm of NaCl

Procedure :-

1) Standardization of EDTA : Pipette 50 ml of standard solution zinc and transfer in a clean beaker. Add NH4OH until permanent precipitate is formed, add 10 ml in excess. Dilute with about 400 ml of distilled water. Add few specks of indicator. Titrate the red solution with EDTA to a clear blue colour end point.
2) Determination : Dissolve 0.5 gm of material in about 25 ml water and add 0.1 gm NH_4Cl and 0.5 gm NaCN (Add more NaCN, if precipitate formed, does not dissolve completely). Add approximately 75 ml water and 10-20 ml NH_4OH. Add few specks of indicator. Mix and add 20 ml formaldehyde solution. Immediately titrate with EDTA till clear blue colour end point.

$$\text{Percent zinc in sample} = 12.5 \times \frac{V1}{V_2 \times M}$$

Where,

V_1 = Vol. of EDTA used with sample in determination.

V_2 = vol. of EDTA used in standardization.

M = Mass of sample taken

Reference :-

1. A.K. Gupta (2007). Practical Manual of Agril. Chemistry. pp. 105-106.