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SOIL AND PLANT ANALYSIS MANUAL

By

S. B.Pradhan
Soil Laboratory Consultant

The Agro-Enterprise and Technology Systems Project
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Dedicated to my late father

Mr. Ananta Bahadur Pradhan

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S.B.Pradhan

FOREWORD

The need of a Manual for Soil and Plant Analysis with the methods suitable to our condition has been felt for many years by most of the Soil Scientists engaged in testing work at different soil laboratories of the country. This manual is, therefore, set forth with the hope of arriving at a more uniform set of analytical methods and better co-ordination among the soil laboratories. With uniformity of methods of analysis, the data from the laboratories can be compared directly and conclusions can be drawn easily. The content of the Manual is an addition of Plant Analysis and some more methods of Soil Analysis including the micronutrient analysis to the previous one - **"Soil Test Manual"** by the author. I hope, the additional procedures, will help soil laboratories to carry on diagnostic works which provide more information to the research scientist towards solving nutrient related problems.

Dr. S.L. Maskey
Chief, Soil Science Division

PREFACE

This manual has been prepared to provide a more uniform set of analytical methods and better co-ordination among the soil laboratories in Nepal. With uniformity of analytical methods, data from the laboratories can be compared directly and conclusions can be drawn more easily.

This manual adds plant analysis and additional methods of soil analysis to the previous one " Soil Test Manual " written by the author. The additional procedures will help soil laboratories to conduct diagnostic work more effectively and provide more back up to the research scientists to help solve the problems related to the agriculture production in Nepal.

The author is confident that the methods described in this manual are suitable for the soil laboratories in the country. In some determinations, more than one method are given which may be necessary due to soil factors. The majority of the determinations form the basic routines of the soil laboratory, while some determinations are suited to particular classes of soil. In choosing methods and procedures, the author has used his experience from different laboratories concerning the condition, available facilities and equipment and has not included methods based on the need for relatively expensive equipment except in the case of micronutrient. However, some basic facilities like fume cupboard, exhaust system and moderately expensive equipment like spectrophotometer and flame photometer are necessary which will increase the accuracy and rate of analysis to a greater extent as in the case of potassium determination.

The single extractant method for four micronutrient Zn, Cu, Fe and Mn is a relatively simple procedure and reduces the chances of contamination, which has been a big problem in trace element analysis in the past and justifies the use of an expensive Atomic Absorption Spectrophotometer (AAS).

The determination of trace elements needs to be confined for the time being to a place where AAS is available and where the contamination problem can be controlled. This state of affairs may change and AAS may also be used in the future for the determination of Ca & Mg. In case of boron (B) analysis the previous curcumine method is replaced by the azomethine-H method as it is simpler and is useful even in the presence of a wide variety of salts.

As the scientists and technicians working in the laboratory have to deal constantly with the chemicals and reagents, some information about the reagents and chemicals, hazardous chemicals and standardization of acid and base are given in first chapter. Preparation of soil sample and the determination of its physical properties are included in second chapter. Chemical analysis of soil are given in third chapter. The analytical methods for cation exchange capacity and exchangeable cations are given in the fourth and fifth chapters whereas the sixth chapter deals with the methods for plant analysis.

I shall be grateful if the reader and users would send suggestions for improvement.

Shanker B. Pradhan

CHAPTER I

Chemical and Standardization of Acid and Base

Chapter I

Chemicals and Standardization of Acid and Base

Some information about the reagents and chemicals, list of hazardous chemicals and standardization of acid and base are described in this chapter.

1) Grades of Reagents and Chemicals

Chemicals are supplied commercially in different grades, the purest being Aristar, followed by Analytical Reagent or Reagent Grade or Guaranteed Reagent, Chemically Pure or Extra Pure, Technical or United States Pharmacecopea B.P., & Laboratory Reagent.

Grades of the Chemicals are abbreviated as follows

Analytical Reagent (BDH Company of UK or India) = AR.
Chemically Pure (" " ") = CP.
Laboratory Reagent (" " ") = LR.
Guaranteed Reagent (E. Merck W. Germany or India) = GR.
Extra Pure (" " ") = EP.
United States Pharmacecopea (U.S. Companies) = USP.

Each of the various grades has a distinct purpose and range of uses for which it is satisfactory. For standards and primary reagents, Reagent Grade or AR or GR Chemicals should be used.

2) Strengths of concentrated Acids and Bases

Manufacturers express the strength of concentrated acids and bases of liquid form in terms of specific gravity. However, in the laboratory, their strength is best expressed on the basis of Chemical equivalence or normality. Given below is the normality of some concentrated acids and bases - Table 1.1.

Table 1.1 Strength of "Concentrated" acids and bases.

Reagent	Concentration		Approximate Specific Gravity
	Normality	Percent by wt.	
Hydrochloric Acid	11.6	37-38	1.19
Sulphuric Acid	35-36	97-100	1.84
Glacial Acetic Acid	17.5	99.5	1.13
Nitric Acid	16	70-71	1.42
Perchloric Acid	9-11.6	60-70	1.51-1.67
Phosphoric Acid	45	85	1.71
Ammonium Hydroxide	15	28-29	0.91

Solutions:

1. Molar solution: Dissolve one molecular weight of the chemicals in one liter of solvent e.g 74.5 gm. Potassium Chloride (KCl) dissolved in 1 liter of water - 1N KCl.

2. 10 % solution: Dissolve 10 gms. of substance in 100 ml. water or alcohol or any other solvent.

3. Normal solution: Dissolve one gm. equivalent weight of chemicals per liter of solvent [e.g. 74.5 gm. KC1 per liter. or 50.05 gm $CaCO_3$ per liter water].

Calculation of Normality of a reagent

$$\text{Normality} = \frac{\text{Sp. gr.} \times \%\ \text{purity} \times 10}{\text{Gramme - equivalent}}$$

Table 1.2 Equivalent and Molecular weights of some common reagents

	Eq.wt.	Mol.wt
1. KCl (Potassium Chloride)	74.5	74.5
2. $K_2Cr_2O_7$(Potassium Dichromate)	49.04	294.22
3. $KMnO_4$(Potassium Permangnate)	31.6	158.0
4. $CaCO_3$(Calcium Carbonate)	50.05	100.1
5. $MgCO_3$(Magnesium Carbonate)	42.16	84.32
6. $Na_2C_2O_4$(Sodium Oxalate)	67.0	134.0
7. NaOH (Sodium Hydroxide)	40.0	40.0
8.$KHC_8H_4O_4$ (Potassium acid phthalate)	204.228	204.228

Cleaning Solutions:

1. 1:1 HCl - Dilute 100 ml. concentrated hydrochloric acid with 100 ml. water.

2. Chromic Acid - Dissolve 5.0 gm. $K_2Cr_2O_7$ or $Na_2Cr_2O_7$ in minimum amount of water & add one liter L.R. H_2SO_4.

3. Aqua Regia - Mix 300 ml. of conc. HCl with 100 ml. of conc. HNO_3.

3. Standardization of Acid

Principle:

Dilution of the concentrated acid to required strength will be approximate only. Exact strength of the diluted acid should be determined by titrating it with primary standard base or alkali of known strength.

a) Using primary standard base:

Apparatus:

1) Oven
2) Balance analytical
3) Watch glass
4) Erlenmeyer flask 250ml
5) Burette

Reagents:

1) **Sodium Carbonate:** Oven dry AR or GR quality anhydrous sodium carbonate contained in watch glass to 120^{0}C for 2hours and cool in desiccator.

2) **Hydrochloric acid:** Dilute 8.5ml of concentrated hydrochloric acid to 1L (approximately 0.1N)

3) **Methyl Orange indicator:** Dissolve 0.1gm methyl orange in 100ml distilled water.

4) **Reference solution:** Add 4 drops of methyl orange solution to 80 ml distilled water.

Procedure:

Accurately weigh enough dried anhydrous sodium carbonate (about 0.21gm) to titrate about 40ml HCl, transfer into 250ml erlenmeyer flask and dissolve in about 40ml distilled water. Add 4 drops of methyl orange indicator and titrate with hydrochloric acid in burette until color begins to deviate from reference solution. Boil the solution gently for 2 minutes and titrate again until the color is barely different from reference solution. Repeat the titration with separate lot of Na_2CO_3 till the result is constant.

Calculation:

$$\text{Normality of the acid} = \frac{\text{gm } Na_2CO_3 \times 1000}{\text{ml acid used} \times 52.997}$$

where, 52.997 = Equivalent weight of Na_2CO_3.

b) **Using Sodium Hydroxide:**

Sodium hydroxide is deliquescent chemical and can not be used as primary standard. To use NaOH for the standardization of HCl, it has to be standardized first.

Apparatus:

1) Burette
2) Pipette 20ml
3) Erlenmeyer flask

Reagents:

1) **Standard NaOH solution:** Prepare approximately 0.02N NaOH and standardize using potassium acid phthalate ($KHC_8H_4O_4$) as described below.

2) **Hydrochloric acid:** Prepare approximately 0.02N HCl by diluting 8.5ml concentrated hydrochloric acid to 1L.

3) **Phenolphthalein indicator.**

Procedure:

Pipette 20ml aliquot of HCl in three 250ml erlenmeyer flasks. Add about 20ml distilled water, boil gently for 2 minutes and add 2-3 drops of phenolphthalein indicator. Titrate it with NaOH until the solution just turns pink which persists for 1 minute. Repeat the titration until the titration value does not differ by more than 0.1ml.

Calculation:

$$\text{Normality of acid} = \frac{\text{Volume of NaOH consumed x normality of NaOH}}{\text{Volume of aliquot (acid)}}$$

4. Standardization of Base

Apparatus:

1) Erlenmeyer flask 250ml
2) Burette 50ml
3) Pipette 20ml
4) Beaker 250ml
5) Desiccator
6) Watch glass

Reagents:

1) **CO_2-free distilled water**: Boil H_2O for 20 minutes and cool with soda-lime protection
2) **Sodium hydroxide solution:**
   a) Prepare 10% NaOH by dissolving 10gm NaOH in 100ml distilled water. Set aside the 10% NaOH solution, closed with rubber stopper until Na_2CO_3 has settled leaving perfectly clear liquid (about 10 days).

   b) Dilute 10.8-11.0 ml of 10% NaOH solution to 10L (approximately 0.02N)
3) **KH phthalate:** Dry AR/GR/Extra pure/NBS Standard reagent at 120°C for 2 hours and cool in desiccator containing H_2SO_4.
4) **Phenolphthalein indicator:** Dissolve 0.1gm phenol- phthalein in 100ml ethanol.

Standardization:

Accurately weigh separately, enough dried $KHC_8H_4O_4$ to titrate about 40ml NaOH in three 250ml flask that has been swept free from CO_2. Add about 50ml cool and CO_2 free H_2O. Stopper the flask and swirl gently until sample dissolves. Add 3 drops of phenolphthalein and titrate with NaOH solution till the color just turns pink which persists for 1 minute. Titration is repeated until the volume of NaOH consumed does not differ more than 0.1ml.

Calculation:

$$\text{Normality } N = \frac{\text{gm. } KHC_8H_4O_4 \times 1000}{\text{ml. NaOH used} \times 204.228}$$

where, 204.228 = Equivalent weight of $KHC_8H_4O_4$

N = Normality of NaOH

5. Common Hazardous Chemicals

Chemical	Life Hazard	Fire Hazard	Storage
1. Acetic Acid	May cause painful burns of skin Na_2O_2 or HNO_3, yields moderately flammable vapors above flash point 104°F.	Dangerous in contact with chromic acid, materials	Isolate from oxidizing
2. Acetone	Toxicity of comparatively low order	A volatile liquid. Gives off vapor which form with air flammable & explosive mixture.	Keep away from flame
3. Bleaching Powder (Calcium Hypochlorite)	Corrosive. Irritating to skin, eyes,lungs.	Not combustible but evolves Cl_2 and at higher temp. O_2.	Store in cool, dry & well ventilated place.
4. Bromine	Corrosive; at ordinary temp. gives off poisonous suffocating vapors	Causes oxidizing affect resulting in heating & may cause fire when contact with organic matter.	Isolate,safeguard against mechanical injury.
5.Calcium Oxide		Heats upon contact with water or moisture. Swells when moist & may burst container.	Isolate; store in dry place.

Chemical	Life Hazard	Fire Hazard	Storage
6. Hydrochloric Acid	Aqueous solution is corrosive, irritating & poisonous. Fumes are corrosive & irritating to mucous membrane.	In contact with metals, H_2 is evolved which may form explosive mixture with air.	Safeguard against mechanical injury Keep away from oxidizing agents, particularly HNO_3 chlorates.
7. Hydro fluoric Acid	Vapors highly toxic & irritating to skin, eyes & respiratory tract Fumes produced by contact with NH_3 & many metals is poisonous. May be neutralized with chalk, $NaHCO_3$.	Not combustible, but volatile & reacts with glass & most substances except Pt.	Isolate,Safeguard against mechanical injury.
8. Hydrocyanic Acid	Poisonous. Few breaths may cause unconsciousness & death.Avoid contact with skin.	Forms flammable & explosive mixture with air.	Isolate. Keep away from any source of heat. Safeguard against mechanical injury.
9. Hydrogen Peroxide	Prolonged exposure to vapor irritating.	May cause ignition of combustible matter if left standing in contact with it. May decompose violently if contaminated with Fe, most metals & their salts.	Store in a cool place ventilated containers remote from combustible materials, catalytic Cu, Cr & materials, Fe, Cu, Cr.

Chemical	Life Hazard	Fire Hazard	Storage
10. Hydrogen Sulphide	Toxic 0.05-0.07% by vol. in air causes dangerous illness in 1/2 to 1 hour.	Flammable gas. Forms flammable & explosive mixture with air or O_2.	Store in ventilated place away from fuming HNO_3 & oxidizing materials.
11. Lead Nitrate	Poisonous	Oxidizing material.	Isolate,safeguard against mechanical injury.
12. Nitric Acid	Corrosive, causes severe burns by contact; deadly if inhaled.	May cause ignition when in contact with combustible materials.	Safeguard against mechanical injury
13. Phenol	Poisonous	When heated yields flammable vapors	Never store with or above food products.
14. Potassium Chlorate (Sodium-Chlorate)	Dangerous under fire condition	Explosive when in contact with combustible materials.	Isolate from combustible material, acids and sulphur.
15. Potassium Cyanide (Sodium Cyanide)	Highly poisonous when taken internally. Evolves hydrocyanic acid (poisonous) on contact with acids or moisture.	Not flammable	Isolate,safeguard containers against mechanical injury.
16. Potassium Permanganate		Oxidizing material. Explosive when treated with sulphuric acid, and in contact with alcohol,ether,flammable gases & combustible materials.	Isolate from chemicals noted under fire hazard.

Chemical	Life Hazard	Fire Hazard	Storage
17. Potassium Nitrate (Sodium-Nitrate)		In contact with organic materials causes violent combustion on ignition.	Store in dry place prevent contact with organic material.
18. Potassium Perchlorate (Sodium-Perchlorate)		Combustible in contact with organic materials. Explosive in contact with concentrated sulphuric acid.	Store in dry place away from acid and combustible material.
19. Potassium Peroxide (Sodium-Peroxide)	Strong caustic reaction and dangerous under fire conditions. Avoid breathing, and wear goggles to protect eyes.	Does not burn or explode per se but mixed with combustible substances are explosive and ignite easily even by friction or on contact with a small amount of water. Reacts vigorously with water and in large quantity this reaction may be explosive.	Store remote from organic substance and water.
20. Silver Nitrate	Corrosive and poisonous	Oxidizing material.	Store in cool and dry place away from combustible materials.
21. Sulphuric Acid	Corrosive; dangerous fumes under fire condition.	May cause ignition by contact with combustible materials. Corrodes metal.	Safeguard against mechanical injury, isolate from salt peter, metallic powder, carbides, chlorates & combustible materials.

CHAPTER II

Soil Analyses: Physical

Chapter II

Soil Analysis-Physical

This chapter deals with the preparation of soil samples and the determination of its physical properties.

1. Preparation of Soil Sample:

The soil sample arrived at the laboratory should first be labelled with the laboratory number on a piece of long lasting material or paper, with water proof ink or paint, since the label has to remain permanently with the sample. The sample container usually a plastic bag, should also be marked with same number. The soil sample is then spread on tray, the stones and undecomposed organic matter are discarded and large aggregates are broken. Labelled tag should be with the sample and the plastic bag underneath the tray to ensure the identification of sample after drying. Sample tray is left in the room or shade to air dry the soil.

After air drying, soil sample is crushed gently with a wooden pestle and mortar and sieved through 2 mm sieve. Part of 2 mm soil is again sieved through 0.2 mm sieve for organic matter determination and packed in a piece of paper. The finer soil in the paper is kept at the top of the soil sample in the plastic bag.

2. Hygroscopic Moisture of Soil

Principle:

Direct or indirect measures of soil water content are needed in practically every type of soil study. In the laboratory, determination and reporting of many physical and chemical properties of soil necessitates knowledge of water content because the soil water content varies according to humidity and therefore, the calculation of data should be on the basis of dry soil. In soils work, water content traditionally has been expressed as the ratio of the mass of water present in a sample to the mass of the sample after it has been dried to constant weight or as the volume of water present in a unit volume of the sample. Oven drying method of evaporating water has been accepted for the water content determination.

Apparatus:

(1) Oven with means for controlling temperature to 100-110°C.
(2) Desiccator (Mg-perchlorate or calcium sulphate)
(3) Spatula
(4) Aluminum can with lid or weighing bottle.
(5) Balance, (Analytical)

Procedure:

Weigh about 10 gm of soil in dry, cool aluminum can or weighing bottle with lid. Place it with lid half open in the oven. Adjust the temperature of the oven at 105°C and heat it over night at constant temperature. Let it cool in desiccator with lid slightly open for an hour and weigh in analytical balance. Heat it again in the oven at 105°C for 2hrs, cool in the desiccator for an hour and weigh as before. Repeat the heating, cooling and weighing till the weight is constant.

Calculation:

$$\text{Hygroscopic moisture \%} = \frac{\text{Weight of fresh soil - oven dry wt. of soil}}{\text{Oven dry wt. of soil}} \times 100$$

3. Particle Size Distribution (Mechanical Analysis)

Principle

Mechanical analysis separates the inorganic mineral portion of soil into classified grades according to particle size and determine their relative proportions by weight. In full analysis, the whole sample of soil and gravel would be examined, but in the procedures given below only material less than 2mm diameter is considered. Two main systems of classification of the particle size, namely USDA system and International system, are common, out of which USDA system procedure is described here.

United States Department of Agriculture (USDA) System.

Classification	Particle Size (mm)
Very coarse sand	2-1.0
Coarse sand	1-0.5
Medium sand	0.5-0.25
Fine sand	0.25-0.10
Very fine sand	0.10-0.05
Silt	0.05-0.002
Clay	<0.002

For routine analysis Hydrometer method is convenient, rapid and accurate enough. In this method, soil samples, after treating with dispersing agent is allowed to settle freely. According to Stoke's law, settling rate for the soil particles is proportional to the square

of the diameter of particles at a particular temperature, at a particular place. Therefore special soil hydrometer, calibrated to read the percent of solid gives silt and clay in 40 sec. reading and clay at 3hrs. Organic matter, calcium carbonate and soluble salt, if present in appreciable amount, should be removed by treating with hydrogen peroxide and hydrochloric acid before dispersing soil for analysis.

Apparatus

1) Soil hydrometer ASTM graduated (-5 to 60)
2) Hydrometer Jar
3) Mechanical Stirrer
4) Dispersion cup
5) Beakers 250 ml
6) Pipette, 10 ml.

Reagents:

1) **Sodium Hexametaphosphate:** Dissolve 101 gm of Sodium Hexametaphosphate in 1 L water.

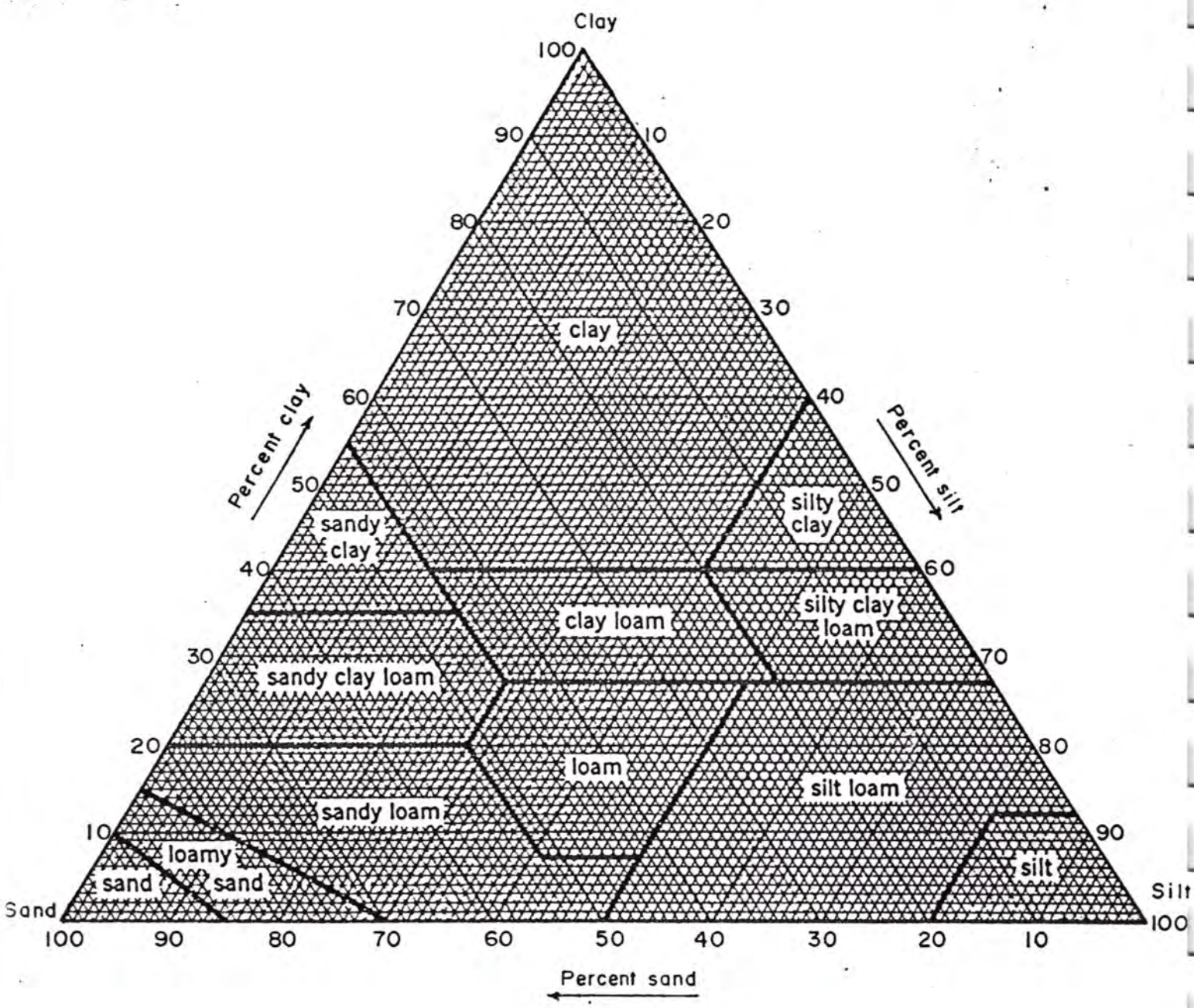
Procedure:

Weigh 100 gms of soil in a 250 ml beaker and add sufficient water to cover the soil. Then add 20ml of sodium hexametaphoshate solution, stir well with a glass rod and leave overnight. Transfer it in a dispersion cup and add sufficient water to fill two-third of the cup. Stir for 10 minutes in the mechanical stirrer, transfer in the hydrometer jar and make up the volume to the mark with the hydrometer in it. Remove the hydrometer and shake the jar upside down several times closing the mouth either by hand or cork. When the soil is well dispersed keep it in the table and note the time immediately. Immerse the hydrometer in the jar and read it at 40 sec. and after 3 hours.

(Once in the table it should not be disturbed through out the experiment). Note the temperature of the suspension at the time of taking hydrometer readings. Correct the hydrometer reading by subtracting 0.3 for every °C below 20°C or by adding if the temperature is above 20°C.

Calculation:

(Silt + Clay) % = Hydrometer reading at 40 sec + 0.3 x (t - 20)°C
Clay % = Reading at 3 hrs + 0.3 x (t-20)°C
% Sand = 100 - % (Clay + Silt)
% Silt = % (Clay + Silt) - % Clay.
Determine the texture from the triangular chart.



TRIANGULAR CHART FOR TEXTURE DETERMINATION

CHAPTER III

Soil Analyses: Chemical

CHAPTER III

Soil Analyses: Chemical

Chapter III

Soil Analyses-Chemical

Chemical analyses of macro and micro-nutrients are dealt in this chapter.

1. p^H Determination

Principle:

The p^H value of the solution surrounding soil particles in the natural state fluctuates because of changing soil-solution relationships brought about by climate, cultivation, crop growth and other factors.A sample of soil may have a particular p^H value at the time it is taken in the field but this changes in the sample as it is dried and prepared for analysis. In the laboratory, the soil is subjected to re-wetting processes with water and with certain salt solutions to establish the probable range of pH values it would have in its natural state.

A measured quantity of soil is shaken with a convenient volume of water or salt solution under consistent conditions and the p^H of the suspension is determined electronically on a direct- reading p^H meter, using a glass electrode with a saturated potassium chloride-calomel reference electrode. Almost any soil:water ratio and conditions can be employed but some ratios have been found suitable for this work and the p^H values so obtained can be utilized for useful interpretation. For this 1:1 soil: water ratio is being used in most of the laboratories.

Standard Buffer:

1. **p^H 4.0**: Dry potassium biphthalate ($KHC_8H_4O_4$) for 2 hours at 105° C and dissolve 10.21 gm. of it in hot distilled water, and dilute the solution to 1L with distilled water. As a preservative, add 1.0 ml. of chloroform or toluene. This solution has a p^H value of 4.00 at temp. from 15-30°C.

2. **p^H 6.86:** Dry the two salts KH_2PO_4 and Na_2HPO_4 for 2 hrs. at 105°C and dissolve 3.44 gm. of KH_2Po_4 and 3.55 gm. of Na_2HPO_4 in distilled water and dilute to 1L. As a preservative add 1.0 ml. of chloroform or toluene. This solution has p^H6.90 at 15°C, p^H 6.88 at 20°C and 6.85 at 30°C.

Apparatus:

1) p^H meter
2) Beaker 50 ml
3) Mechanical stirrer
4) glass rod.

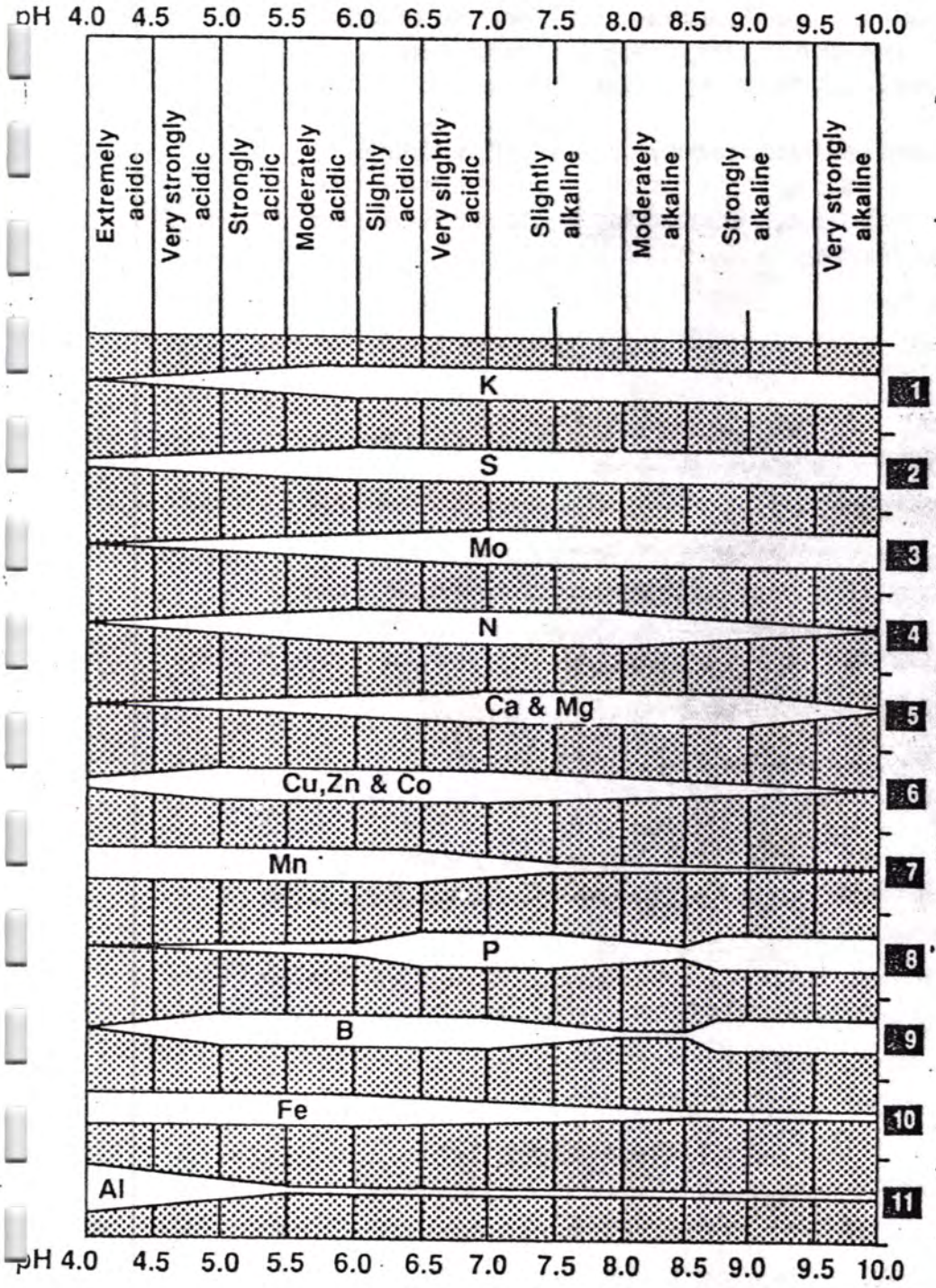
Procedure:

Weigh 20 gms. of air dried soil samples (<2 mm) in 50 ml. beaker and add 20 ml. of distilled water. Shake for about 1 minute in a mechanical stirrer and leave for about an hour. The pH meter is calibrated by using standard buffer solution of pH 4.0 & 6.86. The soil water suspension is stirred well with a glass rod just before immersing the electrode. The pH of soil water suspension is then measured with the calibrated pH meter.For the peat and muck soil, volume of water may be increased. (1:2 soil : water ratio)

Table 3.1 Rating of soil according to pH

Soil reaction (pH)	pH range
1. Extremely Acidic	<4.5
2. Very strongly Acidic	4.5-5.0
3. Strongly Acidic	5.0-5.5
4. Moderately Acidic	5.5-6.0
5. Slightly Acidic	6.0-6.5
6. Nearly Neutral	6.6-7.5
7. Slightly Alkaline	7.5-8.0
8. Moderately Alkaline	8.0-8.5
9. Strongly Alkaline	8.5-9.5
10.Very Strongly Alkaline	9.5-10.0
11.Extremely Alkaline	>10.0

Effect of pH on availability of common elements in soils. Adapted from Truog (1948).



1. Deficiencies likely at low pH.
2. Some reduction at low pH, but S bacteria still active
3. Similar to K.
4. Bacterial fixation curtailed below about pH 5.5.
5. May be deficient in acidic soils. Not available at very high pH.
6. May be toxic in acidic soils and deficient where pH > 7.0.
7. Similar to Cu, Zn, and Co.
8. Liable to be fixed by Fe, Al, Mn at low pH; insoluble forms at high pH, also Ca inhibition.
9. Over-liming may cause deficiency. Toxicity danger at very high pH.
10. Similar to Cu, Zn, and Co.
11. Liming to pH 5.5 recommended to avoid toxicity dangers at low pH.

2. Lime Requirement of Soil

Principle:

The term lime requirement means the amount of calcium carbonate or its equivalent that must be applied to soil to increase the p^H to 7.0 or some other desired value. Addition of lime to a base unsaturated soil raises the p^H; increases the proportion of calcium on the exchange complex and can immobilize by precipitating certain elements such as iron, manganese and aluminum which may be present in soil in excess.

The results obtained by a given lime requirement method are often multiplied by a "Liming factor" which takes into account the chemical composition and fineness of the limestone (lime should have 90% $CaCo_3$ equivalent and should pass 50-60 % through 100 mesh and 95% through 8 mesh sieve)

Apparatus:

1) p^H meter
2) Beaker 100 ml
3) Mechanical stirrer
4) Glass rod / policeman

Reagents:

Buffer solution: Dissolve 1.8 gm p - nitrophenol, 2.5 ml triethanolamine,3.0 gm potassium chromate, 2.0 gm calcium acetate, and 53.1 gm calcium chloride ($CaCl_2$ $2H_2O$) in 1L of distilled water and adjust the P^H to 7.0 with dilute HCl or NaOH.

Procedure:

Weigh 10 gm. of soil in 50 ml. beaker and add 10 ml. distilled water. Shake for 1 min. with mechanical stirrer and read the p^H after 30 min. If the p^H is 6.3 or less, add 20 ml. of above buffer solution to the soil - water suspensions and stir intermittently for 20 minutes. Read the p^H of soil - buffer suspensions immediately and use Table 3.2 to determine the lime requirements.

Table 3.2 Lime required to bring the soil to an indicated p^H according to soil - buffer p^H.

Soil Buffer p^H	Lime required to bring soil to indicated p^H tons/acre					
	Pure $CaCO_3$			Agricultural ground lime* (to nearest half ton)		
	p^H 6.8	p^H 6.4	p^H 6.0	p^H 6.8	p^H 6.4	p^H 6.0
6.7	1.4	1.2	1.0	2.0	1.5	1.5
6.6	1.9	1.7	1.4	2.5	2.5	2.0
6.5	2.5	2.2	1.8	3.5	3.0	2.5
6.4	3.1	2.7	2.3	4.5	4.0	3.0
6.3	3.7	3.2	2.7	5.0	4.5	4.0
6.2	4.2	3.7	3.1	6.0	5.0	4.5
6.1	4.8	4.2	3.5	6.5	6.0	5.0
6.0	5.4	4.7	3.9	7.9	6.5	5.5
5.9	6.0	5.2	4.4	8.5	7.5	6.0
5.8	6.5	5.7	4.8	9.0	8.0	6.5
5.7	7.1	6.2	5.2	10.0	8.5	7.5
5.6	7.7	6.7	5.6	10.5	9.5	8.0
5.5	8.3	7.2	6.0	11.5	10.0	8.5
5.4	8.9	7.7	6.5	12.5	10.5	9.0
5.3	9.4	8.2	6.9	13.0	11.5	9.5
5.2	10.0	8.6	7.4	14.0	12.0	10.0
5.1	10.6	9.1	7.8	14.5	12.5	11.0
5.0	11.2	9.6	8.2	15.5	13.5	11.5
4.9	11.8	10.1	8.6	16.5	14.0	12.0
4.8	12.4	10.6	9.1	17.0	14.5	12.5

* Agricultural ground lime as used here is grade of lime with sieve analysis showing at least 95% passing an 8 mesh screen and 50-60% passing through 100 mesh screen and an average neutralizing power of 90% as that of pure $CaCO_3$.

3. Electrical Conductivity

Principle

The conductivity of a soil is the specific conductivity at 25°C of a water extract obtained from a soil and water mixture of a definite ratio. It is measured on a conductivity meter and is normally expressed in milliSimens/cm, the value giving information on the total amount of water soluble salts present in soil, i.e., on the degree of salinity.

The most easily interpretable conductivity values are those on a saturation extract, prepared from a saturated soil paste. It is also useful to obtain routine conductivity readings on soil: water mixture at other ratios, usually 1:1, 1:2, 1:5 separating soil as much as possible by setting. When extraction is other than saturation, following formula may be used to calculate the conductivity (L) at sat. extraction.

$$L_{sat.\ ext} = L_{1:2} \times \frac{200}{\%\ \text{water in soil saturation}}$$

Following formula is used to calculate the salt concentration in the soil.

$$\%\ \text{salt in soil} = 0.064 \times L_{mS/cm} \times \frac{\%\ H_2O \text{ in soil at extraction}}{100}$$

The specific conductance of the saturation extract can be interpreted directly in terms of plant growth, by means of the following scale.

Table 3.3 Salinity Scale

Specific conductance of the saturation extract of soil, mS/cm

0 2 4 8 16

Non Saline	Very Slightly Saline	Moderately Saline	Strongly Saline	Very Strongly Saline
Salinity effects mostly negligible	Yields of very sensitive crops may be restricted.	Yields of many crops restricted Alfalfa, cotton, sugarbeats cereals and grain sorghum adapted	Only tolerant crops yield satisfactorily; Bare spots appear because of injury to germination	Only a few tolerant crops yield satisfactorily. Only salt tolerant grasses, herbaceous plants, shrubs and tree grow

0 0.1 0.3 0.5 1.0

(Percentage of salt in moisture saturation extract)

Apparatus:

1. Conductivity Bridge with platinum cell.
2. Mechanical Shaker.
3. Buchner Funnels.
4. Filtering flask.
5. Vacuum pump.
6. Beaker 1000 ml.
7. Spatula.

Reagent:

1. **0.02M KCl:** Dissolve 1.49 gm. dried Potassium chloride in 1 liter distilled water. The specific conductance L, of this solution is 2.39 mS/cm at 18°C and 2.768 mS/cm at 25°C.

Procedure (Saturation Extract):

Weigh 500 gm. of soil in a beaker and add about two-third of the water needed down the side of the beaker and leave it. The soil should not be disturbed during this process because water movement through puddled soil is very slow. Add water by increments until the soil mass is fully wetted by capillarity. Allow sufficient time for movement of water through capillary, before adding more to the sample. The soil is then stirred with a spatula and more water or soil added to give the final adjustment of water content. The water is right when the soil hardly flows together in a hole made by spatula, the mixture slides off the spatula and the soil surface is wet enough to glisten. Free water should not collect in the depression on the surface on standing for few minutes. If free water stands on the surface, too much water has been added, and a little more soil is added to blot up the excess water.

Cover the beaker and leave for 2 hours to reach equilibrium.The soil is placed on a suitable size of a buchner funnel with tightly seated Whatman No.42 filter paper and the suction is applied to get saturation extract.

Determine the cell constant of conductance cell by measuring the electrical conductance C of a standard KC1 solution.

$$\text{Cell constant K} = \frac{2.768 \text{ mS per cm}}{\text{KCl reading}} \text{ (at 25°C)}$$

Wash the cell with distilled water and dry with tissue paper or filter paper and read the conductance of soil extract.
Specific conductance of extract = K x R mS/cm
Where, R is the reading for the soil extract in mS/cm and K is the cell constant.

Procedure (1:5 extraction):

Weigh 10 gm soil in 100ml beaker (preferably tall form) and add exactly 50ml distilled water. Stir intermittently for 5 minutes and filter after 30 minutes. Read the conductance of the extract as in saturation extract procedure and multiply by 10.

Note: The temperature of the solution should be taken into account in the calculation of the result. Electrical conductivity of a solution increases approximately 2 percent per degree C.

4. Organic Matter determination (Walkley - Black method)

Principle:

Oxidizable organic matter in the soil is oxidized by chromic acid in the presence of sulphuric acid, the reaction being facilitated by the heat of dilution when 2 volumes of concentrated H_2SO_4 are mixed with 1 volume of IN $K_2Cr_2O_7$ solution. The excess chromic acid is determined by titration with ferrous ammonium sulphate solution and the quantity of the substance oxidized is calculated from the amount of dichromate reduced.

The highest temperature attained by heat of dilution of sulphuric acid is about 120°C, which is sufficient to oxidize the active forms of soil organic C, but not the more inert forms of C that may be present. From the experiments, 77 percent carbon were found to be recovered and same figure was used in calculation. Also, the organic matter is considered to contain 58 percent carbon, and therefore multiplying factor 1.72 was used to convert organic carbon to organic matter. Since no external heat is being utilized, finer soils passing through 0.2 mm sieve is taken for the analysis.

This determination is affected by the presence of easily oxidizable substances like chloride, higher oxides of manganese and ferrous compounds. Whereas Fe^{+2} and Cl give positive or high value, the oxides of manganese give negative or low value. Soil samples air dried for 1-2 days contain insignificant amount of soluble ferrous compound and chloride can be easily eliminated by adding silver sulphate.

According to Walkley - Black, normal soil contains invariably small quantity of reducible oxides of Mn. Only in highly manganiferrous soils, small fraction of all oxides is present in the active state capable of competing with chromic acid.

Apparatus:

1. 50 ml. Burette.
2. 10 ml. bulb pipette.
3. Measuring cylinder 25 ml. or Acid Dispenser or pipette 20 ml. capacity.
4. Conical flask 500 ml. capacity.

Reagents :

1. **Sodium fluoride.**

2. **1N Potassium dichromate solution**: Dissolve 49.04gms. of A.R. Potassium dichromate (dried at 105℃) in distilled water and dilute to the mark in 1L volumetric flask.

3. **0.5N Ferrous ammonium sulphate (FAS):** Dissolve 196 gms. of $Fe(NH_4)_2(SO_4)_26H_2O$ in 800ml distilled water and 20ml concentrated sulphuric acid and dilute to 1L.

4. **Diphenylamine indicator:** Approximately 0.5 gm. of reagent grade diphenylamine is dissolved in 20 ml. of distilled water, add 100 ml. of conc. H_2SO_4.

Procedure:

Weigh 1 gm. soil sample, passing through 0.2 mm sieve and add exactly 10 ml of IN $K_2Cr_2O_7$ solution to it, in a 500 ml. conical flask. Add 20 ml. of concentrated sulphuric acid and mix by gentle rotation for 1 minute, to insure complete contact of the reagent with the soil, but with care to avoid throwing up soil to the sides of the flask. The mixture is allowed to stand for 30 minutes. A standardization blank (without soil) is run in the same way. After half an hour, add about 200 ml of distilled water, 30 drops of diphenylamine indicator and about 0.2 gm. of sodium fluoride. Back titrate the solution with ferrous ammonium sulphate solution. The color is dull green with chromos ion in the beginning, then shifts to a turbid blue as the titration proceeds. At the end point this color sharply shifts to a brilliant green. If over 8 ml out of 10 ml. chromic acid has been consumed during the titration, the determination is repeated with a small quantity of soil or by adding double or treble amount of $K_2Cr_2O_7$ and H_2SO_4.

Calculation

$$\text{O.M. \%} = \frac{(B-S) \times N \times 3 \times 100 \times 100 \times 100}{\text{wt of soil} \times 1000 \times 77 \times 58} = \frac{(B-S)\,N}{\text{wt of soil}} \times 0.67$$

where, B = Volume of FAS used up for blank titration
S = " " " sample "
N = Normality of FAS from blank titration
wt = wt. of soil sample

Note: i) Organic matter contains 58 percent carbon. ii) Recovery factor for this method is 77 percent.
iii) Equivalent weight of carbon = 3.

5. Total Nitrogen

Principle:

Most of nitrogen in the soils is in organic form. Relatively small amounts ordinarily occur in ammonium and nitrate form. Most widely used procedure for N determination is Kjeldhal method in which organic N compounds are converted into ammonium sulphate by digestion with concentrated H_2SO_4. The ammonium ions in the soil also react with the acid but nitrate and nitrite ions are lost. To include nitrate, salicylic acid and sodium thiosulphate must be added. The digestion of the soil with sulphuric acid is facilitated by using sodium or potassium sulphate (raises boiling point) and copper sulphate (catalyses the reaction). The digested solution liberates the ammonia on treating with alkali, which is collected in the boric acid solution and titrated with standardized dilute acid using mixed indicator.

Apparatus:

1. Kjeldhal digestion flask - 50 ml or block digester tubes.
2. N - Digestion apparatus.
3. Distilling apparatus.
4. Volumetric flask - 100 ml.
5. Conical flask - 125 ml.
6. Acid dispenser or measuring cylinder.
7. Pipettes, - 10, & 20 ml.

8. Burette - 25 ml.
9. Kjeldhal flask holder.
10. Asbestos Glove.

Reagents:

1. **Digestion Mixture (Catalyst):** Grind and mix 10 gms. of copper sulphate with 200 gms. of sodium sulphate.

2. **Concentrated sulphuric acid - L.R.**

3. **Sodium hydroxide:** Dissolve 400 gms. of sodium hydroxide (flakes or pellets L.R.) in one liter of distilled water and cool.

4. **Mixed indicator:** Dissolve 0.5 gm. bromo cresol green and 0.1 gm. methyl red in 100 ml. of 95 percent ethanol.

5. **Boric acid 4%:** Dissolve 40 gm. boric acid crystal (H_3BO_3) in one liter distilled water.

6. **0.01N HCl:** Dilute 17 ml. conc. HCl with distilled water to 2L-(A) Standardize 20 ml of solution (A) with $NaCO_3$ and calculate its normality. Dilute the solution (A) according to its strength to give 0.01N HCl. [or - Dilute 100 ml. of solution (A) to IL. and calculate its strength from the above normality of (A)].

Procedure:

Weigh 1 gm. soil in 50 ml. Kjeldhal digestion flask and add 2 gm. catalyst digestion mixture followed by 10 ml. conc. H_2SO_4 and few pieces of broken porcelain. For a fine textured soil add 10 ml. of distilled water and leave it for 30 minutes before adding digestion mixture and sulphuric acid. Mix the soil with sulphuric acid by swirling the flask and heat in the low heat until frothing stops. Gradually increase the heat until the acid boils. Swirl the flask at intervals and digest until the color of the mixture changes to green- blue or grey color and continue it for 1-1.5 hours more. Care should be taken during digestion not to allow the flame to touch the flask above the part occupied by the liquid.

With Block Digester Tecator, add 10ml concentrated sulphuric acid to 1 gm soil and 2gm digestion mixture in 250ml digestion tube and heat in preheated Block Digester at 410°C for 45 minutes. If exhaust system is used, adjust its rate to maximum in the beginning of digestion and reduce it after about 10 minutes (when the evolution of dense fumes of sulphuric acid decrease) so that the acid fume will be condensed at about two-third of the digestion tube.

Cool the flask and add about 20ml. of distilled water before the solution starts crystallizing. Transfer the solution in a 100 ml. volumetric flask, leaving the sand in the digestion flask and make up the volume. Take 20 ml. aliquot in the distilling flask and add 20 ml. of 40 % NaOH and distil it, collecting the liberated NH_3 in 10 ml. 4% boric acid solution containing 2 drops of mixed indicator in 125 ml. conical flask. Titrate it with O.OIN HCl. Run a blank for each batch of 12 samples.

Calculation:

$$\% N = \frac{7 \times n \times (T-B)}{S}$$

Where, n = Normality of acid.
T = Vol. of acid used in titration.
B = Vol. of acid used in Blank.
S = Sample wt.

Interpretation:

a) Terai

Low..... <0.075 % N
Medium..... 0.075 - 0.150 % N
High..... >0.150 % N

b) Hills

Low..... <0.1% N
Medium..... 0.1 - 0.30 % N
High..... >0.30 % N

6. Available Phosphorus

Principle:

Many different solutions have been proposed for the extraction of the available phosphorus that can be correlated with the field crop response. As the phosphate ions in the soil solution are present in small amount, the plant available phosphorus includes some of the insoluble soil phosphorus also. The insoluble soil phosphorus present are mainly di and tri calcium phosphates in neutral and alkaline soils and aluminum and ferric phosphates in acid soils. Three of those extracting methods to include insoluble phosphates have been described in this manual.

Modified Olsen's-Bicarbonate (p^H 8.5) extracting solution is best suited for calcareous and alkaline soil but also recommended for the moderately acid soils.

Bray and Kurtz No.1 (0.03N NH_4F in 0.025N HCl) extracting solution is said to remove "adsorbed" phosphorus and is recommended for acid soil.

Bray and Kurtz No. 2 (0.03N NH_4F in 0.1N HCl) extracting solution is said to remove "absorbed" and "acid soluble" phosphorus and is recommended for acid as well as neutral or even up to calcareous soil.

The extraction of available phosphorus from the soil is affected by:

1. Fineness of the particle
2. Soil: Extractant ratio
3. Shaking period

Several methods are available for color development. The choice of the method for determining P depends upon the concentration of P, concentration of interfering substances and the acid system involved in the procedure.

The molybdenum blue methods are the most sensitive and as a result, they are widely used for the soil extracts containing small amount of P as well as total P determination in soils. These methods are based on the principle that in an acid molybdate solution containing orthophosphate ions, phosphomolybdate complex forms that can be reduced by stannous chloride or other reducing agents to a molybdenum blue color. The intensity of blue color varies with the P concentration (follows Beers Law) but is affected by other

factors such as acidity, arsenates, silicates and substances which influence the oxidation-reduction conditions of the system.

(a) Modified Olsen's bicarbonate Method

Principle:

This method uses 0.5N $NaHCO_3$ solution of p^H 8.5 as an extractant which controls the activity of Ca, Al and Fe by precipitating calcium as carbonate and aluminum and iron as hydroxides. The organic matter dissolved by the extractant must be removed by the use of activated charcoal.

Apparatus:

1. 100 ml polythene bottles
2. Shaker
3. Funnel
4. Whatman No. 42 filter paper
5. Volumetric Flask 50 ml.
6. Pipettes 5 and 10 ml.
7. Beakers 50 ml.

Reagents:

1. **Extracting solution (0.5N $NaHCO_3$ p^H 8.5):** Dissolve 210 gm of CP $NaHCO_3$ in 5L distilled water. Adjust the pH to 8.5 with 0.5N NaOH or H_2SO_4. As the p^H of the solution tends to increase on exposed to atmosphere, few drops of liquid paraffin should be added and the pH should be checked monthly.

2. **5N H_2SO_4:** Dilute 35 ml concentrated A.R. H_2SO_4 to 250 ml.

3(a) **Ammonium Molybdate:** Dissolve 12.0 gm of A.R. ammonium molybdate in 250 ml distilled water. In 100 ml distilled water dissolve 0.2908 gm of antimony potassium tartrate. Add both the solutions to 1000 ml of 5N H_2SO_4 (141 ml of concentrated H_2SO_4 per liter water), mix thoroughly and make to 2L. Store in a pyrex glass bottle and keep it in a dark and cool temperature.

3(b) Dissolve 1.056 gm of ascorbic acid in 200 ml of ammonium molybdate solution [Reagent (3a)]. This reagent should be prepared as required since it can not be kept for more than 24 hours.

4. Activated Charcoal (Darco G-60)

5. Standard P Solution

(a) **Primary standard 50 ppm P:** 0.2195 gm of A.R. KH_2PO_4, dried at 40°C, is dissolved in about 400 ml of distilled water in one liter volumetric flask. Add 25 ml of 7N H_2SO_4 to it and make the volume to IL. Thus preserved with H_2SO_4, the solution keeps indefinitely but it should be stored in a weathered soft glass bottle (rather than one of pyrex) to minimize contamination with arsenic.

(b) **Secondary standards, 2 and 20 ppm P:** Dilute 20 ml of 50 ppm P stock solution to 500 ml in a volumetric flask for the 2 ppm P. Similarly for the 20 ppm P, dilute 100 ml of 50 ppm P stock solution to 250 ml in a volumetric flask. These dilute standard solutions do not keep well, even with toluene added, and must be made up if fungus growth in the solution is noticed.

6. p-nitrophenol indicator 0.25%: Dissolve 0.25 gm indicator in 100 ml of distilled water.

Procedure:

Weigh 2.5 gm soil sample (air dried <2 mm) in a 100 ml polyethylene bottle. Add one teaspoon of activated charcoal (Darco G-60) and 50 ml of 0.5N $NaHCO_3$ extracting solution. Shake for 30 minute in a shaker and filter through whatman No. 42 filter paper. Pipette 10 ml aliquot of the filtrate in a 50 ml volumetric flask and acidify with 5N H_2SO_4 to pH 5.0 using p-nitrophenol indicator till the yellow color just disappeared. Shake gently after each addition of acid. Further add acid, dropwise this time until the color changes from yellow to colorless. Add distilled water washing down the sides of volumetric flask to 40 ml followed by 8 ml of reagent 3b. Make up the volume to the mark and shake well. Maximum intensity of the blue color is obtained in 10 minutes and remains stable up to 24 hrs. Include a blank in every batch by shaking the extracting solution without soil. It should include all the reagents added in every step. Measure the

color intensity in a colorimeter after 10 minutes using red filter (660 mu). Prepare the standard curve by taking 0,1,2,4,6,8,10,12 and 15 ml of 2 ppm P standard solution in 50 ml volumetric flask, add $NaHCO_3$ extracting solution and proceed exactly like in test solution.

Calculation:

$$\text{ppm P in soil} = \text{ppm P in solution} \times \frac{50}{10} \times \frac{50}{2.5}$$

$$= \text{ppm P in solution} \times 100$$

$$P_2O_5 \text{ Kg/ha} = \text{ppm P in soil} \times 2.24 \times 2.3$$

Where,

2.24 = conversion factor for ppm in soil to Kg/ha in soil

2.3 = conversion factor for P to P_2O_5

Interpretation

Low..... < 26 Kg/ha P_2O_5
Medium.....26 - 55 Kg/ha P_2O_5
High..... > 55 Kg/ha P_2O_5

b). Bray and Kurtz No. 1

Principle:

The fluoride has the special property of complexing Al^{+3} and Fe^{+3} in acid solution, with consequent release of P held in the soil by these trivalent ions. The combination of HCl and NH_4F is designed to remove easily acid soluble P forms, largely calcium phosphates, and a portion of the aluminum and iron phosphates.

Apparatus:

Same as in Olsen's bicarbonate method.

Reagents:

(1) **Extracting solution (0.03N NH_4F-0.025N HCl):** Dissolve 22.2 gm of solid NH_4F in 180 ml of distilled water, filter and add to it 18L of water containing 83.3 ml of 6N HCl. Make up the volume to 20L.

(2) **10N HCl:** Dilute 1300 ml of AR HCl with 200 ml of distilled water. Cool and make up the volume to 1500 ml.

(3) **2N HCl:** Dilute 50 ml of concentrated CP HCl to 300 ml with distilled water.

(4) **(1:5)NH_4OH:Water:** Dilute 50 ml of concentrated ammonium hydroxide to 300 ml with distilled water.

(5) **Boric acid (0.8N):** Dissolve 50 gm of crystal H_3BO_3 in 1L of distilled water.

(6) **Chloromolybdic Acid (1.5 percent):** Dissolve exactly 15.0 gm of AR or CP ammonium molybdate $[(NH_4)_6Mo_7\ O_{24}.4H_2O]$ in about 300 ml of distilled water, warm to about 50°C. If necessary, filter the solution to remove sediments. Cool the solution and add 350 ml of 10.0N HCl gradually with stirring. After this solution has cooled again to room temperature, dilute with distilled water in 1L volumetric flask, mix thoroughly and store in a stoppered amber glass. Replace this reagent every 2 months.

(7) **Stannous chloride:**

a. **Stock Solution:**Dissolve 10 gm of A.R. stannous chloride, $[SnCl_2.2H_2O]$ in 25 ml concentrated hydrochloric acid, HCl. Keep it in a dark tightly stoppered bottle. This solution has to be replaced every 2 months.

(b) Working Solution: Dilute 1 ml of stannous chloride stock solution to 333 ml with freshly boiled and cooled distilled water. This working solution should be prepared for every 4 hours work.

(8) **Standard phosphorus solution 2 ppm P :** Dilute 20 ml of 50 ppm P stock solution (Olsen's bicarbonate method) to 500 ml with distilled water in a volumetric flask.

Procedure:

Weigh 2.85 gm of air dry soil sample (<2 mm) into a 100 ml polyethene extraction bottle. Add to it 20 ml of the extraction solution (0.03N NH_4F - 0.025N HCl) from a pipette or automatic dispenser. Stopper the bottle and shake for 1 minute in a mechanical shaker. Filter the suspension immediately through Whatman No. 42 filter paper. If the filtrate is not clear, quickly pour back through the same filter.

Take 5 ml aliquot of the clear filtrate into a 50 ml volumetric flask and add 15 ml of boric acid solution to minimize fluoride ion interference. Since the boric acid does not completely prevent fluoride interference, the appropriate amount of extracting solution 5ml and boric acid 15ml should be added to the blank as reference standards.

Adjust the p^H of the solution in 50ml volumetric flask to 3.0 with 1:5 NH_4OH and 2N HCl using 2,4 - dinitrophenol as indicator. Add distilled water to about 30 ml mark followed by 10 ml of ammonium molybdate and mix well. Finally add 5 ml of freshly diluted stannous chloride and make up the volume upto 50 ml mark and shake to mix.

Read the color of the solution within 5 to 15 minutes in a colorimeter using red filter (660 mu). Include a blank in every batch by shaking the extracting solution without soil. It should include all the reagents added to the sample. Prepare the standard curve by taking 0,1,2,4,6,8 and 10 ml of 2 ppm P standard in 50 ml volumetric flask, add the extracting solution and proceed exactly like in test solution.

Calculation:

$$\text{ppm P in soil} = \text{ppm P in solution} \times \frac{50 \times 20}{5 \times 2.85}$$

$$= \text{ppm P in solution} \times 70$$

$$P_2O_5 \text{ Kg/ha} = \text{ppm P in soil} \times 2.24 \times 2.3$$

Where, 2.24 = conversion factor for ppm in soil to Kg/ha in soil
2.3 = conversion factor for P to P_2O_5

Interpretation

Low..... < 35 Kg/ha P_2O_5
Medium.....35 - 105 Kg/ha P_2O_5
High..... > 105 Kg/ha P_2O_5

(c) **Bray and Kurtz No. 2**

This method has higher concentration of HCl and includes more phosphorus of the soil apatite in near neutral or calcareous soil compared to Bray and Kurtz No. 1 method.

Apparatus:

Same as in Bray and Kurtz No. 1

Reagents

(1) **Extracting solution (0.03N NH_4F and 0.1N HCl) :** Dissolve 11.10 gm of solid NH_4F in 200 ml of distilled water, filter and add to it 5L of distilled water containing 166.7 ml of 6N HCl and make up the volume to 10L.

(2) Other reagents same as in Bray and Kurtz No. 1.

Procedure:

The procedure is the same as in Bray and Kurtz No. 1 except the extracting solution of 0.03N NH_4F and 0.1N HCl.

7. Available Potassium

Principle:

The potassium extracted by normal neutral ammonium acetate is considered to be available to plants. For most soil the potassium removed is largely that associated with the clay and humus complex as exchangeable ions but in some saline soil, there may be a fair amount of water soluble potassium. In the assessment of availability, exchangeable and water-soluble potassium ions are not differentiated, but measured together in the soil extract, usually by flame photometer.

In routine analysis it is accurate enough to shake soil with ammonium acetate solution in 1:10 soil:extractant ratio, a procedure which normally removes 90-95 percent of the exchangeable potassium and all the water-soluble potassium. Alternatively, soil may be leached with ammonium acetate solution.

Although exchangeable and water soluble potassium values are usually reported in meq.per 100 gms, available potassium values are always reported in ppm or kg/ha in soil.

a). Flame Photometer method :

Apparatus:

(1) Flame photometer.
(2) Shaking apparatus.
(3) 100 ml. Erlenmeyer flask.

Reagents:

(1) IN Ammonium acetate pH 7.0: Dissolve 77.0 gm. of ammonium acetate in 1L of distilled water.

or

To 58 ml of glacial acetic acid add 500 ml. of distilled water followed by 65 ml.of liquor ammonium and dilute to 1L. Adjust the pH to 7.0 $\pm$ 0.02 with dilute NH_4OH or acetic acid.

(2) K standard (stock solution): Dissolve 0.1905 gm. dried KCl in 1L volumetric flask and make up the volume -- 100 ppm K

Take 0,5,10,15,20 & 25 ml. of 100 ppm K solution in 100 ml volumetric flask and dilute with 1N ammonium acetate pH 7.0 solution to the mark-0,5,10,15,20 & 25 ppm. K.

Procedure:

Weigh 2.0 gm. air dried soil in a 125 ml. conical flask (or 100 ml polyethylene bottle with leak proof cap), add 20 ml. normal neutral ammonium acetate, shake for 5 minutes in a mechanical shaker and filter through Whatman No. 42, 12.5 cm filter paper. Prepare a standard curve of K by aspirating 0,5,10,15,20 and 25 ppm K after adjusting full scale

deflection of flame photometer with 25ppm K, note the reading and draw graph. Aspirate the soil solution, note its reading and determine K in the soil solution from the graph.

Calculation:

$$K_2O \text{ Kg/ha} = R(\text{ppm}) \times \frac{20}{2} \times 1.2 \times 2 \times 1.12 = R \times 26.88$$

where,	R=	Potassium of soil extract in ppm. from the standard curve.
	1.2=	Conversion factor for K to K_2O
	2x1.12=	Conversion factor for ppm to Kg/ha
	20/2 =	Dilution factor

Interpretation

Low.....< 110 Kg/ha K_2O
Medium..... 110 - 280 Kg/ha K_2O
High.....> 280 Kg/ha K_2O

b). <u>Turbidimetric method:</u>

Apparatus:

1. Colorimeter with colorimeter tubes.
2. Hypodermic syringes 2 ml. with 18 gauge needle.

Reagents:

1.(a) **Standard Potassium stock solution:** Dissolve 1.905 gm AR KCl dried at (105°C) in 1L. distilled water. - 1000ppm K.

1.(b) **Working Solution** - Take 10,20,30,40 & 50 ml. of this stock solution and dilute to 1L with Morgan's reagent. This will give 10,20,30,40 and 50 ppm K.

2. **Morgan's Soil extractant**:- Dissolve 100gm pure sodium acetate in 500 ml water. Add 30 ml. of glacial acetic acid and dilute with distilled water to 1L.

3. **Sodium Cobaltinitrite solution:-** Dissolve 50 gm of $Co(NO_3)_26H_2O$ and 300 gm of sodium nitrite $NaNO_2$ in 600ml. distilled water. Add 25ml. of glacial acetic acid and dilute to 1L with distilled water. Keep over night, filter and store in a cool dark place in a glass stoppered brown bottle. If the sodium cobaltinitrite itself is available, prepare 20% solution by dissolving 20 gm of $Na_3CO(NO_2)_6$ in 80 ml of cold distilled water (5°C) and make up the volume to 100 ml. After 24 hours, the solution is filtered to remove insoluble matter. Store the filtered solution in the refrigerator. It can be used upto 3 weeks.

4. **Alcohol mixture** :- Mix 100ml pure methyl alcohol with 100ml isopropyl alcohol and store in a glass bottle.

Procedure:

Preparation of standard curve: All apparatus and reagents are cooled to 60-66°F (15.5-19°C) before use. Take 2 ml. of alcohol mixture in each of the test tubes and cool it. Add 6 drops of sodium cobaltinitrite solution with a dropper directly in the alcohol mixture and shake well. Deliver 2 ml. of each of working potash standard solution i.e. 0,10,20,30,40 & 50 ppm K. by means of the hypodermic needle into the alcohol mixture in the different tubes, holding the needle vertically at a distance of about 1 inch from the

surface of the solution in the tubes. Shake the solution in the tubes vigorously for 20 seconds, to develop a uniform turbidity in the reagent. A blank is prepared in the same way using 2 ml. of Morgan's solution without potassium. Keep the tubes in the water at 66°F (19°C) for 10 minutes and read the turbidity with the Colorimeter, using 660 mu. (red) filter and setting the instrument at zero with the blank. Plot the colorimeter reading against ppm K. present in the standard solution.

Determination in soil sample:

Weigh 5 gm. of air dried soil passing 2 mm sieve in a 125 ml. conical flask or polyethylene bottle and add 25 ml. of soil extractant. Shake for 5 minutes in a mechanical shaker, filter and develop the turbidity as above, using 2 ml. of the filtrate and read on the colorimeter.

Calculation:

$$K_2O \text{ Kg/ha} = R(\text{ppm}) \times \frac{25}{5} \times 1.2 \times 2 \times 1.12 = R \times 13.44$$

where,

R	=	K ppm in the soil extract from the standard curve.
1.2	=	Conversion factor for K to K_2O
2x1.12	=	Conversion factor for ppm to Kg/ha
25 / 5	=	Dilution factor

8. Available Sulphur

Principle:

The specific forms of sulphur in soil that are available to the plants are not completely understood. Although it has been demonstrated in nutrient cultures that organic S compounds may be utilized by plants, it is generally accepted that most of the S in soils is absorbed by plants in sulphate form. Numerous reagents have been proposed for measuring extractable SO_4^{-2} in soils. These include water, salt solutions and acidic solutions of the salts. Extracting soils with water, acid or alkaline buffered solutions frequently results in colored media which are unsatisfactory for the turbidimetric method of determination. But the turbidimetric determination of sulphate as $BaSO_4$ is well adapted to measuring the small amount of sulphate which occur in many soils. Also the

turbidimetric method is suitable for the laboratories having simple equipment like colorimeter. The method utilizes the most characteristic reaction of the SO_4^{-2} ion with Ba^{+2} to form $BaSO_4$. The method presented here overcomes the above mentioned problem of color media.

Apparatus:

1. Colorimeter or spectrophotometer.
2. Erlenmeyer flask 100 ml.
3. Shaker.
4. Pipette 10 ml, 1 ml.

Reagents:

1. **Extracting solution**: Dissolve 39 gm of NH_4OAC in 1L of 0.25N acetic acid.

2. **Activated charcoal (Darco G-60 or Norit "A"): Wash the charcoal with** extracting solution until it is free of sulphate. (Test with barium chloride solution)

3. **Standard sulphur solution:**

   a). Dissolve 0.5434 gm of dried AR K_2SO_4 in 1L of distilled water - 100 ppm.S as K_2SO_4.

   b). Working standard of S is prepared by diluting 0,2,5,10,20,25,30 and 40 ml. of 100ppm S solution with extracting solution to 100ml, to give 0,2,5,10,20,25,30 and 40 ppm S.

4. **Acid "Seed" solution**: Dilute 260 ml of concentrated HCl to 400 ml with distilled water and add exactly 100 ml of 100 ppm S, cool and dilute to 500 ml volume (6N HCl containing 20 ppm S)

5. **Barium chloride ($BaCl_2$ $2H_2O$) crystal 20-60 mesh.**

6. **Whatman No. 42 filter paper 12.5 cm. diameter**

7. **A.R. K_2SO_4.**

Procedure:

Weigh 10 gm soil in 125 ml. Erlenmeyer flask and add 25 ml of ammonium acetate extracting solution. Shake it for 30 minutes. Add 0.25 gm of activated charcoal (Darco G-60) and continue shaking for 3 minutes. Filter it through Whatman No. 42 filter paper. Take 10 ml of the clear filtrate in another Erlenmeyer flask, add 1 ml of acid "seed" solution, swirl to mix and add 0.5 gm of $BaCl_2$. $2H_2O$ crystal. Let it stand for 1 minute and then swirl the solution frequently until the crystals are dissolved. After the crystals are dissolved, read the calorimetric reading at 420 mu within the time interval of 2 to 8 minutes.

Run a blank and standards of 0,2,5,10,20,25,30,and 40 ppm sulphur as above and plot the graph.

Calculation:

$$\text{ppm S} = \frac{R}{4}$$

where,

R = ppm S in the aliquot from the graph.

9. Micronutrient analysis

Principle:

The major categories of micronutrient extractants presently in use are dilute acids and solutions containing chelating agents like DTPA or EDTA. Other less commonly used or specific extractants include hot water (B), acidic ammonium or sodium acetate (Zn), Na-citrate or Na-dithionate (Fe) and ammonium oxalate (Mo).

i). Dilute acid extractants:

Dilute acids (0.025 - 0.1 M) have been used as extractants for micronutrients Zn, Cu, Fe & Mn for many years (primarily on acidic soils). However, their applicability is confined to acidic soils because they generally are not sufficiently buffered to extract meaningful levels of micronutrients from calcareous soils. Acidic extractants do not have a particular sound, theoretical basis, but, due to their extensive use in field and laboratory studies, a well-developed database exists, relating extractable levels of most micronutrient to crop response.

ii). **Extractants that use chilating Agents:**

One of the major advances in micronutrient soil testing has been the developing of extracting solutions containing the chelating agents, primarily DTPA and EDTA. These chelates reduce the activity of free-metal ions in solution through the formation of soluble metal-chelate complexes. The quantity of metal ions extracted by a chelate reflects both the initial concentration in the soil solution and the ability of the soil to maintain this concentration. Thus, chilating agents simulate nutrient removal by plant roots and replenishment from labile solid phase in the soil. The DTPA soil test, developed for near-neutral and calcareous soils by Lindsay and Norvell, illustrates the evolution of a soil test extractant from theoretical principles derived from soil chemistry for verification through greenhouse and field calibration studies. The DTPA extractant was selected because it offered the most favorable combination of stability constant necessary for simultaneously extracting four micronutrient cations (Fe, Nn, Cu and Zn). Results such as those obtained in the FAO study, and those reported by others on acidic soils reduced soils, and metal contaminated soils, have intensified the interest in DTPA as a universal micronutrient extractant.

Because of the three factors (a) Usefulness as single extractant for multi-elements (micro) (b) the only good extractant for calcareous soil and (c) almost equally good extractant for acidic soils, the DTPA method is getting great popularity among the soil testing laboratories.

Apparatus:

1. Atomic Absorption Spectrophotometer.
2. Acetylene gas cylinder.
3. Paraffin wax paper.
4. Shaker.
5. Pipette 20 ml.
6. Conical flask 125 ml.

Reagents:

1. **Diethylene triamine penta acetic acid (DTPA) extracting solution:** Dissolve 149.2 gm of reagent grade triethanolamine (TEA), 19.67 gm of DTPA and 14.7 gm of $CaCl_2$ $2H_2O$ in deionized distilled water (Glass double distilled). (Since DTPA is not very soluble in water, place it in a small amount of water and then dissolve in the TEA solution.) When the DTPA has dissolved, dilute to approximately 9 liters. Adjust the pH to 7.3

$\pm$ 0.05 with 1:1 HCl (approximately 83 ml of 1:1 HCl are required). Make to 10L final volume. The pH should be checked periodically, because a pH of 7.3 is critical for the extraction. Thus prepared 0.005 M DTPA, 0.01M $CaCl_2$ and 0.1M TEA solution is stable for several months.

2. **Glass double distilled water or deionized distilled water (DDW).**

3. **Standard Zinc solution:**

(i) **Stock Solution:** Dissolve exactly 0.4399gm AR $ZnSO_4.7H_2O$ in glass double distilled water, add 25ml 1N HCl and dilute to 1L in volumetric flask- 100 ppm Zn.

(ii) **Working Standard:** They are prepared by diluting 50 ml of 100ppm Zn to 250 ml (20ppm Zn) and then further dilution of 0,2.5,5,10,15 and 25ml of this 20 ppm Zn to 250ml with DTPA extracting solution to give 0,0.2,0.4,0.8,1.2 and 2ppm of Zn.

4. **Standard Fe solution:**

(i) **Stock Solution:** Dissolve 0.7023gm AR $Fe(NH_4)_2(SO_4)_2.6H_2O$ in deionized distilled water (DDW) and add 20ml of 1N H_2SO_4. Oxidize it by adding 25ml 1% $KMnO_4$ slowly and then dropewise till the pink color just stays. Dilute to exactly 1L - 100 ppm Fe.
(ii) **Working Standards:** They are prepared by diluting 0,5,10,15,20 & 25ml of 100ppm Fe to 100ml with DTPA extracting solution to give 0,5,10,15,20 & 25 ppm Fe.

5. **Standard Copper solution:**
(i) **Stock Solution:** Dissolve 0.3929gm AR $CuSO_4.5H_2O$ in 500 ml DD water, add 20ml 1N H_2SO_4 and dilute to 1L in volumetric flask-100 ppm Cu.
(ii) **Working Standards:** They are prepared by diluting 50ml of 100 ppm Cu to 250ml (20ppm Cu) and then further dilution of 0,2.5,5,10,15 & 25 ml of this diluted (20ppm Cu) to 250ml with DTPA extracting solution to give 0,0.2,0.4,0.8,1.2 and 2.0 ppm of Cu.

6. **Standard Mn solution:**

(i) Stock Solution: Dissolve 0.2877 gm potassium permanganate in 500ml DD water and add 25ml 1N H_2SO_4. Boil for few minutes and carefully add 1.2 gm sodium sulphate crystal ($Na_2SO_3.7H_2O$). Boil again to remove SO_2, cool and make up the volume to 1L-100 ppm Mn.

(ii) Working Standards: They are prepared by diluting 0,5,10,15,20 & 25ml of 100ppm Mn solution to 100ml with DTPA extracting solution to give 0,5,10,15,20 and 25 ppm Mn.

Extraction Procedure:

Weigh 10 gm of air dried soil in a 125ml Erlenmeyer flask and add 20 ml of the DTPA extracting solution. Cover the flask with parafilm or polyethylene stoppers and secure upright on a horizontal shaker with a stroke of 8.0cm and a speed of 120 cycle/min. After exactly 2 hours of shaking, filter the suspension by gravity through Whatman No.42 filter paper. The shaking time is very important because extracting is not complete in 2 hours. (The labile and nonlabile quantities of the trace elements will continue to dissolve even after 2 hours). The filtrate may be used to analyze for the trace elements Fe, Cu,Mn,Zn with AAS after standardization for particular element.

(a). Available Zinc:

Place Zinc Hollow Cathode Lamp in the working lamp turret and let it warm for specified period as per the instruction of the manufacturer. Light the burner using air and acetylene gas, adjust the slit, wavelength and burner height as per the instruction of AAS manufacturer. Let it warm for 5 minutes and standardize AAS using 0 and 2 ppm Zn standard. Run series of Zinc standards, note the absorbance reading and draw graph.

Aspirate the soil extract, note the absorbance reading and determine zinc in solution from the graph.

Calculation:

ppm Zn in soil = 2 x (R-B)

where,

R is the ppm Zn in soil extract from the graph.

B is the ppm Zn in Blank extract from the graph.

Interpretation:

Factors affecting the interpretation of the soil test data are pH, $CaCO_3$ percent, phosphorus, organic matter, clay percent, CEC.

Typical range in critical level for DTPA extractable Zn is reported to be 0.2 to 2.0 Zn mg/kg (ppm) and average critical value is 0.8 Zn mg/kg.

(b). Available Copper:

Place copper Hollow Cathode Lamp in the working turret, proceed as in the zinc determination, note the absorbance reading of copper standards and draw graph.

Aspirate the soil extract, note the absorbance reading and determine Cu in solution from the graph.

Calculation:

ppm Cu in soil = 2 x (R-B)
where,
R is the ppm Cu in soil extract from the graph.
B is the ppm Cu in blank extract from the graph.

Interpretation:

Factors influencing the interpretation of the soil test data are pH, organic matter, $CaCO_3$ percent and crop.

Typical range in critical level for DTPA extractable Cu is reported to be 0.12 to 0.25 Cu mg/kg (ppm).

(c) Available Iron:

Place Iron Hollow Cathode Lamp in the working turret proceed as in the Zn determination, note the absorbance reading of Fe standards and draw graph.

Aspirate the soil extract, note the absorbance reading and determine Fe in solution from the graph.

Calculation:

ppm Fe in soil = 2 x (R-B)
where,
R is the ppm Fe in soil extract from the graph.
B is the ppm Fe in blank extract from the graph.

Interpretation:

Factors influencing the interpretation of the soil test data are pH, organic matter, $CaCO_3$ percent, aeration, soil moisture and CEC.

Typical range in critical level for DTPA extractable Fe in USA and Tropics is reported to be 2.5 to 5.0 Fe mg/kg (ppm).

(d). Available Manganese:

Place Mn Hollow Cathode Lamp in the working turret, proceed as in Zn determination note the absorbance reading of Mn standards as above and draw graph.

Aspirate the soil extract, note the absorbance reading of AAS and determine Mn in solution from the graph.

Calculation:

ppm Mn in soil = 2 (R-B)
where,
R is the ppm Mn in soil extract from the graph.
B is the ppm Mn in blank extract from the graph.

Interpretation:

Factors influencing the interpretation of the soil test data are pH, organic matter, $CaCO_3$ percent and texture.

Typical range in critical level for DTPA extractable Mn is reported to be 1.0 to 5.0 Mn mg/kg (ppm) and average critical value is 1.4 mg/kg.

(e). Available Boron (Hot Water):

Principle:

Although there are several chemical soil test methods for predicting crop response to B, the hot water extraction procedure is the most widely used one. Current method typically includes dilute electrolyte instead of water which provides clear and colorless extract and thus eliminates the need of activated charcoal. Because of the low B concentrations encountered in soils, and availability of the apparatus in the laboratories, the calorimetric procedure is commonly used. Out of the four recommended calorimetric methods, a relatively new procedure that employs azomethine-H as a complexing agent for boric acid in aqueous media is given here.

Developed by Samina et al, modified by Basson et al, further modified by Wolf and improved by John et al, the azomethine-H method is very simple, little or no interference from a wide variety of salts and is useful for high volume routine analysis of B. Over a concentration range of 0.5 to 10 ug of B/ml, azomethine-H solutions form a stable complex with H_3BO_3 at pH 5.1. The complex formation is very rapid that retains proportional absorbance-concentration properties (maximum absorbance at 420 mu) for several hours. This technique is not only rapid but also valuable and more convenient to use than the traditional procedures employing carmine, calcimine or quinalizarin.

Apparatus:

1. Spectrophotometer.
2. Polypropylene test tube tubes - 10 ml capacity.
3. Reflux condenser.
4. Evaporating dishes, porcelain.
5. Low-B glass wares.
6. Pipettes 1 ml, 2 ml and 5 ml.

Reagents:

1. **Calcium hydroxide suspension:** Add 0.4 gm of reagent grade $Ca(OH)_2$ to 100 ml of distilled water.

2. **Hydrochloric acid 0.1N:** Dilute 8.1 ml of AR concentrated HCl to about 900 ml with distilled water, mix, cool and adjust the volume to exactly 1L.

3. **Calcium chloride 0.02N**: Dissolve 1.11 gm of anhydrous $CaCl_2$ in 500 ml of distilled water and dilute to exact 1000 ml.

4. **Buffer solution:** Dissolve 250 gm of NH_4OAc and 15 gm of EDTA - disodium salt in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix.

5. **Azomethine-H reagent:** Dissolve 0.45 gm of azomethine-H (Pierce Chemical Co, Rockford, I11.,USA) in 100 ml of 1 percent L-ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.

6. **Boron standard solution**: Dissolve 0.114 gm of A.R H_3BO_3 in distilled water and dilute to 1L -- 20 ppm B/ml.

7. **Working standard boron solution:** Dilute 10, 20, 30, 40 and 50 ml of stock standard solution to 100 ml with distilled water. This solution will have 2,4,6,8 and 10 ppm B/ml respectively.
8. **Deionized distilled water.**

Procedure:

Weigh 20 gm of air dried soil in a 250 ml low-boron erlenmeyer flask (corning) and add 40 ml of $CaCl_2$ solution. Attach a water cooled reflux condenser to the flask and heat the suspension until initiation of boil. Then reflux the suspension for precisely 5 minutes and cool the flask without removing the condenser. Filter or centrifuge the suspension for 15 minutes at 1500-2000 rpm. Transfer a 20 ml aliquot of the filtrate to an evaporating dish, add 2 ml of $Ca(OH)_2$ suspension and evaporate to dryness on steam hot plate. Heat the evaporating dish gently over a flame (just enough) to destroy organic matter. Cool the dish and add 5 ml of 0.1N HCl. Triturate the residue thoroughly with a policeman. Run a blank as above but without soil. Take an appropriate aliquot (usually 1 ml) of sample, blank and standard solution into a 10 ml polypropylene tube, add 2 ml of buffer and mix. Add 2 ml of azomethine-H reagent, mix and read the absorbance after 30 minutes at 420 mu. Refer these readings to that of a standard curve prepared with 0,2,4,6,8 and 10 ppm B/ml solutions to convert readings to boron concentrations in the test sample.

Calculation:

$$\text{ppm B in soil} = \frac{R}{2}$$

Where,

R = ppm B in 1 ml of solution tested.

Interpretation:

Factors influencing the interpretation of the soil test data are pH, organic matter, soil moisture, texture, soil type and crop yield goal.

Typical range in critical level for hot water soluble B is reported to be 0.1 to 2.0 B mg/kg (ppm) and average critical value is 0.5 mg/kg.

(f). Available Molybdenum

Principle:

The acid ammonium oxalate (AAO) procedure first developed by Grigg (1953) is perhaps still the most commonly used soil test extractant for Mo. Popularity of the procedure is not a result of its unerring ability to predict Mo deficiency, but rather no better method has been developed.

In the AAO procedure, Mo ion is extracted from soil by shaking with 0.2N oxalic acid buffered at pH 3.3 with ammonium oxalate. The oxalate ion in the extract is destroyed by ignition and taken up in dilute acid.

The molybdate ion in the solution is reduced (Mo^{-7} to Mo^{-5}) by stannous chloride in presence of thiocyanate to form an amber-orange colored complex between the thiocyanate and 5-valent Mo. Because of small amount of Mo in most soil extract, the colored complex is dissolved out of the aqueous phase into a small volume of immiscible organic solvent. The most suitable organic solvent is prepared by mixing CCl_4 and isoamyl alcohol.

When the thiocyanate - Mo complex is formed, the acidity (as HCl) should be near 1N and the thiocyanate concentration should be at least 0.5 percent (1% as the K salt). Considerable variation in the concentration of stannous chloride is, however, allowable; a final concentration of 1-2 percent is usually used. The presence of at least 1mg of iron

insures full color development of thiocyanate-Mo complex and there is no adverse effect from large amount; thus about 1mg of Fe (ferrous or ferric may be used) is added, although some may be present already in the soil extract. The $SnCl_2$ reduces ferric ion and so prevents the formation of red ferric thiocyanate.

Interferences are possible from tungsten, titanium, vanadium and platinum but none of these is likely to be present in acid oxalate soil extracts in amount sufficient to cause serious errors.

Apparatus:

1. Erlenmeyer flask 250 and 500ml.
2. Measuring cylinders 10 and 250ml.
3. Shaker.
4. Separating funnel, 50ml, preferably with plastic stopcock and having short exit tubes (Squibb type).
5. Stand for separator funnel.
6. Bulb pippets 1,2 and 5ml.
7. Spectrophotometer, having cells or tubes of 1.0-1.5cm cross section and a capacity of 5ml or less.
8. Furnace.
9. Basins, porcelain 30-35ml
10. Filter paper whatman No.40;15cm
11. Funnel 75cm diameter.

Reagents:

1. **Glass double distilled water or deionized distilled water (DDW):**

2. **Extracting solution (AAO), 0.2N $H_2C_2O_4.2H_2O$ with 2.5 percent $(NH_4)_2C_2O_4.H_2O$, pH3.3:** Dissolve 250gm ammonium oxalate and 126gm oxalic acid in 10L DD water. Check that pH value is near 3.3

3. **Hydrochloric acid 5N:** Dilute 435ml of concentrated HCl to 1L.

4. **Ferrous ammonium sulphate, approximately 0.01N:** Dissolve 4gm $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 800 ml DD water, add 15ml of 1N H_2SO_4 and dilute to 1L.

5. **Potassium thiocyanate, 30%:** Dissolve 30gm potassium thiocyanate (or sodium thiocyanate) in 100 ml DD water.

6. **Stannous chloride, 30% in 1N hydrochloric acid:** Add 30gm $SnCl_2.2H_2O$ to 20ml 5N HCl and dilute to 100 ml. Filter if turbid and store in the refrigerator.

7. **Organic solvent:** Mix equal volume of Carbon tetrachloride and iso-amyl alcohol.

8. **Standard Mo. solution, 1000ppm:**

   a) Dissolve exactly 1.5gm molybdenum trioxide, MoO_3, in 5ml of 5N sodium hydroxide, dilute to about 500 ml and just acidify with about 2ml of 5N HCl. Finally dilute it to 1L.

   b) Working Mo standard, 10ppm: Dilute 5ml of 1000ppm Mo solution to 500ml. Prepare only as required.

Procedure:

Weigh 25gm soil in 500ml Erlenmeyer flask, add 250ml acid oxalate extracting solution (AAO), shake for 12-16 hours (overnight) period and filter through Whatman No.40 filter paper.

Evaporate a total of 200ml of extract to dryness adding a little at a time in porcelain basin whose top has been lightly greased with vaseline to prevent creeping of the oxalate over the edge. Evaporate the same amount of extracting solution in separate basin for the blank. Ignite the residue at 450°C for 3-4 hours in a muffle furnace to destroy oxalate.

Cool the basin and add 5ml of 5N HCl to dissolve the salt. Transfer the solution to a 50ml separator funnel, bringing the volume to 20-21ml. If the residue does not dissolve completely in the acid, the liquid may be filtered through a small filter paper into the separating funnel, washing the paper well, although, slight turbidity is not harmful. Then add 2ml ferrous ammonium sulphate solution to the solution in the separator funnel. Prepare a blank (in addition to the blank on the extracting solution) and standards by transferring 0,1,2,3,4 and 5ml of working molybdenum standard (10ppm Mo) to six separarory funnels, adding 5ml of 5N hydrochloric acid and 2ml of ferrous ammonium sulphate to each and making the final volume to 22-23ml.

Add 2-3ml organic solvent and shake well for two minutes to saturate the aqueous phase. A preliminary treatment of the solution with the organic solvent prior to addition of thiocyanate and reducing agent ensures that aqueous phase is saturated with it and therefore there is no subsequent volume change of organic phase when thiocyanate-Mo complex is finally extracted quantitatively. Allow the phases to separate, tapping the funnel or swirling the liquid to produce a clean boundary line; then run off the lower (organic) phase.

Add 1ml of 30 percent potassium thiocyanate solution to the aqueous phase, mix, add 1 ml of 30 percent stannous chloride solution and mix again. Then add exactly 5ml organic solvent and shake well for two minutes. Invert the funnel in a stand so that the stopcock is uppermost, dry the exit tube and stopcock bore with absorbent paper or suction. After 15 minutes, shake the liquids again quickly and allow them to separate once more, with the funnel supported in the normal position.Make sure the exit tube is still dry and then run off the colored organic phase into a suitable spectrophotometer tube or cell.
Read the absorbance of standards as well as samples at 470 mu wavelength, as compared with the blank containing no molybdenum.

Calculation:

$$\text{ppm Mo} = (\text{R-B}) \times \frac{250}{200} \times \frac{1}{25} = \frac{(\text{R-B})}{20}$$

where, R = ppm Mo in the soil extract from the graph.
B = ppm Mo in the blank (AAO) from the graph.

N.B: In Table 5, a summary is given.

Interpretation:

Factors affecting the interpretation of the soil test data are pH and crop. Typical range in critical level for Acidic Ammonium Oxalate (AAO) extractable Mo is reported to be 0.1 to 0.3 Mo mg/kg (ppm) and level higher than 0.2 Mo mg/kg has been identified as adequate

Table 3.4. Soil test methods, soil factors influencing their interpretation,and typical ranges in critical levels for micronutrients.

Element	Interacting factors	Method	Range in critical level mg/kg.
Boron	Crop yield goal, pH,soil moisture texture, organic matter, soil type	Hot-water soluble	0.1-2.0
Copper	Crop,organic matter,pH, percent $CaCO_3$.	DTPA	0.1-2.5
Iron	pH,percent $CaCO_3$,aeration, soil moisture, organic matter, CEC.	DTPA	2.5-5.0
Manganese	pH,texture, organic matter,percent $CaCO_3$.	DTPA	1.0-5.0
Molybdenum	pH, crop	Ammonium Oxalate, pH 3.3	0.1-0.3
Zinc	pH,percent $CaCO_3$, P, organic matter, percent clay, CEC.	DTPA	0.2-2.0

CHAPTER IV

Cation Exchange Capacity

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Cation Exchange Capacity

Different methods of the determination of cation exchange capacity of soils are described in this chapter.

Principle:

Soil minerals and organic colloidal particles have negative charges that hold dissociable cations. The negative charges in soil may be either of permanent nature due to isomorphous substitution within the silicate layer structure or of variable nature due to the broken bond at the mineral edges and external surfaces, dissociation of acidic functional groups in organic compounds and the preferential adsorption of certain ions on the particles surfaces. The cation exchange capacity determination involves the measurement of total quantity of negative charges per unit weight of the soil. Cation exchange capacity (CEC) is usually expressed as milliequivalent per 100 gm. of soil (m.e/100 gm). Many methods for determining CEC are available by using different combinations of soil saturation, washing and extraction procedures, with different cations for saturation and replacement cations at different p^H. Most methods used may be categorized as one of four given below:

1. The sum of the exchangeable cations displaced with a saturating solution is taken as CEC.

2. After the soil has been saturated with an index cation, the adsorbed and the small excess amount of saturating cation after centrifuging is displaced directly by another salt solution without washing. The saturated cations and an excess are then determined in the resulting extract, and their difference is taken as the CEC of the soil.

3. After the exchange soils have been saturated with index cation, the soil is washed free of excess saturating cations, and the cation adsorbed by soil is determined by displacement.

4. After the exchange sites have been saturated with index cation, the radioactive isotope of the saturating cation is used to determine the adsorbed cations by isotope dilution method.

The different reagents commonly used for saturating exchange complex are normal neutral ammonium acetate, normal sodium acetate at pH 8.2 and $BaCl_2$ - triethanolamine at pH 8.0. The normal neutral ammonium acetate and normal alkaline NaoAc method are described here and the guide to methods of CEC determination is given below. While reporting CEC of soil, it is important to mention the method used for its determination.

Guide to Methods of Cation Exchange Capacity Analysis

(Soil) **Saturating cations and pH of solution**

Soil Types	Ammonium 7.0	Sodium 8.2	Barium-TEA 8.0
Acid soil pH (1:5) less than 6.0			X
Calcareous			X
Slightly acid to neutral		X	X
Saline and/or gypsiferous	X		
Organic (73%C)	X	X	X
Method for determination of CEC	Distillation	Flame photometer	Gravimetric
Suitability for Exch. cations	X		Exch. Acidity.

The suggested pH values and carbon percentage are approximate.

a). Ammonium acetate method

Principle:

Cation exchange sites of the soil are saturated with ammonium during the leaching of the soil with ammonium acetate. After removing the excess of saturating cations, the adsorbed ammonia is determined by distillation with alkali.

There are two important advantages of the ammonium acetate method. Firstly the determination of ammonium is easy. Secondly the leachate can be used to determine the exchangeable cations.

The disadvantages with this method are (i) Calcareous soils present a problem due to solubility of $CaCO_3$ in NH_4OAc solution (ii) NH_4 is trapped in expanding clay (iii) low results obtained in high organic matter and kaolin content soils.

Apparatus:

1. Vacuum pump.
2. Buchner funnel.
3. Volumetric flask 250 ml.
4. Distillation apparatus with 500 ml. or 800 ml. flask.
5. Erlenmeyer flask 250 ml. & 500 ml.
6. Beaker 500 ml.
7. Filtering flask 500 ml.

Reagent :

1. **1N ammonium acetate pH 7.0**: Dilute 575 ml. of glacial acetic acid to 8L with distilled water. Add 700 ml. of concentrated NH_4OH and dilute to 10 L. Adjust the pH to 7.0 with NH_4OH or HOAC.
2. **Ethanol:** 95 percent ethanol.
3. **Mixed indicator**: Dissolve 0.5 gm Brom cresol green and 0.1 gm methyl red indicators in 100 ml. of 95 percent ethanol.
4. **Boric acid 4%**: dissolve 40 gm crystal H_3BO_3 in one liter distilled water.
5. **Magnesium oxide (Light).**
6. **0.1N HCl**: Dilute 16 ml of 6N HCl to 1L and standardize with Na_2CO_3.

Procedure :

Weigh 10 gm soil in 250 ml Erlenmeyer flask and add 50 ml of 1N neutral ammonium acetate solution. Shake for 30 minutes and leave overnight. Fix Whatman No. 42 filter paper into the Buchner funnel by wetting and applying gentle suction. Transfer the content of the Erlenmeyer flask quantitatively into the Buchner funnel and collect the leachate in 500 ml suction flask. After the liquid has passed through, leach the soil with about 15-20 ml portions of saturation solution, allowing each portion to drain through before adding the next until 200-220 ml of the solution has been collected. Transfer the leachate into 250 ml volumetric flask make the volume and save for the determination of exchangeable cations.

Wash the soil in the Buchner funnel with 10 ml portions of ethanol, draining between each addition, until a total of 75-100 ml of liquid has passed through. Do not let the soil dry after this step.

Transfer the well drained soil along with filter paper into distillation flask and add distilled water to about 200 ml in (500 ml) distillation flask. (350 ml if 800 ml kjeldahl flask is used). Add about 2 teaspoonful of MgO and heat to boil. Heat should be controlled in the beginning to avoid frothing.Collect the distillate into 50 ml of 4% boric acid containing 4 drops of mix indicator, previously placed under the condenser. Collect 150 to 200 ml of distillate and titrate with 0.1N HCl.

Carry out a blank determination including all the reagents and the process as in sample.

Calculation:

$$\text{CEC me/100gm} = \frac{(T-B) \times N}{W} \times 100$$

Where,

T = Volume of acid used in the titration of sample.
B = Volume of acid used in titration of blank.
N = Normality of the acid.
W = Oven dry wt. of soil.

b). Sodium acetate method

Principle:

Saturation of the exchange sites is effected with sodium acetate and the excess of salt is removed with ethanol. Then the adsorbed sodium is replaced by ammonium, using ammonium acetate and the sodium in solution is determined with flame photometer.

This method is suitable for arid land soils because the saturating solution of sodium acetate does not dissolve $CaCO_3$ and $CaSO_4$, which is a problem with ammonium acetate method.

Apparatus:

1. Flame photometer.
2. Vacuum pump.
3. Buchner funnel.
4. Filtering flask 500 ml.
5. Volumetric flask 100 ml.
6. Pipette.

Reagents:

1. **1N sodium acetate, pH 8.2**: Dissolve 1360 gm NaOAc in 10L of distilled water and adjust the pH to 8.2 with 1N NaOH or HOAc.

2. **Ethanol 95 percent.**

3. **1N ammonium acetate pH 7.0**: Prepare as given in ammonium acetate method.

4. **Sodium standard solution 50 m.e/L**: Dissolve 1.4612 gm dried AR NaCl in exactly 500 ml distilled water.

5. **Working Na standard:** Dilute 2,4,6,8 and 10 ml of 50 m.e/L stock solution to 600 ml. Add 40 ml of 1N NH_4OAc to each and dilute to 1L. The solutions will be 0.1,0.2,0.3,0.4 and 0.5 m.e/L Na in 0.04N NH_4OAc.

Procedure:

Weigh 10 gm of soil in 250 ml Erlenmeyer flask and add 50 ml of 1N sodium acetate pH 8.2. Shake for 30 minutes and leave overnight. Fix Whatman No. 42 filter paper into the Buchner funnel by wetting and applying gentle suction. Transfer the content of the flask quantitatively into the Buchner funnel and collect the leachate in 500 ml suction flask. After the liquid has passed through, leach the soil with about 15-20 ml portion of saturating solution (NaOAc), allowing each portion to drain through before adding the next, until 200-220 ml of the solution has been collected. Wash the soil in the Buchner funnel with 10 ml portions of ethanol, draining between each addition, until a total of 75-100 ml of liquid has passed through. Place a new filtering flask and leach the soil with 1N ammonium acetate pH 7.0 solution. Continue the leaching as above until the leachate is about 200-220 ml. Transfer the leachate into 250 ml volumetric flask and make up the volume. Run the blank as above but without soil. Dilute 10 ml of ammonium acetate leachate to 150 ml with distilled water. Calibrate the flame photometer with the sodium working standards and determine the reading for the diluted extracts.

Calculation:

$$\text{CEC m.e/100 gm} = (R-B) \times \frac{250}{10} \times \frac{250}{W} \times \frac{1}{10}$$

Where,

R = m.e Na in diluted extract from graph.

W = Oven dry wt. of soil.

B = m.e Na in blank from graph.

c) **Ba-triethanolamine method**

Principle:

Barium chloride buffered with triethanolamine at pH 8.0 is used as saturating solution. Barium chloride alone is used at the end of the saturating stage and the excess is washed out with water. Barium is finally displaced by treatment with ammonium chloride also buffered at pH 8.0 and determined gravimetrically as sulphate.

Apparatus:

1. Buchner funnel for 7cm diameter filter paper.
2. Filtering flask 500ml
3. Beaker 250ml
4. Vacuum pump

5. Filter paper Whatman No.42 or 54, 7cm diameter
6. Volumetric flask 250ml
7. Balance
8. Policeman
9. Pipette bulb 50ml
10. Pipette graduated 5ml
11. Measuring cylinder
12. Water bath
13. Wash bottle glass
14. Wash bottle plastic
15. Muffle furnace
16. Drying oven
17. Desiccator

Reagents:

1) Barium chloride 0.5N - triethanolamine buffered at pH 8.0: Dissolve 305 gm $BaCl_2.2H_2O$ to about 3 liter of distilled water. In a separate vessel, dilute about 35ml triethanolamine to about 150ml with distilled water and add about 120ml of 1N HCl.

Mix the two solutions and make up to 5L with distilled water. Check the pH and adjust if necessary with diluted triethanolamine or HCl to pH 8.0. Protect the solution from carbon dioxide.

2) Barium Chloride 0.1N: Dissolve 12.2 gm $BaCl_2.2H_2O$ to 2L with distilled water.

3) Ammonium Chloride 1N pH 8.0: Dissolve 535gm ammonium chloride to nearly 9L and add 100ml 5N ammonia solution and make up the volume to 10L. Check and adjust the pH to 8.0.

4) Hydrochloric acid 5N: Dilute 105ml concentrated HCl to 250ml with distilled water.

5) Sulphamic acid.

Procedure:

Weigh 10gm air dry soil passing through 2mm sieve in 250ml beaker and add 50ml Barium chloride triethanolamine solution, stir, cover with watch glass and leave overnight. Fix Whatman No.42 filter paper into the Buchner funnel by wetting and applying gentle suction. Pour the supernatant solution from the beaker in the Buchner funnel without or gentle suction. After the liquid has passed through, transfer the soil to the funnel with saturating solution and allow to drain. Leach the soil with four lots of 25ml 0.1N barium chloride, allowing each portion to drain through before adding the next. The leaching process should take at least one hour. Wash the soil in the Buchner funnel with three lots of 25ml distilled water. Transfer the filtrate in 250ml volumetric flask and make up the volume (This solution may be used for the determination of exchangeable acidity).

Transfer the well drained soil (saturated with Ba) to a 250 ml beaker with 50ml ammonium chloride solution, stir,cover with watch glass and leave overnight. Decant the supernatant liquid in a clean set of Buchner funnel and filtering funnel and proceed as before washing the soil with four lots of 25ml of ammonium chloride. Transfer the filtrate quantitavely into 250ml volumetric flask and make up the volume.

Pipette 50ml aliquot out of 250ml ammonium chloride leachate to a 250ml beaker, dilute to 100-125ml with water and add 1.5ml 5N HCl. Also add 1gm sulphamic acid and heat on a water bath. Continue heating for 30 minutes after the precipitate of barium sulphate begins to appear. Filter the barium sulphate immediately through a Whatman No.42 filter paper or preferably fine filtering crucible (tared), cleaning the beaker thoroughly with a policeman. Wash the precipitate with minimum quantity of hot water until the filtrate is free of chloride (test with silver nitrate). If a filter paper is used, fold it carefully round the precipitate and place it in a tared porcelain or silica crucible. Dry this (or filtering crucible if used) in an oven at 105^0C. Transfer to a muffle furnace and ignite slowly, finally increasing the temperature to 800^0C for an hour. Remove the crucible from furnace, cool in a desiccator to room temperature and weigh to 0.1mg accuracy. Repeat heating, cooling and weighing till the weight is constant.

Calculation:

$$\text{Cation exchange capacity} = \frac{8.567 \times W \times V \times 100}{A \times D}$$

Where,

A =	Aliquot taken out of final ammonium chloride leachate
V =	Total volume of ammonium chloride leachate
D =	Weight of soil
W =	Weight of barium sulphate precipitate
8.567=	Conversion factor from barium sulphate to milliequivalent.

CHAPTER V

Exchangeable Cations

Chapter V

Exchangeable Cations

In this chapter, the analytical methods for the estimation of exchangeable cations in the soils are given.

Principle:

Exchangeable cations are those that can be exchanged by a cation of the salt solution added to the soil. Calcium, magnesium, sodium and potassium are the exchangeable cations that occur commonly in soils.

In most agricultural soils Ca occurs in larger quantity followed by magnesium, potassium and sodium in order. Soils having magnesium material in the parent material may have lower Ca than Mg. Similarly saline soil may have lower Na content than Mg and K.

The determination of exchangeable cations is straight forward when the soil contains only small amount of water soluble salts of the cations concerned. But the extracts of saline soils contain Ca, Mg, Na and K from the salts deposited and therefore exchangeable cations determination in such soils are never very accurate whatever the method is used. The most commonly used method for the determination of exchangeable cations is saturation with normal neutral NH_4oAc. With this method cation Ca extraction is not accurate in soils having free $CaCO_3$ and gypsum and cation K extraction is doubtful in soils dominated by mica and vermiculite.

Ammonium acetate method of replacement

Principle:

In the ammonium acetate extract of soils, Ca and Mg may be determined by versenate titration and sodium and potassium can be determined directly by flame photometer.

Apparatus:

1. Flame photometer.
2. Same as in CEC determination except distillation apparatus.
3. Porcelain basin 100 ml.
4. Burette 50 ml.
5. Pipette 10 ml graduated.

Reagents:

1. **Normal neutral ammonium acetate** solution as in CEC determination by NH_4oAc method.

2. **K standard** as in available K determination.

3. **Na standard** as in CEC determination by sodium acetate (NaoAc) method.

4. **0.02N versenate (EDTA) solution**: Dissolve 2 gm of EDTA (Ethylene diamine tetra acetic acid disodium salt) in 500 ml distilled water. Add approximately 50mgm of $MgCl_2$ $6H_2O$ to the solution and dilute to 1L.

5. **Standard Ca solution**: Weigh 0.5005 gm of dried AR $CaCO_3$ and dissolve in 10 ml of approximately 3N HCl. Boil the solution to expel the CO_2 and dilute to 1L. The solution is 0.01N with respect to Ca.

6. **Sodium hydroxide 10% solution**: Dissolve 10 gm of reagent grade NaOH in 100 ml distilled water.

7. **Calcon indicator:** Dissolve 20 mgm of calcon in 50 ml of methanol. Prepare a fresh solution every week.

8. **Murexide indicator**: Thoroughly mix 0.1 gm of Murexide indicator with 20 gm of powdered potassium sulphate.

9. **Eriochrome Black T indicator (EBT)**: Dissolve 0.5 gm of EBT with 4.5 gm of (NH_2OH. HCl) hydroxylamine hydrochloride in 100 ml of methanol.

10. **Potassium cyanide solution:** Dissolve 1 gm of KCN in 100 ml of distilled water.

11. **Ammonium hydroxide - ammonium chloride buffer solution**: Dissolve 67.5 gm of NH_4Cl in 200 ml of distilled water and 570 ml of concentrated ammonium hydroxide and dilute to 1L.

12. **Triethanolamine, Reagent Grade (TEA).**

13. **Potassium ferrocynide solution:** Dissolve 4 gm of $K_4Fe(CN)_6$. $3H_2O$ in 100 ml of distilled water.

Standardization of the Versenate

1. With EBT : Pipette 10 ml aliquot of Ca standard solution in the porcelain basin and dilute with about 50 ml of distilled water. Add to it 10 ml of NH_4OH - NH_4Cl buffer solution followed by 10 drops each of KCN, TEA, $K_4Fe(CN)_6$ and EBT indicator. Titrate the solution with EDTA to a permanent blue color. Titrate three distilled water blanks with every standardization.

2. With Murexide or Calcon indicator : Pipette 10 ml aliquot of Ca standard solution in three porcelain basins and dilute with about 50 ml of distilled water. Add 5 ml of 10% NaOH solution, 10 drops each of KCN, TEA and about 50 mg of Murexide indicator. Stir the solution to dissolve the indicator and titrate with versenate from the red to purple color. When close to end point, the versenate should be added at the rate of two drops every 5 or 10 seconds, as the color change is not instantaneous. [If calcon indicator is used, titrate the solution from red to blue with the versenate solution].

Procedure:

Weigh 10 gm soil in 250 ml Erlenmeyer flask and extract the soil as in CEC determination by NH_4OAc method and make the volume to 250 ml.

Determination of:

a). Potassium:

Calibrate the flame photometer with potassium standard and aspirate the above extract directly and note the flame photometer reading. If the K concentration in the solution is high, dilute the extract with ammonium acetate and note the reading. Note the dilution of the extract for the calculation. While diluting the solution take care to have the flame photometer reading between 20-80 in 0-100 scale.

Calculation:

$$\text{K m.e/100 gm} = (R-B)\ X\ \frac{D.F}{W}\ X\ \frac{250}{10}$$

Where, R = m.e K in the extract from graph.
B = m.e K in the blank from graph.
W = Oven dry wt. of sample.
DF = Dilution factor.

b). Sodium:

Calibrate the flame photometer with sodium standard and proceed as in exchangeable potassium determination.

Calculation:

$$\text{Na m.e/100 gm} = (R-B)\ X\ \frac{D.F}{W}\ X\ \frac{250}{10}$$

Where,

R = m.e Na in the extract from graph.
B = m.e Na in the blank from graph.
W = Weight of oven dry soil.
DF = Dilution Factor.

c). Calcium:

Take 20 ml aliquot from the ammonium acetate extract solution in a porcelain basin and dilute with about 50 ml of distilled water. Add 10 drops each of KCN, TEA, NH_2OH. HCl and enough of 10% NaOH to raise the pH to 12 (about 10 ml). Add about 50 mg of murexide indicator (or 5 drops of calcon indicator) and titrate the solution from red to purple color with EDTA solution. When close to end point, the EDTA should be added at the rate of about 2 drops every 5 or 10 seconds, as the color change is not instantaneous.

[If calcon indicator is used, the color change is from red to blue at the end point]

Calculation

$$\text{Ca m.e/100gm} = \frac{(T-B)\ N}{20}\ X\ \frac{250}{W}\ X\ 100$$

$$= \frac{(T-B)N\ X\ 1250}{W}$$

Where,

T = Volume of EDTA used up for sample titration.
B = Volume of EDTA used up for Blank titration.
N = Normality of EDTA.
W = Oven dry wt. of soil.

d). Magnesium:

Take 20 ml aliquot from the ammonium acetate extract solution in a porcelain basin and dilute with about 50 ml of distilled water. Add 15 ml of NH_4OH - NH_4Cl buffer solution followed by 10 drops each of KCN, NH_2OH HCl, $K_4Fe(CN)_6$ and TEA. Heat the solution to near boiling point for several minutes and add 10 drops of EBT indicator. Titrate the solution from a red to blue color with EDTA solution. Titrate the blank in the same manner.

Calculation:

$$(Ca + Mg)\ m.e/100\ gm = \frac{(T-B) \times 250 \times 100 \times N}{20 \times W}$$

$$= \frac{(T-B)N \times 1250}{W}$$

Where,

T = Volume of EDTA used up for sample titration.
B = Volume of EDTA used up for Blank titration.
N = Normality of EDTA.
W = Oven dry wt. of soil.

Therefore, Mg m.e/100 gm = (Ca + Mg)m.e/100 gm - Ca m.e/100 gm.

CHAPTER VI

Plant Nutrient Analyses

CHAPTER VI

PLANT NUTRIENT ANALYSES

Introduction:

Plant analysis aids in the characterization of soil chemical properties in terms of soil fertility and mineral nutrition of plants. It has been used as a tool in interpreting the results of research, confirming visual symptoms of nutrient deficiencies, excess or imbalances in plants and guide in fertilizing soils. It is an analysis of soil by means of the plants. In this chapter the analysis of nutrient elements in plants and different methods involved are described.

Purposes of plant testing:

Plant testing may be done for three purposes: diagnostic, monitoring, and predictive or prognostis testing.

a) Diagnostic testing (sometime called 'troubleshooting') is under taken to diagnose the cause of poor crop vigour (e.g. patches of poor growth in an otherwise healthy crop, pasture or orchard) or to conform a diagnosis made on the basis of plant symptoms or soil tests.

b) Monitoring samples are collected to assess the adequacy of current fertilizer practices and related management factor (e.g.irrigation technique). Monitoring can be done to compare the nutritional status of crops grown in successive years and allow the fertilizer use to be adjusted according to trends in the chemical composition of the plant. It can also be used to monitor the nutritional status of an individual crop or pasture during its development (i.e.crop logging) and thus ensure that its nutrient requirements are being met satisfactorily.

c) Predictive or prognostic testing may be used in three ways.

   i) Analysis of samples collected during early crop development is used to predict the likelihood of nutrient deficiencies occurring before crop maturity is reached.

   ii) Analysis of fruit is used to predict its likely behaviour in storage.

   iii) Analysis of seeds or grain is used to predict likely deficiencies in succeeding crops.

1. **Preparation of Plant Samples**:

Plant samples collected from the field should first be cleaned of soil particles with minimum amount of distilled water or moist tissue paper. The sample is dried immediately in a forced draft oven at 70°C (60-80°C). While drying the samples should be thinly spread in the oven. The dried sample can be ground in grinding mill for the major nutrients determinations, but if the trace elements have to be determined, it should be

ground with the pestle and mortar. The ground plant should be fine enough to pass through 0.5 mm sieve.

2. Total Nitrogen

Principle:

Organic nitrogen is converted into ammonium by hydrolysis during the digestion of the plant tissue with concentrated sulphuric acid. The digestion mixture hastens the reaction by raising the boiling temperature as well as acting as catalyst. The ammonium in the digested solution is liberated during the distillation with alkali which is being absorbed in boric acid. The adsorbed ammonium reacts with standard acid during titration.

Apparatus:

1. Digestion flasks or block digester tubes
2. Digestion apparatus
3. Distillation apparatus
4. Volumetric flask - 100ml
5. Conical flask - 250ml
6. Acid dispenser
7. Pipette - 10 & 20ml
8. Measuring cylinder - 25ml
9. Burette - 25ml
10. Asbestos gloves

Reagents:

1. **Concentrated sulphuric acid (LR)**

2. **Sodium hydroxide 40%**: Dissolve 400 gms sodium hydroxide (LR or flakes) in one litre of distilled water and cool.

3. **Boric acid 4%**: Dissolve 40 gms boric acid crystal in one litre of distilled water.

4. **Mixed indicator:** Dissolve 0.5 gms brom cresol green and 0.1 gm methyl red indicators in 100 ml 95 percent ethanol.

5. **Digestion mixture:** Grind and mix 10gms of copper sulphate, one gm of selenium and 100gms of potassium sulphate or sodium sulphate.

6. **0.05N HCl**: Dilute 17ml of concentrated hydrochloric acid in two litre of distilled water (A). Standardize 20ml of this solution (A) with 0.5N sodium hydroxide or sodium carbonate. Dilute the solution (A) according to the strength to have 0.05 N HCl.

Procedure:

Weigh 0.20 gm of plant sample in a filter paper and drop as package into a 100 ml digestion flask or tube. Add 2 gm of digestion mixture and 10 ml of concentrated H_2SO_4. Digest the sample in low heat until the frothing has stopped. Raise the temperature to about 400°C taking care that the acid condenses at about one third the way up the neck of the digestion flask. [With Block Digester Tecator, slow heating is not necessary. The digestion tube can be placed in the preheated digester]. Swirl the flask at intervals and continue the digestion till the carbonaceous particle is present and the colour changes to green-blue. Continue the digestion for one to one and half hour more after the colour has changed to greenish blue. Cool the flask and add about 40 ml of distilled water before the solution starts crystallizing. Transfer the solution in a 100 ml volumetric flask washing the digestion flask with 3-4 lots of small amount of distilled water and make up the volume. Take 20 ml of 4 percent boric acid in 125 ml. Erlenmeyer flask and add 4 drops of mixed indicator. Place the flask under the condenser.

Take 20 ml aliquot of the digested solution in a distilling flask and add about 100 ml distilled water. Pour 20 ml of 40 percent sodium hydroxide solution down the neck holding the flask at 45° angle so that it runs to the bottom of the flask without mixing. Attach the flask quickly to the distillation unit and swirl to mix. Heat the distillation flask to boiling but avoid sucking back of boric acid. Continue distillation till the distillate is about 75ml. Determine the nitrogen by titrating the distillate with 0.05 N Hcl. The colour of the mixed indicator just changes from blue to reddish at the end point. Run the blank with all the chemicals and process except the plant sample, for each batch of 20 samples.

Calculation:

$$\% \ N = \frac{(S-B) \times n \times 14 \times 100 \times 100}{W \times 1000 \times 20} = \frac{(S-B) \times n \times 7}{W}$$

Where, S = Volume of standard acid (ml) used up by sample

B = " " " Blank

n = Normality of the standard acid.

W = Oven dry weight of sample.
14 = Equivalent weight of nitrogen
20 = Aliquot.

3. **Plant Tissue Extract**

For the determination of mineral nutrients in the plant tissue samples, the organic matter has to be oxidized and the mineral elements should be released. This may be done by either dry ashing or wet oxidation by means of oxidizing acids such as the ternary acid mixture (HNO_3 - H_2SO_4 - $HClO_4$) or acid digestion in H_2SO_4 and H_2O_2.

a) Wet Oxidation:

Wet oxidation with $HClO_4$ avoids the loss of K through volatilization and gives a clear solution of all constituents. It has been reported that phosphorus and boron can be lost during $HClO_4$ digestion if excessive temperature (much over 200°C) is used. Danger of explosion with $HClO_4$ can be overcome by i) predigestion with concentrated HNO_3 ii) inclusion of H_2SO_4 in $HClO_4$ - HNO_3 solution iii) exhaustion of the $HClO_4$.

Apparatus:

1. Erlenmeyer flask 500ml.
2. Electric hot plate with thermostat for temperature control.
3. Digestion chamber with exhaust system.
4. Steam bath.
5. Sand tray to cover the hot plate.

Reagents:

1. **Concentrated nitric acid.**

2. **Ternary acid:** Mix 100 ml of concentratedHNO_3,10 ml of concentrated H_2SO_4 and 40 ml of 60 percent $HClO_4$ and cool.

(i) Predigestion in HNO_3:

Weigh 1 gm of dried and powdered plant sample in a 500 ml conical flask and add 5ml of concentrated HNO_3 for each gram of plant tissue. Swirl the flask to moisten the entire mass of plant, place it on a steam bath for 30 minutes and then on hot plate at 180°C as measured in a beaker of glycerol standing on the hot plate. The suspension is boiled until taken nearly to dryness. Run the blank digestions (in duplicate) on the reagents added in the same amounts as employed in the determinations. Carry out all steps parallel to the sample.

ii. Digestion in Ternary Acid HNO_3 - H_2SO_4 - $HClO_4$:

After predigestion, cool the conical flask slightly and add ternary acid mixture. The amount of acid mixture should be 5 ml for each gm of tissue sample. Digest the sample at 180 to 200°C until dense white fumes of $HClO_4$ and H_2SO_4 are evolved. A brown or greenish scum of MnO_2 may appear while $HClO_4$ is present, but this redissolves in the concentrated H_2SO_4 at the end of the digestion. Continue the digestion at 180° to 200°C until the acid liquid is largely volatilized. If the acid liquid turns brown with caramelized organic matter as the volume becomes low, add 5 ml more of the ternary mixture of acids and continue the digestion as before until a clear solution remains after the acids are largely volatilized. Stop the digestion when the residues in the flask are clear and white and only slightly moist with H_2SO_4. The $HClO_4$, at this point, has been largely removed. Cool the flask and add 5 ml of concentrated HCl. Swirl, police and pour in 100 ml volumetric flask through Whatman No 42, 12.5 cm filter paper. Repeat the process with another 5 ml of concentrated HCl and filter through the same filter paper. Rinse the

flasks with two small portions of 6N HCl and transfer into the flask through the same filter paper and make the volume up to mark with 6N HCl and shake well. Label the filtrate as plant tissue extract - (A)

b. Dry Ashing (Alternate procedure)

Dry ashing by ignition is an alternative to wet oxidation of plant tissue for the release of mineral elements. This method has been a popular and very satisfactory however, significant amount of potassium may be volatilized at the usual ignition temperature of 550°C to 600°C. Both P and K may be lost at temperatures over 600°C. Phosphorus is not lost by volatilization in dry ashing if the ash is alkaline but some may be lost if the ash is acid. Occasionally charring occurs with large samples resulting into incomplete ashing. Also, significant errors may occur in the determination of P and the trace elements due to a portion becoming occluded or insoluble in the ash. Dry ashing is often more time consuming than wet oxidation. For boron determination, dry ashing method is recommended as it can be lost during wet digestion.

Apparatus:

1. Muffle furnace
2. Hot plate
3. Porcelain crucible 30 ml.
4. Volumetric flask 50 ml.

Reagents:

1. **5N Nitric acid**: Dilute 320 ml of concentrated nitric acid, specific gravity 1.42 to 1L with distilled water.

2. **Concentrated hydrochloric acid AR.**

3. **4N Hydrochloric acid:** Dilute 172 ml of concentrated hydrochloric acid, specific gravity 1.19 to 500 ml with distilled water.

Procedure:

Weigh 1.0 gm of plant sample in 30 ml porcelain crucible. Place it in a cool muffle furnace and raise the temperature to 500°C $\pm$ 25°C. Ignite the sample at this temperature for 5 hours and remove the crucibles from the furnace. If the samples have unashed carbon, add 3 ml of 5N HNO_3 and evaporate to dryness. Place it again in a cool furnace at 400°C for about 15 minutes. If the sample still appear black after this treatment, the colour is due to manganese. Take out the sample from furnace, cool, moisten with distilled water and add 3ml of concentrated hydrochloric acid. Evaporate to dryness on low heat hot plate and allow to bake for one hour to dehydrate the silica. Remove from hot plate, add 5ml of 2N HNO_3, heat to warm and stir with policeman to dissolve the residue of salts. Filter the solution through Whatman No 31, 11 cm filter paper into 50 ml volumetric flask, washing the crucible and funnel with hot distilled water. Cool and make up the volume and mix. Transfer the filtrate into a polythene bottle and take this solution to determine Ca, Mg, P, K, Na and trace elements. Label the filtrate as plant tissue extract - (B)

(c) **Acid -Peroxide Digestion (H_2SO_4 and 30% H_2O_2)**

This method of digestion of plant tissue was developed by Barber (1972) and confirmed by Parkinson and Allen (1975) and used successfully by Wolf (1982) for N determination as well as other major elements and micro nutrients in plant tissue. This method of digestion is quick and less dangerous than ternary acid digestion.

Apparatus:
1. Beaker 600 ml
2. Watch glass or Funnel
3. Hot plate or Block digester.
4. Thermometer 360° C

Reagents:
1. **Hydrogen peroxide (H_2O_2) 30% by Volume**
2. **Concentrated H_2SO_4**

Procedure:

Weigh 0.5 gm plant tissue sample (20 mesh sieve) into beaker or digestion tube. Add 3.5 ml of concentrated H_2SO_4 and let stand for 30 minutes. Add 3.5 ml of 30% H_2O_2 and cover the beaker with watch glass or place a funnel into the mouth of the digestion tube. Place the beaker on a hot plate or digestion tube into a port of the digestion block. Heat at 350°C for 30 minutes. Remove the beaker or digestion tube from hot plate or digestion block and let cool. Add further 2ml aliquot of 30% H_2O_2 and repeat the digestion until the cool digest is clear. Once the digest is clear, dilute to 25 ml with distilled water. The digest is ready for elemental analysis -Solution (C).

4. **Flame photometric determination of K and Na.**

Apparatus:

1. Flame Photometer
2. Beakers

Reagents:

1. **Potassium standard stock solution**: Dissolve 1.907 gm of AR KCl,dried at 40°C,in distilled water and dilute to 1L. -1000 ppmK. Use this stock solution for preparing the working solution.

1a. **Working standard K solution**: Prepare 100 ppm K solution by diluting 10 ml of 1000 ppm K into 100 ml volumetric flask. Take 0,1,2,4,6,8 and 10 ml of 100 ppm K in 100ml volumetric flask. Add to it 10 ml of 1N H_2SO_4 or 1N HNO_3 depending upon the acid system of sample and dilute to the mark with distilled water to give 0,1,2,4,6,8 and 10 ppm K in the acid system.

2. **Sodium standard stock solution:** Dissolve 2.541 gm of dried AR NaCl to 1L distilled water -- 100 ppm Na. Dilute this 1000 ppm Na standard stock solution to prepare the working solution.

2a. **Working standard Na solution:** Prepare 100ppm Na solution by diluting 10ml of 1000 ppm Na into 100 ml volumetric flask. Take 0,2,4,6,8,10,15 and 20 ml of 100 ppm Na in 100 ml volumetric flask. Add to it 10 ml of 1N HNO_3 or H_2SO_4 depending upon the acid system of the sample and dilute up to the mark with distilled water to give 0,2,4,6,8,10,15 and 20 ppm Na in the acid system.

Procedure:

Read the series of standards, with the highest standard to correspond to 100 reading, using the K filter and determine the readings of other standards and draw a graph.

Dilute the plant tissue extract (B) from dry ashing method to contain 2 to 8 ppm K or Na per ml and determine the flame photometer reading after calibration with known standard. Determine the K or Na content from the standard curve.

In the case of plant tissue extract (A) from ternary acid digestion method, and (C) from acid peroxide digestion, take 5 ml aliquot in a beaker and evaporate to dryness. Dissolve the residue in 5 ml of 2 N HNO_3 and make up the volume to 50 ml. Dilute this solution to contain 2 to 8 ppm K or Na per ml and proceed as above to determine its flame photometer reading.

Calculation:

$$\text{Na or K \%} = \frac{R \times 100}{W \times 10^6} \times DF$$

Where, R = ppm K or Na in the solution
W = Oven dry weight of sample
DF = Dilution Factor.

5. <u>Versenate method of determination of Calcium & Magnesium</u>

Apparatus:

1.	Burette	50ml.
2.	Pipette	10ml.
3.	Pipette graduated	10ml
4.	Porcelain basin	100ml

Reagents:

1. **0.02N Versenate solution**: Dissolve 2 gm of EDTA (Ethylene diamine tetra acetate di sodium salt) in 500ml distilled water. Add approximately 50 mgm of $MgCl_2.6H_2O$ to the solution and dilute to 1L.

2. **Standard Calcium solution:** Weigh 0.5005 gm portion of dried AR $CaCO_3$ and dissolve in 10ml of approximately 3N HCl. Boil the solution to expel the CO_2 and dilute to 1L. The solution is 0.01N with respect to Ca.

3. **Sodium hydroxide 10% solution**: Dissolve 10gm of reagent grade NaOH in 100ml distilled water.

4. **Murexide indicator**: Thoroughly mix 0.1 gm of Murexide indicator with 20 gm of powdered potassium sulphate.

4(a) **[Calcon indicator:** Dissolve 20mg of Calcon in 50ml methanol. Prepare a fresh solution weekly].

5. **Eriochrome Black T indicator (EBT):** Dissolve 0.5 gm of Eriochrome Black T indicator with 4.5 gm of $NH_2OH.HCl$ (hydroxylamine hydrochloride) in 100ml of methanol.

6. **Potassium cyanide solution:** Dissolve 1 gm of KCN in 100ml of distilled water.

7. **Ammonium chloride ammonium hydroxide buffer solution:** Dissolve 67.5 gm of NH_4Cl in 200 ml of distilled water and add 570 ml of concentrated ammonium hydroxide and dilute to 1L.

8. **Triethanolamine, Reagent Grade (TEA).**

9. **Potassium ferrocynide solution:** Dissolve 4gm of $K_4Fe(CN)_6.3H_2O$ in 100ml of distilled water.

<u>Standardization of Versenate:</u>

a) <u>With Eriochrome Black T</u>: Pipette 10ml aliquot of Ca standard solution in three porcelain basins and dilute with about 50 ml of distilled water. Add to it 10 ml of NH_4OH - NH_4Cl buffer solution, 10 drops each of KCN, TEA, $K_4Fe(CN)_6$ and 4 drops of EBT indicator. Titrate the solution with versenate to a permanent blue colour.

Titrate three distilled water blanks with every standardization.

b) **With Murexide indicator:** Pipette 10ml aliquot of Ca standard solution in three porcelain basins and dilute with about 50 ml of distilled water. Add to it 5 ml of 10% NaOH solution, 10 drops each of KCN, TEA and about 50 mg of Murexide indicator. Stir the solution to dissolve the indicator and titrate the solution from the red to purple colour. When close to end point, the versenate should be added at the rate of about two drops every 5 or 10 seconds, as the colour change is not instantaneous. [If calcon indicator is used, titrate the solution from red to blue with the EDTA (versenate) solution].

If the end point is difficult to obtain because of the presence of phosphate, to a new sample add 10 drops each of KCN & TEA and a known excess of EDTA. Slowly add 5ml 10% NaoH stirring continuously to bring the p^H up to 12. Then heat the solution to near boiling for several minutes, cool and add Murexide indicator and titrate with standard Ca solution from purple to red colour.

(a) **Calcium Determination:**

Take 5ml aliquot of the plant extract solution (A) or (B) or (C) in a porcelain crucible and dilute to about 30 ml with distilled water. Add 10 drops each of KCN, NH_2OH HCl and TEA and enough of 10% NaOH to raise the p^H to 12 [10ml for solution (A) and 5ml for solution (B) or (C)]. Add about 50mg of Murexide indicator and titrate the solution with (EDTA) versenate solution from red to violet colour.

If the end point is difficult to obtain due to the presence of phosphate, take a new aliquot and add 10 drops each of KCN & TEA followed by a known excess of versenate. Slowly bring the p^H to 12 with 10% NaOH. Then heat the solution to near boiling for several minutes to speed up the reaction. When the solution has cooled, add Murexide indicator and titrate with standard Ca solution from purple to red. [If Calcon indicator is being used in the versenate titration, the colour change is from red to blue.]

Calculation: Ca meq/100gms $= \dfrac{(S-B)N}{W} \times \dfrac{50}{5} \times \dfrac{100}{}$

$$\text{Ca \%} = \text{Ca meq \%} \times \frac{20}{1000}$$

Where W = Wt of oven dry sample.
S = Vol. of Versenate used for sample titration
B = " " " Blank
50/5 = dilution factor.
N = Normality of EDTA (Murexide Indicator)

(b) Calcium Plus Magnesium Determination:

Take 5ml aliquot of the solution (A), (B) or (C) in a porcelain crucible and dilute to about 30ml, with distilled water. Add enough of NH_4Cl-NH_4OH buffer solution [15ml for solution (A) and 10ml for solution (B) & (C)] and 10 drops each of $KCN, K_4Fe(CN)_6$, TEA and 4 drops of EBT indicator. Titrate the solution with versenate, stirring, to a permanent blue colour.

$$\text{Calculation: } (Ca+Mg)\text{meq}/100\text{gm} = \frac{(S-B)N}{W} \times \frac{50}{5} \times \frac{100}{}$$

Where W = Wt of oven dry sample.
S = Volume of Versenate used for sample titration.
B = " " " Blank
50/5 = dilution factor.
N = Normality of EDTA (EBT indicator).

Mg meq/100gm = (Ca+Mg) meq/100gm - Ca meq/100gm

[Mg % = Mg meq/100gm x 12/1000.]

6. Total Phosphorus

Vanadomolybdophosphoric Yellow Method

Principle:

The exact nature of the yellow chromogen of the vanadomolybdo- phosphoric system is not known, but the colour is attributed to substitution of oxyvanadium and oxymolybdenum radicals for the oxygen of PO_4 to give a heteropoly compound that is chromogenic. This method is very simple and good for samples having high phosphorus content. Besides simplicity, it has greater tolerance to the interfering elements than any other methods. The acid concentration in the determination is not so critical however, final concentration of 0.3N to 0.8N acidity is recommended. The sensitivity varies almost 10 fold between the wavelengths 400 and 490 mu with the higher sensitivity at the lower wavelength. Therefore, for the determination of yellow colour of P, the 420 mu wavelength is recommended.

Apparatus:

1. Spectrophotometer
2. Volumetric flask 50ml
3. Pipette 5ml.

Reagents:

1. **Ammonium molybdate-vanadate solution:** Dissolve 25gm of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_20$] in 400ml of distilled water. Dissolve 1.25gm of ammonium metavanadate (NH_4Vo_3) in 300ml of boiling distilled water. Cool and add to it 250ml of conc. HNO_3. Cool and mix the two solutions and dilute to 1L with distilled water.

2. **Standard P. Solution:** Dilute 100 ml of 50 ppm stock P solution to 250 ml. (Olsen's-Bicarbonate method) - 20ppm.

Procedure: (Dry ash extract):

Take 5ml aliquot (0.05 to 1.0 ppm P) of extract solution (B) in 50ml volumetric flask and dilute to 35ml. Add 10 ml of vanadomolybdate reagent and dilute to 50 ml with distilled water and mix. Measure the yellow colour after 20 minutes at 420 mu. and compare with that of the phosphorus standards.

Prepare a standard curve by taking 0,2,4,6,8,10,12 and 15 ml of 20 ppm P standard in 50ml volumetric flask and dilute to 35ml and mix. Add 10ml of vanadomolybdate reagent and dilute to 50ml mark. Mix and measure the yellow colour after 20 minutes at the same wavelength as the sample.

Calculation: ppm P in plant tissue = $\frac{\text{ppm P in solution}}{\text{W}} \times 10$

where: W = Oven dry wt of plant sample and 5ml aliquot is taken from 50ml dry ashing extract.

% P in Plant tissue = ppm P in plant tissue x $\frac{100}{10^6}$

Procedure:(Wet digestion extract):

Pipette 10ml aliquot of the wet digestion extract (A) or 5 ml of acid peroxide extract (C) in 100ml beaker and evaporate to dryness. Dissolve the residue in 5ml of 2N HNO_3, warming if necessary. Transfer it to 25ml volumetric flask with distilled water and make up the volume. Take 10ml aliquot of this diluted solution in the 50ml volumetric flask and proceed to determine phosphorus by vanadomolybdate phosphoric yellow method as in dry ashing extract.

Calculation:

$$\text{ppm P in plant tissue} = \frac{\text{ppm P in solution}}{W} \times 25 \text{ for (A)}$$

or

$$\frac{\text{ppm p in Solution}}{W} \times 12.5 \text{ for(C)}$$

where, W = Oven dry wt of plant sample and 10ml of (A) or 5 ml of (C) extract is being used to control the acidity & diluted to 25ml volume, out of which 10ml aliquot is taken for colour development.

$$\% \text{ P in the plant tissue} = \text{ppm P in plant tissue} \times \frac{100}{10^6}$$

7. Total Zinc:

Apparatus:

1. Atomic Absorption Spectrometer (AAS)
2. Zinc Hollow Cathode Lamp.
3. Acetylene gas in cylinder
4. Volumetric flask
5. Pipettes
6. Glass cups 5ml or beakers 25ml.

Reagents:

1. Standard Zn solution:

a) Dissolve exactly 0.4399gm AR $ZnSO_4.7H_2O$ in glass double distilled water, add 25ml 1N HCl and dilute to 1L in volumetric flask. - 100ppm Zn.

b)Working standard solution are prepared by first diluting 50ml of 100ppm Zn to 250ml to make 20ppm Zn, then further dilution of 0,2.5,5,10,15 and 25ml of this diluted (20ppm Zn) to 250 ml with 0.1N HNO_3 or other acid as in sample.

2) Glass Double Distilled Water or Deionized Distilled Water (DDW).

Procedure:

Place Zinc Hollow Cathode Lamp in the working lamp turret of AAS and let it warm for specified period as per the instruction of the manufacturer. Light the burner using air and acetylene gas, adjust the slit, wavelength and burner height as specified by manufacturer for Zn determination. Let it warm for 5 minutes and standardize AAS using 0 and 2 ppm Zn standards. Run series of Zn standards, note the absorbance and draw graph.

Aspirate the plant digestion extract (A),(B)or(C), note the absorbance reading and determine zinc in solution from the graph.

Calculation:

$$\% \text{ Zinc in plant} = R \times 100 \times \frac{100}{10^6} \text{ for the digestion extract-A.}$$

$$= R \times 50 \times \frac{100}{10^6} \text{ for the digestion extract-B \& C.}$$

where, R = ppm zinc in the extract from the graph.

8. Total Copper:

1. Atomic Absorption Spectrometer (AAS)
2. Copper Hollow Cathode Lamp.
3. Acetylene gas in cylinder
4. Volumetric flask
5. Pipettes
6. Glass cups 5ml or beakers 50ml.

Reagents:

1. Standard Cu solution:

a) Dissolve exactly 0.3929gm AR $CuSO_4.5H_2O$ in 500ml glass double distilled water, add 20ml 1N H_2SO_4 and dilute to 1L in volumetric flask. - 100ppm Cu.

b) Working Cu standards are prepared by first diluting 50ml of 100ppm Cu to 250ml to make 20ppm Cu and then 0,2.5,5,10,15 and 25ml of this diluted (20ppm Cu) to 250 ml with 0.1N H_2SO_4 or HNO_3 depending upon the acid system of sample to give 0,0.2,0.4,0.8,1.2 and 2.0 ppm Cu.

2) Glass Double Distilled Water or Deionized Distilled Water (DDW).

Procedure:

Place Copper Hollow Cathode Lamp in the working lamp turret of AAS and let it warm for specified period as per the instruction of the manufacturer. Light the burner using air and acetylene gas; adjust the slit, wavelength and burner height as specified by manufacturer for Cu determination. Let it warm for 5 minutes and adjust the full scale deflection of AAS using 0 and 2 ppm Cu standards. Run series of Cu standards, note the absorbance reading of AAS and draw graph.

Aspirate the plant digestion extract (A)(B)or(C) after proper dilution, note the absorbance reading and determine Cu in the solution from the graph.

Calculation:

$$\% \text{ Cu in plant} = \frac{R \times 100 \times 100}{10^6} \text{ for the digestion extract A}$$

$$\% \text{ Cu in plant} = \frac{R \times 50 \times 100}{10^6}, \text{ for the digestion extract-B and C.}$$

where, R = ppm Cu in the extract from the graph.

9. Total Iron:

1. Atomic Absorption Spectrometer (AAS)
2. Iron Hollow Cathode Lamp.
3. Acetylene gas in cylinder
4. Volumetric flask
5. Pipettes
6. Glass cups 5ml or beakers.

Reagents:

1. Standard Fe solution:

a) Dissolve exactly 0.7023gm AR $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 500ml double distilled water (DDW), add 20ml 1N H_2SO_4. Oxidise it by adding 25ml 1% $KMnO_4$ slowly and then dropwise till the pink colour just stays. Dilute to exactly 1L with DD water. · 100ppm Fe.

b) Working Fe standards are prepared by diluting 0,5,10,15,20 and 25ml of 100ppm Fe to 100ml with 0.1N H_2SO_4,HNO_3 or HCl depending upon the acid system of sample to give 0,5,10,15,20 & 25 ppm Fe.

Procedure:

Place Fe Hollow Cathode Lamp in the working lamp turret of AAS and let it warm for specified period as per the instruction of the manufacturer. Light the burner using air and acetylene gas; adjust the slit, wavelength and burner height as specified by the manufacturer. Let it warm for 5 minutes and standardize AAS using 0 and 25ppm Fe standards. Run series of Fe standards, note the absorbance reading of AAS and draw graph.

Aspirate the plant digestion extract (A)(B)or(C) after proper dilution, note the absorbance reading and determine Fe in the solution from the graph.

Calculation:

$$\% \text{ Fe in plant} = \frac{Rx100x100}{10^6} \text{ for the digestion extract-A.}$$

$$= \frac{Rx50x100}{10^6} \text{ for the digestion extract-B and C.}$$

where, R = ppm Fe in the digestion extract from the graph.

10. Total Manganese:

1. Atomic Absorption Spectrometer (AAS)
2. Manganese Hollow Cathode Lamp.
3. Acetylene gas in cylinder
4. Volumetric flask
5. Pipettes
6. Small beakers or glass cups.

Reagents:

1. Standard Mn solution:

a) Dissolve exactly 0.2877gm potassium permanganate in 500ml double distilled water, add 25ml 1N H_2SO4, boil for few minutes and carefully add 1.2gm sodium sulphate crystal ($Na_2SO_3.7H_2O$). Boil again to remove SO_2, cool and make up the volume to 1L.-100ppm Mn.

b) Working Mn standards are prepared by diluting 0,5,10,15,20 and 25ml of 100ppm Mn solution to 100ml with 0.1N H_2SO_4,HNO_3 or HCl depending upon the acid system of sample to give 0,5,10,15,20 & 25 ppm Mn.

Procedure:

Place Mn Hollow Cathode Lamp in the working lamp turret of AAS and let it warm for specified period as per the instruction of the manufacturer. Light the burner using air and acetylene gas; adjust the slit, wavelength and burner height as recommended by the manufacturer for Mn determination. Let it warm for 5 minutes and standardize AAS using 0 and 25ppm Mn standards. Run series of Mn standards, note the absorbance reading of AAS and draw graph.

Aspirate the plant digestion extract (A)(B)or(C) after proper dilution, note the absorbance reading and determine Mn in the solution from the graph.

Calculation:

$$\% \text{ Mn in plant} = \frac{Rx100x100}{10^6} \text{ for the digestion extract-A.}$$

$$= \frac{Rx50x100}{10^6} \text{ for the digestion extract-B and C.}$$

where, R = ppm Mn in the extract from the graph.

11. Total Boron:

Principle

The nutrient boron is not very mobile in the plants. Its content varies according to the plant parts. Therefore care should be taken in systematic sampling of plant parts for boron analysis.

The Azomethine-H method described here was developed by Savina et al and used by Derek Plaskett, Murdoch University, Western Australia.

Apparatus:

(1) Muffle furnace
(2) Electric hot plate having low temperature control
(3) Centrifuge
(4) Polystyrene vial or tube
(5) Eppenderf syringe
(6) Colorimeter

Reagents:

(1) **Buffer-masking Reagent**: Dissolve 280 gm NH_4OAC+20gm KOAC+20gm tetra sodium salt of EDTA and 8gm nitrilo triacetic acid in 400 ml of deionised distilled water. After dissolving completely, add slowly 125 ml of concentrated HOAC and dilute to 2 litre. Let it stand overnight and filter through Whatman No.1 filter paper.

(2) **Deionized Distilled Water**
(3) **1N Hydrochloric Acid:** Dilute 97 ml of concentrated hydrochloric acid to 1 litre.

(4) **Azomethine-H Reagent:** Dissolve 0.8 gm of fresh azomethine-H (Merk) and 2 gm ascorbic acid in 60 ml DD Water and dilute to 100 ml. Prepare it at least 24 hours before using and store in polypropylene bottle wrapped in Al foil and place in refrigerator. This reagent is usable for 14 days.

(5) **Mixed Reagent:** Mix two parts (by volume) of the buffer masking reagent with one part of the Azomethine-H reagent. This must be used within 4 hrs.

(6) **Standard boron solution (100 ppm):** Dissolve 0.5716 gm AR boric acid in 1 litre DD Water-100ppm B. This solution is used to prepare a series of working B standards in N HCl (0,1,2,3,4,5 ml of 100 ppm B standard solution diluted to 100 ml will give 0,1,2,3,4 and 5 ppm B).

Procedure:

(1) **Drying Ashing:** Weigh 0.300 gm of oven dried and ground plant tissue material into a tall crucible and place in a cool muffle furnace. Ash it at 500^{0}C for 8 hours and cool. Include blanks with each batch of sample.

Cool the crucible and rinse the walls down with 2 ml 1:1 HCl. Adjust the temperature of the hot plate to 50^{0}C and heat gently for approximately 30 minutes. Cool and transfer the solution from the crucible to 10ml graduated polystyrene vial with small wash of distilled water. Dilute to 10 ml and shake well. Allow the precipitate to settle by standing overnight or centrifuge at 2500-3000 rpm for 4 minutes.

(2) **Colour Development:** Pipette 1 ml of extracted solution into a vial. Add 3 ml of the mixed reagent in a strong jet with an Eppenderf syringe to mix well with sample. Stand for 1 hour and determine the colorimetric reading at 420 mu. Compare the reading with the B standard readings prepared at the same time.

Precaution: All the plastic container are cleaned with distilled water, left in 20% conc HCl overnight, rinsed with distilled water and air dried.

Calculation:

$$\% B = \frac{R}{100\ W}$$

where R = ppm B in solution from standard curve
W = oven dry wt. of plant sample

Calculation:

$$\text{ppm B in plant} = \frac{R}{2}$$

where, R = ppm B in 1 ml of solution tested.

12. Total Molybdenum:

Principle:

For the determination of Mo in plant tissue sample, the Organic matter has to be oxidised and the mineral Mo should be released. This may be done by either of the methods described under Plant Tissue extract or by fusion with Na_2CO_3. However, the solution should contain no actively oxidising acids, such as HNO_3 or $HClO_4$ or H_2O_2 and if present they are removed by evaporation.

The Mo ion in the solution obtained is reduced by $SnCl_2$ in the presence of thiocyanate to develop an amber-orange coloured complex between the thiocyanate ion and 5-valent Mo; the concentration of which is increased by the use of organic solvent.

During the complex formation, the acidity should be maintained near normal and the thiocyanate concentration should be at least 0.5 percent. Although the concentration of $SnCl_2$ can be variable, a final concentration of 1-2 percent is recommended. The presence of at least 1mg of Fe ensures full colour development and there is no adverse effect from large amounts. The $SnCl_2$ reduces ferric ion and presents the formation of red ferric thiocyanate.

Apparatus:

1) Spectrophotometer, having 1 cm diameter tube.
2) Separating funnel, 50ml, preferably with plastic stopcock and having short exit tube for easy drying (Squibb type).
3) Hot plate.
4) Beaker.
5) Bulb pipettes 1,2,5 & 10 ml.
6) Stands for separating funnels.
7) Whatman filter paper No.40, 7cm.
8) Funnel 30mm.

Reagents:

1) **Glass double distilled water or deionised distilled water (DDW)**

2) **Hydrochloric acid 5N:** Dilute 435ml of concentrated HCl to 1L.

3) **Ferrous ammonium sulphate, approximately 0.01N:** Dissolve 4gm $Fe(NH_4)_2SO_4.6H_2O$ in 800ml DD Water, add 15ml of 1N H_2SO_4 and make up the volume to 1L.

4) **Potassium thiocyanate, 30% :** Dissolve 30gm potassium thiocyanate (or sodium thiocyanate) in 100ml DDW.

5) **Stannous chloride, 30% in 1N HCl:** Add 30gm $SnCl_2.2H_2O$ to 20ml5N HCl and dilute to 100ml. Filter if turbid and store in the refrigerator.

6) **Organic solvent:** Mix equal volume of CCl_4 and iso-amyl alcohol.

7) **Standard Mo solution:**

a)Dissolve exactly 0.75gm molybdenum trioxide, MoO_3, in 5ml of 2.5N sodium hydroxide, dilute to about 300ml and just acidify with about 1ml of 5N HCl. Finally dilute to 1L.- 1000ppm Mo solution.

b)Working Mo standard, 10ppm: Dilute 5ml of 1000ppm Mo solution to 500ml.

Procedure:

Pipette an aliquot of 10ml from the plant extract (A) or 5ml from the plant extract (B) or (C) in 100ml beaker and evaporate to dryness. Dissolve the residue in 5ml of 5N HCl and transfer the solution to a 50ml separating funnel, bringing the volume to 20-21 ml. The liquid may be filtered through a small filter paper into the separating funnel, washing the paper well, although, slight turbidity is not harmful. Then add 2ml ferrous ammonium sulphate solution to the solution in the separating funnel. Prepare a blank (in addition to the blank on the digestion process) and standards by transferring 0,1,2,3,4 and 5 ml of working Mo standard (10ppm) to six separatory funnels,adding 5ml of 5N HCl and 2ml of ferrous ammonium sulphate to each and making the final volume to 22-23 ml.

Add 2-3 ml organic solvent and shake well for two minutes to saturate the aqueous phase. A preliminary treatment of solution with organic solvent is to prevent any volume change of organic phase when thiocyanate-Mo complex is finally extracted quantitatively. Allow the phases to separate, tapping the funnel or swirling the liquid to produce a clean boundary line; then run off the lower (organic) phase.

Add 1ml of 30% potassium thiocyanate solution to the aqueous phase, mix,add 1ml of 30% stannous chloride solution and mix again. Then add exactly 5ml of organic solvent and shake well for two minutes. Invert the funnel on a stand so that the stopcock is uppermost, dry exit tube and stopcock bore with absorbent paper or suction. After 15 minutes, shake the liquids again quickly and allow them to separate once more, with the funnel in the normal position. Make sure the exit tube is still dry and then run off the coloured organic phase into a suitable spectrophotometer tube or cell.

Read the absorbence of standards as well as samples at 470 mu wavelength, as compared with the blank containing no molybdenum.

Calculation:

$$\% \text{ Mo} = \frac{(R-B)\times 100\times 100}{10^6 \times 10} = \frac{(R-B)}{1000}$$

where, R = ppm Mo in the plant from the graph.
B = ppm Mo in the blank (plant digestion) from the graph.

Table 6.1

Critical micronutrient concentration for corn, soybean, wheat and alfalfa for diagnostic interpretations of total plant analysis.

Micro-nutrient	Corn* mg kg^{-1}	Soybean** mg kg^{-1}	Wheat + mg kg^{-1}	Alfalfa ++ mg kg^{-1}
B	10	25	15	30
Cu	5	5	5	7
Fe	25	30	25	30
Mn	15	20	30	25
Mo	0.2	0.5	0.3	0.5
Zn	15	15	15	15

* Leaf at or opposite and below ear level at tasselling.
** Youngest mature leaves and petioles after first pod formation.
\+ Whole plant at the boot stage.
++ Upper stem cuttings in early flower stage.

Appendix 1

Function of essential elements in plants (for more detail see Epstein (1972) and Mengel and Kirkby (1982)

Elements	Physiological process	Activator of enzyme	Constituent of metabolite
Nitrogen			Amino acids. proteins, nucleotides, chlorophyll
Phosphorus	Energy transfer, membrane integrity		ATP, nucleotides, phospholipids
Potassium	Translocation, stomatal opening	+	
Sulphur	Protein synthesis and function, structure		Amino acids, co-enzymes, proteins
Calcium	Membrane maintenance	+	Calcium pectates
Magnesium	CO_2 assimilation	+	Chlorophyll
Chlorine	Maintenance electrical neutrality, internal turgor		
Copper	Lignin synthesis		Ascorbic acid oxidase, phenolases, plasto-cyanin
Zinc	Auxin metabolism, nucleotide synthesis	+	Dehydrogenases

continue..

Elements	Physiological process	Activator of enzyme	Constituent of metabolite
Manganese	Oxidation-reduction in electron transport	+	
Iron	Electron transport		Iron porphyrins (leaves), ferrodoxin
Boron	Nucleotide synthesis, assimilate translocation		
Molybdenum	Nitrogen fixation, nitrate reduction		Nitrogenase, nitrate reductase

Appendix 2.

Mobility of nutrients within plants

Mobile	Variably mobile	Immobile
Nitrogen	Sulphur	Calcium
Phosphorus	Copper	Manganese
Potassium	Zinc	Boron
Magnesium	Molybdenum	Iron

Appendix 3.
General description of symptoms of nutrient deficiencies.

Nutrient	Symptoms
Nitrogen	Chlorosis of whole plant often with reddening. Older leaves usually affected first.
Phosphorus	Dark green foliage, reddening or purpling of leaves or petioles (similar to cold effects).
Potassium	Older leaves may show necrotic spots or marginal burn: younger leaves may develop red pigmentation or become interveinally chloritic and show a shiny surface.
Calcium	Growing point dies. In fruit crops, disorders of fruits (e.g. bitter pit in pome fruit, blossom-end rot in tomato and pepper). In leaf crops, disorders such as tip burn.
Magnesium	Marginal or interveinal chlorosis often quite strongly coloured. Green area of leaf may form an 'arrowhead' in woody plants. Strong reddening may border the chlorotic zone. Usually on older tissue first.
Sulphur	Chlorosis of the whole plant, often younger leaves affected first
Copper	Death of young leaves, chlorosis, failure of fertilization and fruit set (S-shaped shoot growth and fruit gumming in citrus).
Zinc	Little leaf, rosetting,chlorotic mottle in less severe cases.
Manganese	Interveinal chlorosis; when severe, necrotic spots or streaks may form. Often occurs first on middle leaves.
Iron	Interveinal chlorosis which in severe cases may mean total bleaching of young foliage followed by necrosis. Occurs first on young leaves.
Boron	Death of growing points. Axillary buds may burst giving a witches broom effect.Some species (e.g.grape) may show leaf distortion characteristic of impaired metabolism of auxin. Fruit may be distorted or show woody pits or cracking of the surface. Petiole cracking in celery and hollowness in some root vegetable species.
Molybdenum	In legumes, general paleness. In nonlegumes, mottled pale appearance, marginal burn of mature leaves (rock melon, maize,sunflower). Whiptail in cauliflower.

Appendix 4.

General description of symptoms of nutrient toxicities.

Nutrient	Symptoms *
Nitrate-nitrogen	Edge burn may be followed by interveinal collapse.
Ammonium-nitrogen	Initial necrosis, blackening around tips and edge of leaves. Root death may occur.
Phosphorus	Interveinal chlorosis in younger leaves (may resemble iron deficiency), necrosis and tip die back may follow in susceptible species. Marginal scorch and shedding of older leaves.
Sodium	Marginal chlorosis and burn.
Chlorine	Bronzing, chlorosis, marginal burn; leaf drop may be premature. In some species the marginal burn is accompanied by upward cupping.
Manganese	Yellowing, beginning at the leaf edge of older leaves, sometimes with upward cupping. Interveinal bronze-yellow chlorosis in beans; orange-yellow marginal and interveinal chlorosis in lemons; brown 'tar spots' in orange leaves; necrosis in apple bark.
Aluminium	Symptoms on shoots may resemble those of phosphorus deficiency. Roots frequently stunted with many short laterals.
Boron	Interveinal necrosis (often spotty at first)
Fluorine	Scorching of leaf tip and margin, extending into interveinal areas.

* Caution: Symptom expression varies from crop to crop. It is important to check the specific symptoms described for a particular crop-see reference list in Appendix 1.

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Conversion Table

1. **Plant nutrition conversion**

Phosphorus (P)x 2.29	= P_2O_5
Potassium (K)x 1.20	= K_2O
ppm x 1 2	= Kg/ha
ppm x 1	= mg/L
ppm x 1	= ug/ml
Ca me/100gm x 0.02	= % Ca
Ca me/100gm x 200	= ppm Ca
Mg me/100gm x 0.012	= % Mg
Mg me/100gm x 120	= ppm Mg
K me/100gm x 0.039	= % Ca
K me/100gm x 390	= ppm K
Nitrogen % x 6.25	= Crude protein %
Total nitrogen % x 20	= Oraganic matter %
Nitrogen Kg/ha x 22400	= N %
Organic Matter x 0.58	= Organic Carbon

2. Yield

lb/ac x 1.12	= Kg/ha
Hundred weight/ac x 1.12	= quintal/ha
bu/ac x 0.87	= hectolitre/ha

3. Length

Mile x 1.609	= Kilometre
Yard x 0.914	= meter
inch x 2.54	= centimetre

4. Area

Square mile x 2.59	= Square Kilometre
Acre x 0.00405	= Square Kilometre
Acre x 0.405	= Hectare

5. Volume

Square mile x 2.59	= Square Kilometre
Cubic foot x 0.2832	= Hectolitre
Bushel x 0.352	= Hectolitre
Bushel x 35.24	= Litre
Quart x 0.946	= Litre

6. Mass

Ton (English) x 0.907	= Metric ton
Hundred weight x 0.454	= Quintal
Pound x 0.454	= Kilogram
Ounce x 28.35	= gram

7. Pressure

lb/sq in x 0.06895	= Bar
Atmosphere x 1.013	= Bar
Atmosphere x 1.033	= Kg/Sq cm
lb/sq in x 0.0703	= Kg/sq cm
lb/sq in x 0.06805	= Atmosphere.

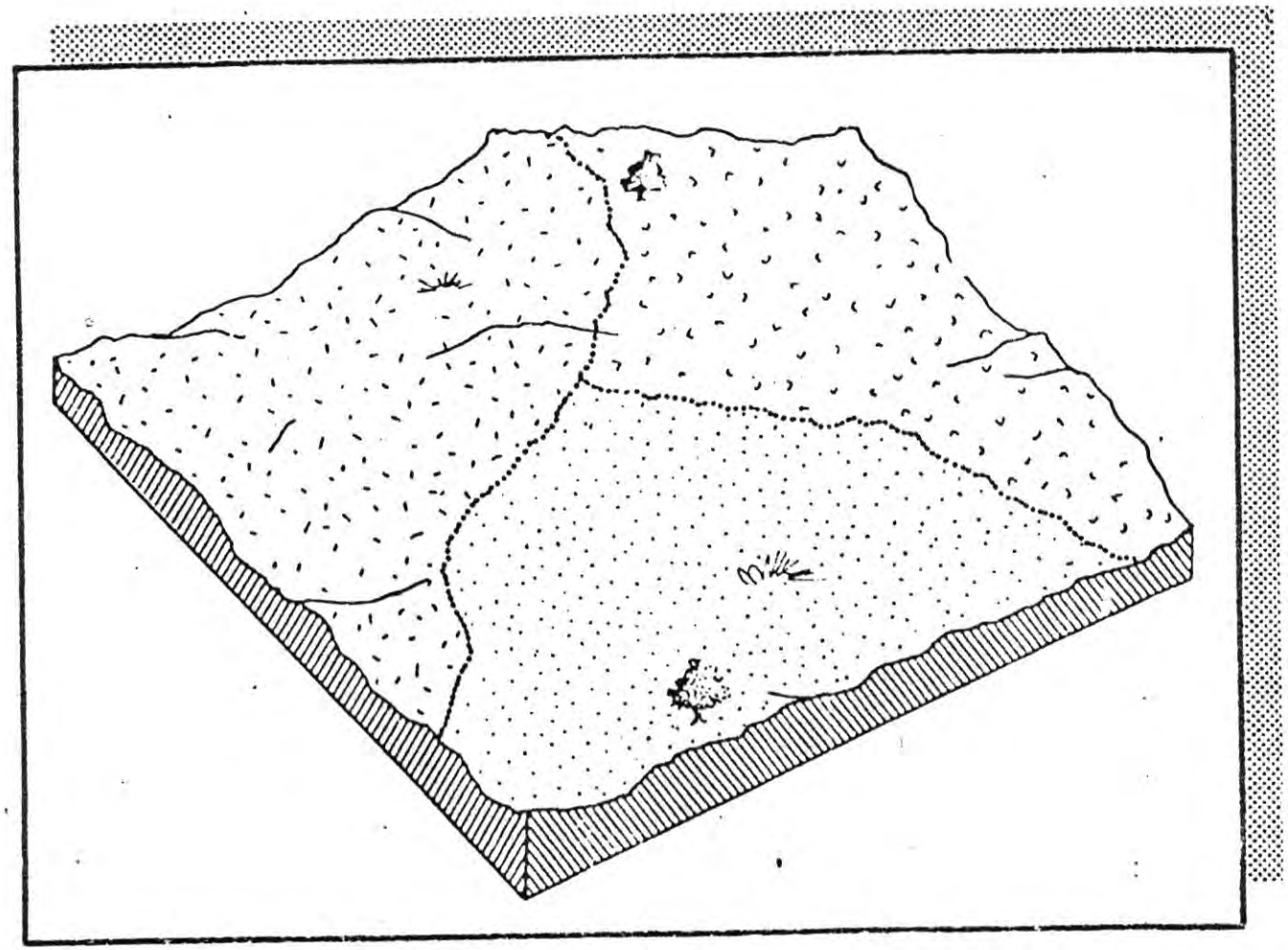
International Atomic Weights

ELEMENTS	SYM-BOL	ATOMIC NUMBER	ATOMIC WEIGHT	ELEMENTS	SYM-BOL	ATOMIC NUMBER	ATOMIC WEIGHT*
Actinium	Ac	89	227	Mercury	Hg	80	200.61
Aluminum	Al	13	26.98	Molybdenum	Mo	42	95.95
Americium	Am	95	[243]	Neodymium	Nd	60	144.27
Antimony	Sb	51	121.76	Neon	Ne	10	20.183
Argon	Ar	18	39.944	Neptunium	Np	93	[237]
Arsenic	As	33	74.91	Nickel	Ni	28	58.69
Astatine	At	85	[210]	Niobium			
Barium	Ba	56	137.36	(Columbium)	Nb	41	92.91
Berkelium	Bk	97	[249]	Nitrogen	N	7	14.008
Beryllium	Be	4	9.013	Nobelium	No	102	[253]
Bismuth	Bi	83	209.00	Osmium	Os	76	190.2
Boron	B	5	10.82	Oxygen	O	8	16.000
Bromine	Br	35	79.916	Palladium	Pd	46	106.7
Cadmium	Cd	48	112.41	Phosphorus	P	15	30.975
Calcium	Ca	20	40.08	Platinum	Pt	78	195.23
Californi	Cf	98	[249]	Plutonium	Pu	94	[242]
Carbon	C	6	12.010	Polonium	Po	84	210
Cerium	Ce	58	140.13	Potassium	K	19	39.100
Cesium	Cs	55	132.91	Praseodymium	Pr	59	140.92
Chlorine	Cl	17	35.457	Promethium	Pm	61	[145]
Chromium	Cr	24	52.01	Protactinium	Pa	91	231
Cobalt	Co	27	58.94	Radium	Ra	88	226.05
Columbium (see Niobium)				Radon	Rn	86	222
Copper	Cu	29	63.54	Rhenium	Re	75	186.31
Curium	Cm	96	[245]	Rhodium	Rh	45	102.91
Dysprosi	Dy	66	162.46	Rubidium	Rb	37	85.48
Einstei	Es	99	[245]	Ruthenium	Ru	44	101.1
Erbium	Er	68	168.94	Samarium	Sm	62	150.43
Europium	Eu	63	152.0	Scandium	Sc	21	44.96
Fermium	Fm	100	[255]	Selenium	Se	34	78.96
Fluorime	F	9	19.00	Silicon	Si	14	28.09
Francium	Fr	87	[223]	Silver	Ag	47	107.880
Gadoli	Gd	64	156.9	Sodium	Na	11	22.991
Gallium	Ga	31	69.72	Strontium	Sr	38	87.63
Germanium	Ge	32	72.60	Sulphur	S	16	32.066
Gold	Au	79	197.0	Tantalum	Ta	73	180.95
Hafnium	Hf	72	178.6	Technetium	Tc	43	[99]
Helium	He	2	4.003	Tellurium	Te	52	127.61
Holmium	Ho	67	164.94	Terbiu	Tb	65	158.93
Hydrogen	H	1	1.0080	Thallium	Tl	81	204.39
Indium	In	49	114.76	Thorium	Th	90	232.05
Iodine	I	53	126.91	Thulium	Tm	69	169.4
Iridium	Ir	77	192.2	Tin	Sn	50	118.70
Iron	Fe	26	55.85	Titanium	Ti	22	47.90
Krypton	Kr	36	83.80	Tungsten	W	74	183.92
Lanthanum	La	57	138.92	Uranium	U	92	238.07
Lead	Pb	82	207.21	Vanadium	V	23	50.95
Lithium	Li	3	6.940	Xenon	Xe	54	131.3
Lutetium	Lu	71	174.99	Ytterbium	Yb	70	173.04
Magnesium	Mg	12	24.32	Yttrium	Y	39	88.92
Manganese	Mn	25	54.94	Zinc	Zn	30	65.38
Mendelevium	Md	101	[256]	Zirconium	Zr	40	91.22

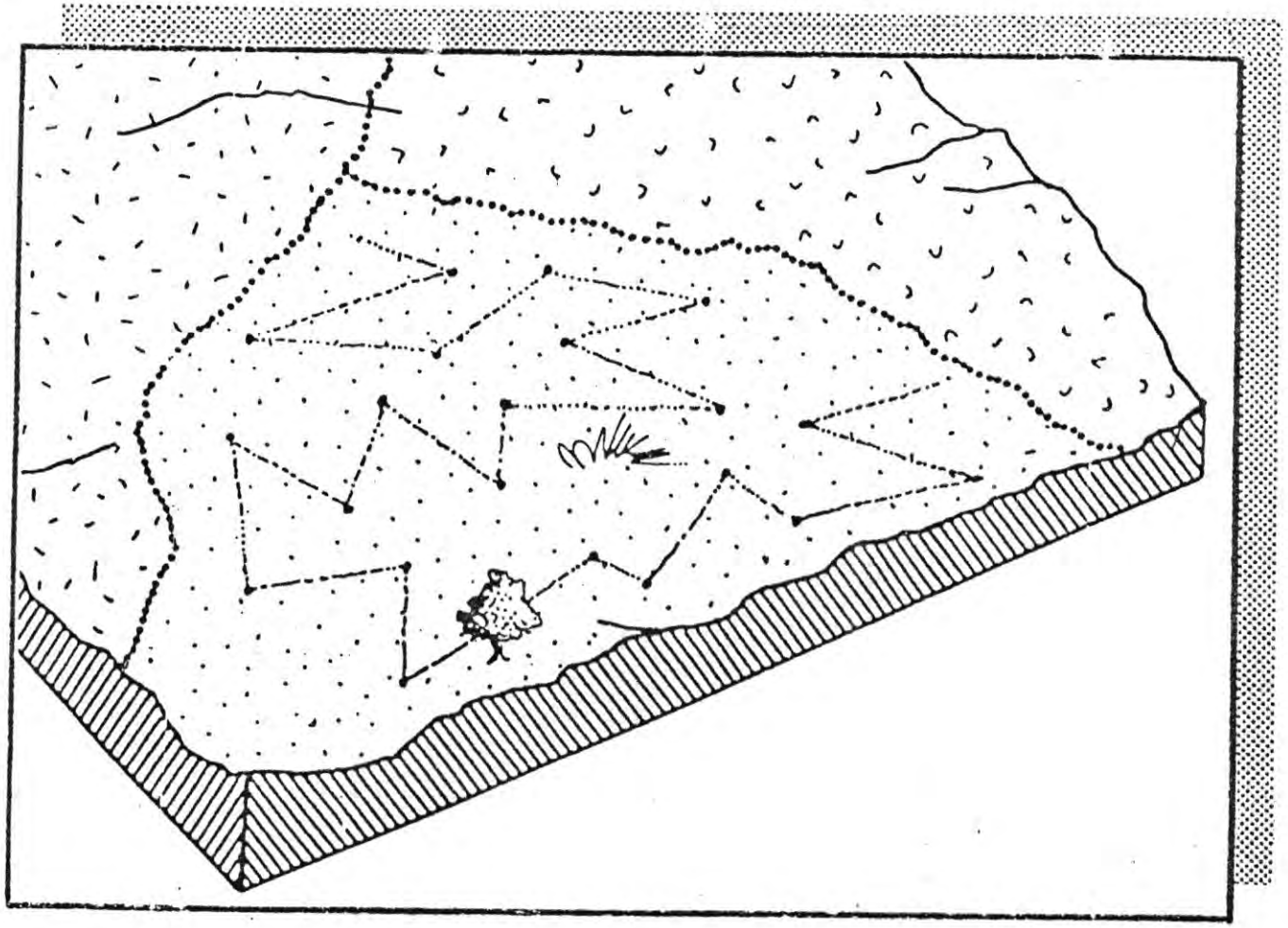
A value given in brackets denotes the mass number of the isotope of longest known half-life.

Soil Sampling Methods

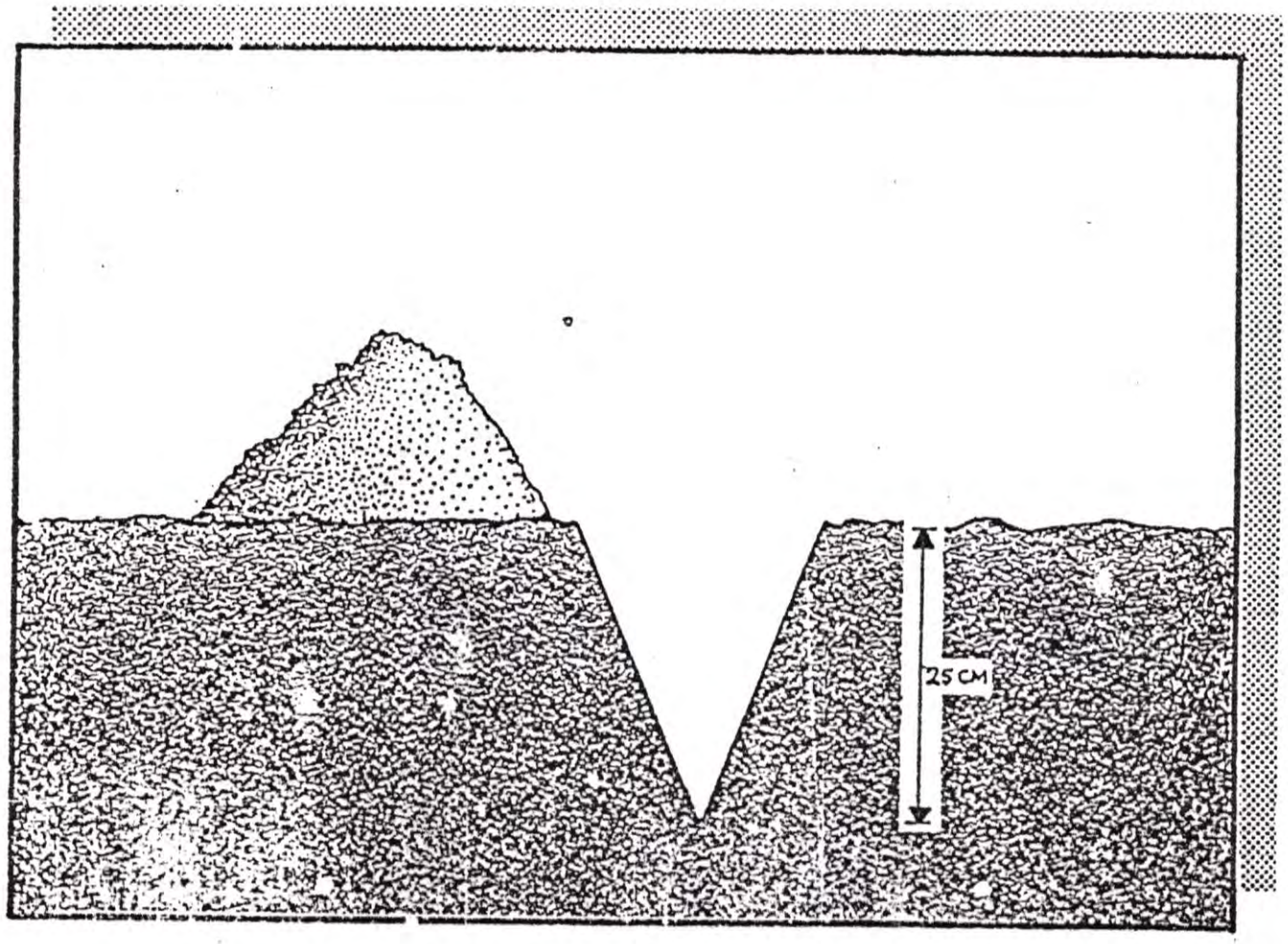
SOIL SAMPLING METHOD



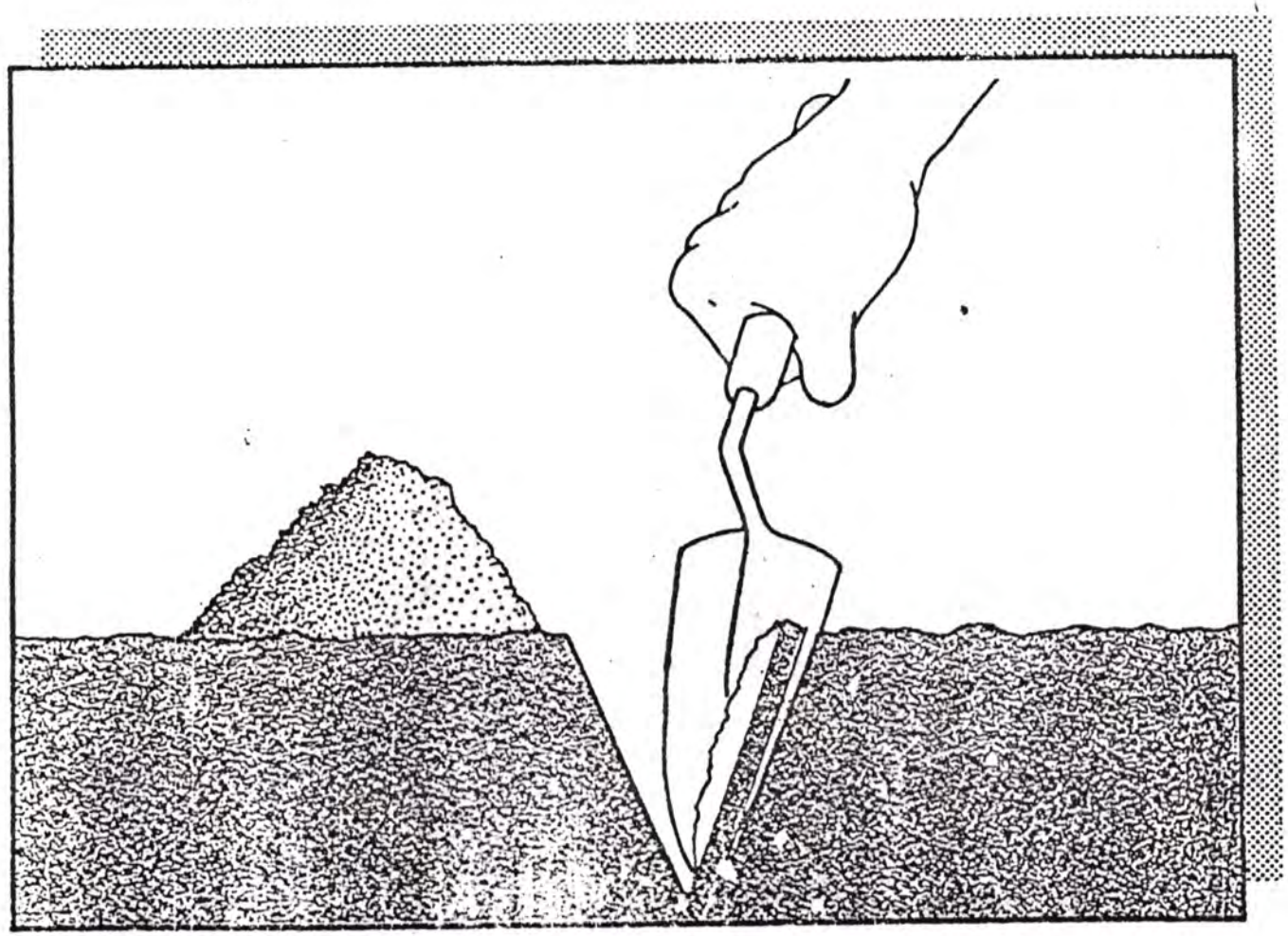
1. DIVIDE FIELD INTO UNIFORM SEGMENTS TOPOGRAPHICALLY



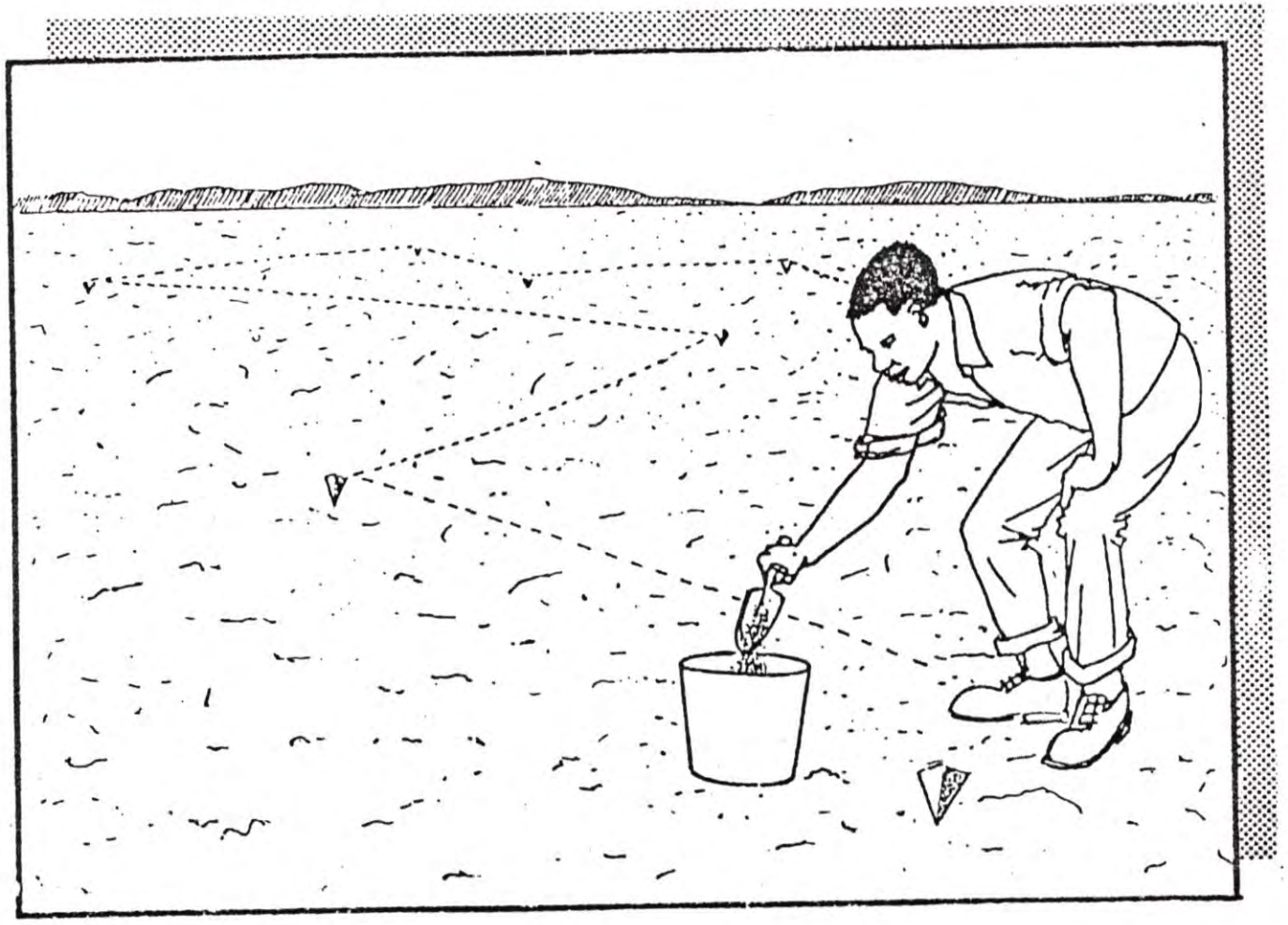
2. SELECT 15 - 20 SPOTS PER SEGMENT.



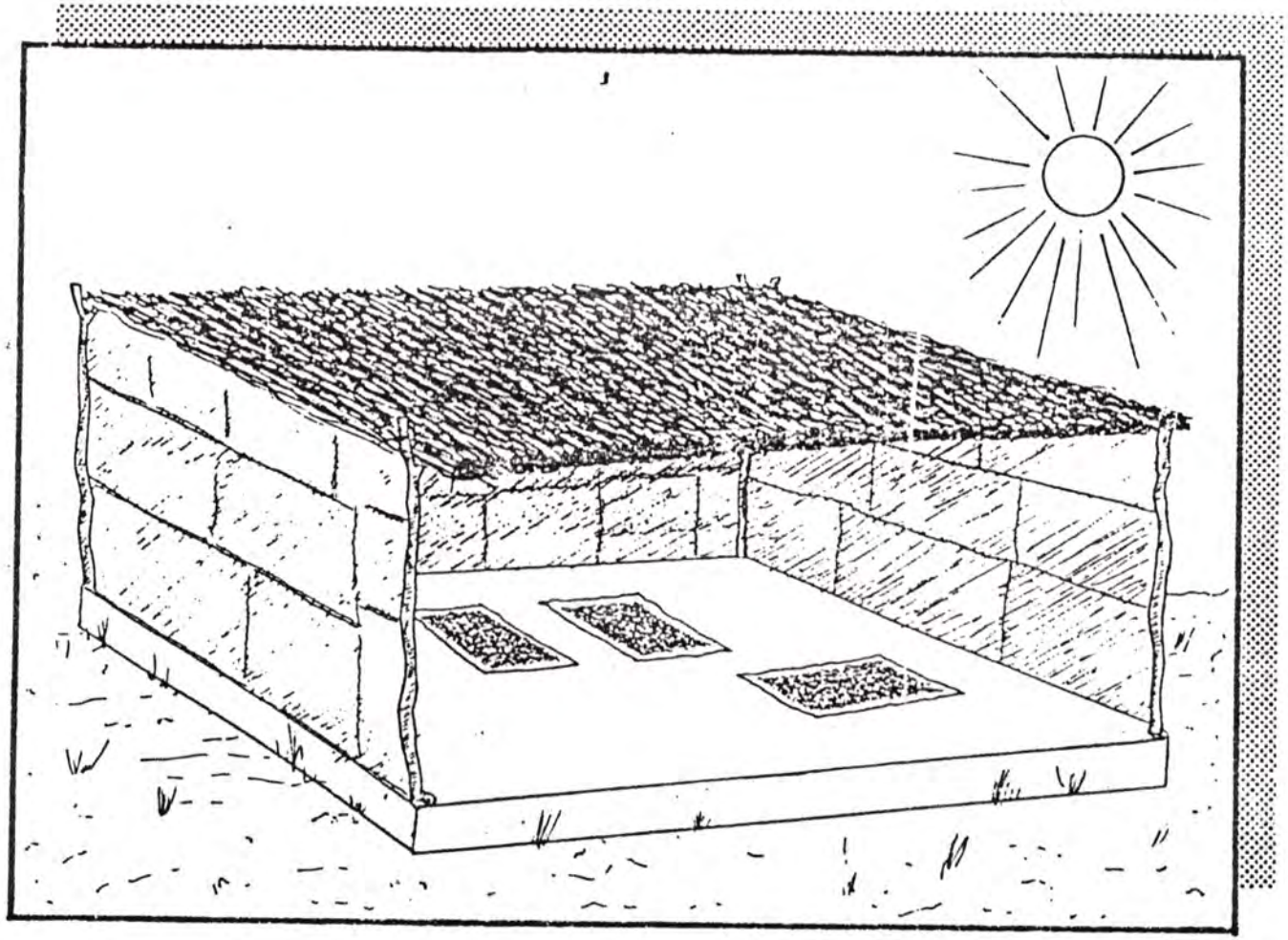
3. MAKE 25 CM "v" SHAPED DITCH.



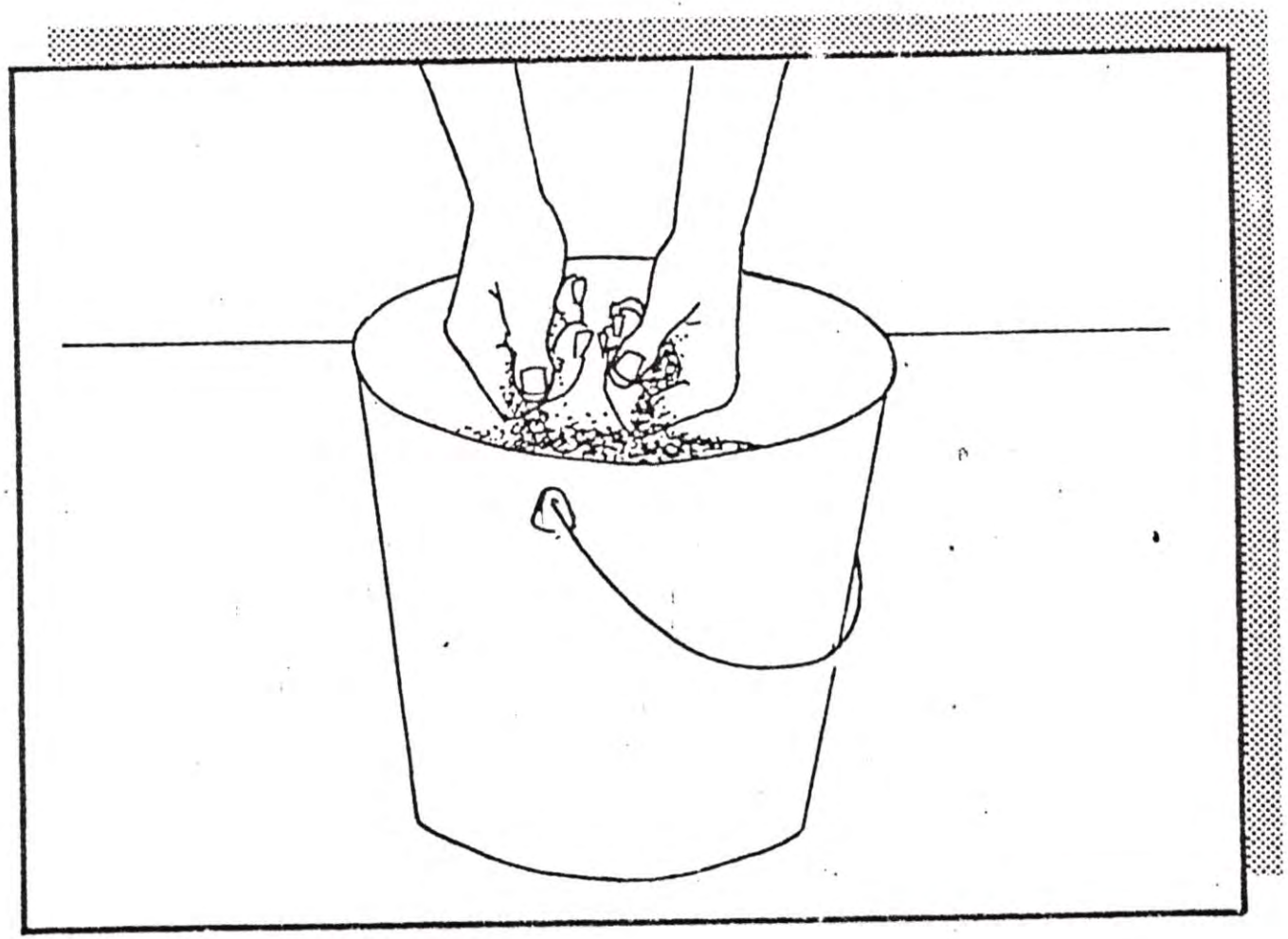
4. CUT A 3CM SLICE.



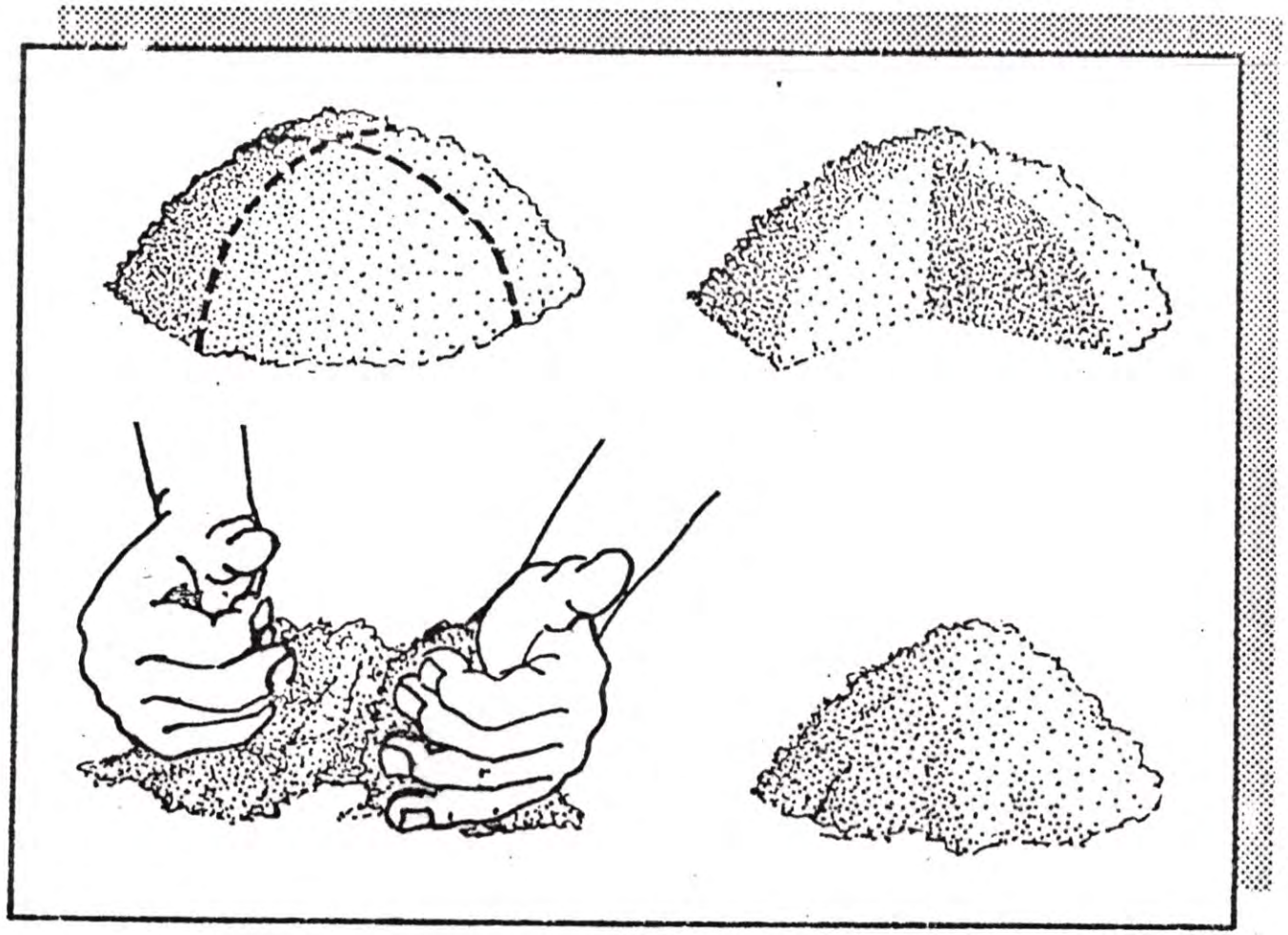
5. COLLECT SAMPLES INTO ONE CONTAINER



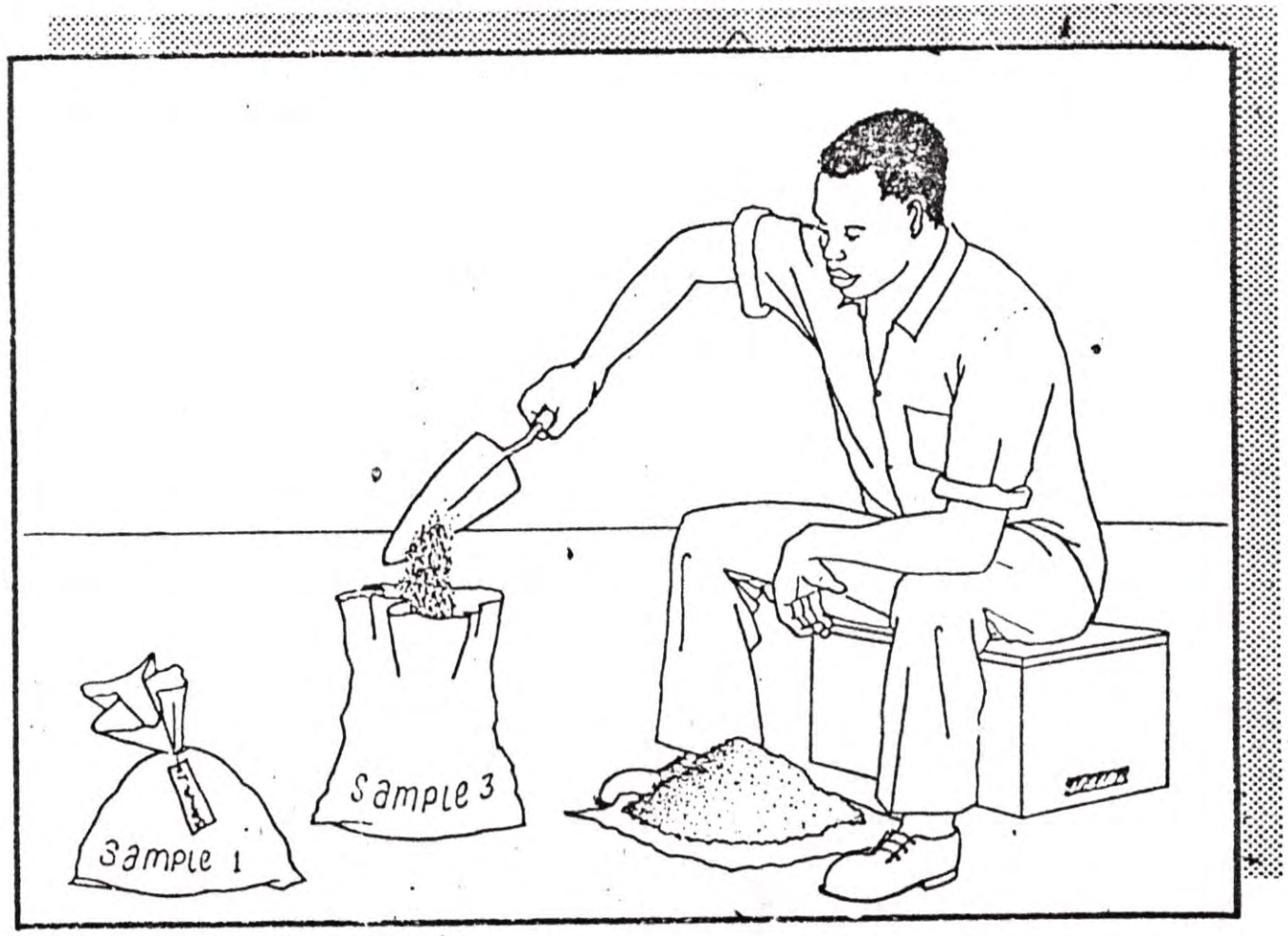
6. DRY IN THE SHADE.



7. MIX THOROUGHLY.



8. REDUCE VOLUME BY QUARTERING TO 1 KG.



9. TRANSFER INTO A BAG AND LABEL.