e-READING MANUAL

ASC-222

Soil Water and Plant Analysis

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B.Sc. (Hons) Horticulture

Prepared by

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e-Reading Manual on

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Forward

Soil and water are vital natural resources, and the sustainability of agriculture depends on these two. The prime concern is to maintain the increasing trend of food grain production for an escalating population and improve the quality of produce. Hence, periodic monitoring is indispensable to understand the status of the quality of soil and water and their impact on agricultural sustainability.

Further, the curriculum of agriculture undergraduate degree programs has been designed by ICAR in consideration of natural resources. Thus, students not only know the importance of soil and water but also the analysis procedure for the determining basic quality parameters of soil and water analysis. It is not only in agriculture but also the importance of basic quality parameters of soil and water are given particular well emphasis in other professional programs such as environment, and health

The practical manual on soil, plant and water analysis is comprehensive and well-designed for the students. The introductory analytical techniques have been adequately covered. Accessible language is used to explain the principles and procedures of soil, plant, and water determination. The chemical equations are used as and when required for the explanation of the principles. The calculations for the determination of different parameters are presented in a very professional and self-explanatory manner.

Hence, I believe the manual will be very useful for undergraduate students as well as postgraduate students and instructors. The authors put their best efforts into the preparation of the manual.

I appreciate the efforts of the authors in bringing out valuable publications for agriculture and other students.

(G.S. Panwar)
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September 23, 2022

Preface

Soil is a vital source of healthy foods, feeds, fibers, etc., for humans and animals. It plays a significant role in the development of civilization and food security. India has witnessed the self-sufficient production of food grains after independence. This appreciable milestone has been achieved at the cost of soil health. The continuous depletion of nutrients, microbes, and soil health is a significant concern for the supply of foods in the future. Hence, periodic monitoring of soil health is imperative to ensure soil sustainability.

The practical manual on soil plant and water analysis is meant to cater to the needs of undergraduate and postgraduate students of agriculture universities and soil scientists. All the standard protocols for the analysis of essential parameters of the soil fertility evaluation are included in the manual and explained in simple and understandable language. The presentation of the analysis is straightforward and easy to understand for the students. Furthermore, the manual also covers the basics of analytical chemistry. The procedure of determination of plant analysis and quality of irrigation water analysis has also been explained in understandable language.

The manual will also be helpful for the competitive examination aspirants of ICAR- JRF in physical science, postgraduate examinations UPCATET and SRF, and NET exams for soil science and agronomy students. Thank the authors for preparing a practical manual for the students.

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September 23, 2022

Index

1. Introduction to Analytical Chemistry

1. **Solution-** A perfect homogenous mixture of a solute in a solvent.
2. **Solute-** Solid part of the substances which is dissolve in solution.
3. **Solvent**- Liquid component of substance which is dissolving the solute.
4. **Standard solution**- A solution contains a known quantity of the reagent (solute) in a definite volume of solution. It is expressed as normality, molarity, ppm etc.
   A. **Primary standard solution**: This solution is also called as stock solution. Concentration of this solution is exactly known. It is non-hygroscopic in nature and available in pure form.
   Examples - Sodium carbonate, Potassium dichromate, Sodium oxalate.
   B. **Secondary standard solution**: Substance which cannot be obtained in pure form. It is hygroscopic in nature and cannot be used as primary standard solution. It cannot be weigh accurately. These solutions standardized against the primary standards for determining the exact strength of solution.
   Examples – HCl, H_2SO_4, NaOH, KOH, $KMnO_4$ etc.

   C. **Working Standard:** These solutions are prepared by taking required quantity of stock solution and dilute to require concentration at the time of reading or analysis.

N1V1=N2V2

Molarity – Molecular weight of substances dissolve per litre of solution. It is expressed by '**M**'.

Molarity= Molecular weight of substances x Molarity required x Volume (litre)

5. **Normality** - Equivalent weight of substances dissolve per litre of solution. It is expressed by '**N**'.
   Equivalent weight = Molecular weight/ valence
   Normality (for solid) = Eq. wt. of substance X Normality required X Volume (litre)
   Normality (for liquid) = $\frac{\text{Eq. wt. of subs}}{\text{Sp. gravity}} \times \frac{100}{\text{Purity}} \times$ Normality X Volume (litre)
6. **Endpoint**: It is usually point in which reaction is completed. For determination of this point in an acid base titration we use indicators. At the possible to the equivalence point indicator changes colour of solution, it means end point has been reached.
7. **Indicator** – A substances that changes the colour in response to a chemical changes.

Table 1: List of important indicators used in soil plant analysis

S.N.	Indicators	pH range	Colour in acid solution	Colour in Alkaline solution
1.	Methyl yellow	2.9-4.0	Red	Yellow
2.	Methyl Orange	3.1-4.4	Red	Orange
3.	Bromo cresol green	3.8-5.4	Yellow	Blue
4.	Methyl red	4.2-6.3	Red	Yellow
5.	Phenolphthalein	8.3-10.0	Colourless	Red

8. **PPM**- this is an abbreviation "parts per million" and it also can be expressed as milligram per litre (mg/l).

1ppm= $1/1000000=1/10^{-6}$

1ppm= 0.0001% or 1 %=10000 ppm

Objective: To prepare 1000 ppm standard stock solution of potassium from the potassium chloride salt

Instruments & Glassware:

- Electronic weighing balance
- Volumetric flask (1000 ml)
- Pipette

Chemical:
Potassium chloride

Calculation:

Molecular weight of Potassium (K): 39
Molecular weight of chloride (Cl): 35.5

Molecular weight of KCl: 39 + 35.5=74.5

For preparation of 1000 ppm of K: 74.5/39= 1.91 g

Procedure:

1. Weigh exactly 1.91 g of dry potassium chloride
2. Dissolve it in 1000 ml volumetric flask by using distilled water
3. Stir the solution until KCl salt disappear in water
4. Make the volume 1000 ml by distilled water
5. This solution will stock solution

Work Sheet

Date:…….

Instructor Signature

2. SOIL PLANT AND WATER SAMPLING, PROCESSING AND STORAGE

Soil, plant and water sampling should be done as per the objective of analysis and accuracy to be required for reporting of the result of laboratory testing parameters and, it should also represent truly the characteristics of soil, plant and water being tested. If soil samples are not taken by standard procedure, the results of the analysis will be futile. The chemical analysis is a time consuming and expensive, hence representative sampling is essential for the accuracy of the results. The heterogeneous condition of soil within one site creates challenges for soil sampling and build up chance to sampling errors.

Soil Sampling Guideline

For soil sampling for horticultural crops following basic things to be adopted.

1. Sample has to be collected preferably, with the help of stainless steel tube auger or with a *Khurpi* / spade.
2. Take the sample separately if there is variation in soil slope, colour, topography within one field. Larger field may be divided into smaller homogenous parts for better sampling.
3. Collect at least one sample from the one acre field.
4. Scrape away the litter and other materials from the surface of soil, if any.
5. Composite sample should be collected randomly in zigzag direction (depicted in figure 1) from different location of the field.
6. Take a uniform samples from the depth 15 cm of every spots of the zigzag by the soil auger or **"V"** shaped cut in soil by *khurpi* and cut uniform layer of 0-15 cm slice and collected it.

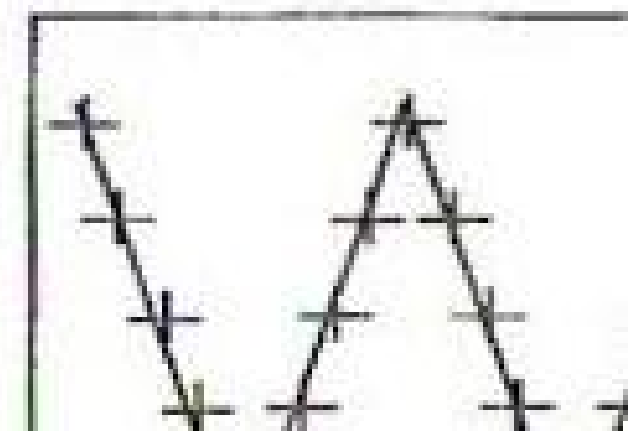


Fig 1: Selection of spots of a field for collection of soil samples

7. The border area of the field adjacent to all sides (2- 3 meter border strip) should be avoided for sampling.
8. Soil sample should not be taken from near road side , fencing , buildings , manure heaps , fertilized plots and adjacent to forest plantation.
9. For plantation crops, the pits of 90 cm depth can be dug and the separate samples should be drawn from 0-30 cm, 30-60 cm and 60-90 cm depths. Presence of canker pan in profile may be recorded.

10. Composite sample should be mixed and reduced upto 500gm by quartering process to make representative sample for laboratory testing.
11. For horticultural crops, sampling is done at depths of 0-15, 15-30, 30-60, 60-90, 90-120, 12-150 and 150-180 cm.
12. In saline-alkali soils, salt crust (visible or suspected) should be sampled separately and sampling depth be recorded.
13. Sampling should be done every year if the field is under intensive cultivation. If one crop per year is grown, sampling once in three years is sufficient. Soil sampling should be done at the same time in each year.

Time of Soil Sampling:

Sampling should be done once in three year interval for monitoring of the soil fertility. However, quick change in fertility can be monitored every year analysis. In general, yearly samples should be collected from the soil after the harvest of Rabi crop. Amendment treated soils, the pH samples reading stabilize after the two years of the treatments. Hence, it would be better to take samples in 2 year interval from amendment treated soils.

Sampling tools

Sampling tools

Soil sampling can be done with the help of following tools:

i) Tube auger

ii) Screw type auger

iii) Post-hole auger

iv) Spade or *Khurpi*

Polythene

Permanent marker/tag

Scale

For sampling soft and moist soil, a tube auger, spade or khurpi is quite satisfactory. A screw type auger is more convenient on hard or dry soil, while the post-hole auger is useful for sampling in excessively wet areas viz. rice fields. Tube Auger is convenient for sampling from

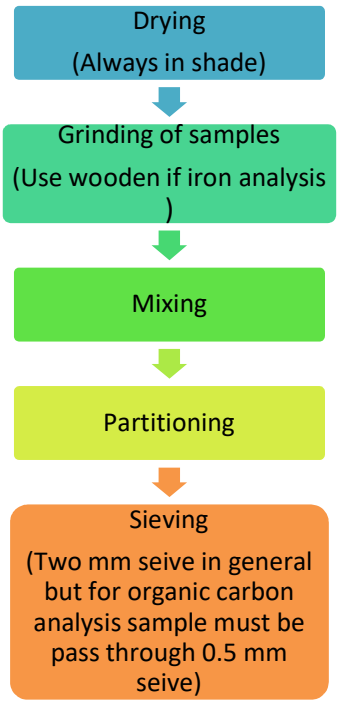
lower depths. If a spade or khurpi is used, a V-shaped cut may be first made up to the plough layer and a uniform 2 cm thick slice is taken out.

Labelling of samples

For identification, label the soil samples. A label of thick paper with identification mark along with the details of the sample should be put inside the sample bag and another label carrying same details tied outside the bag. In addition to location, field number, name of cultivator and relevant information about slope, drainage, previous cropping history, irrigation, fertilizer, manure used etc. must be recorded.

Processing and storage of soil sample

Processing of soil sample needs following steps :-



Storage of Soil sample:- After processing , samples are stored in polythene bags or plastic / glass jars with proper labelling for further use.

Plant Sampling

Perennial horticultural plants are quite different from seasonal crops in their nutritional requirement due to their plant size, rooting pattern, growth and fruiting behaviour. Accumulation of nutrients in plant tissues shows the uptake of the concern nutrient from the

soil to the plant. Total plant analysis is done to find out the amount of nutrient removed from the soil by plant uptake, which represents total nutrient requirement of the plant. While, Leaf analysis is the best method for identifying the deficiency/need of nutrient to be applied for a plant. There is a close relationship between dose of nutrient supplied, leaf nutrient content and yield or quality.
Hence, preparation of plant sampling plan subjected to purpose of analysis to be carried.

Principle of plant sample collection

- The general principle in the index tissue sampling is to collect recently / youngest mature leaf or petiole or for field crops at the time of harvesting dry plant and grain part can collect for the total nutrient analysis.
- Collect a composite sample from all four sides of the plant i.e. north,south, east and west.
- Collect sample prior to irrigation and, manure and fertilizer application.
- Avoid sampling from plants that are in stress of biotic and abiotic conditions.
- Wash sample several times with deionised water or distilled water
- To remove waxy or greasy content on leaf surface,sample may be washed with 0.2 % detergent solution.
- Followed by N/10 HCl solution washing , finally wash with double distilled water.
- After, transport from the field, samples may be stored in a refrigerator at 5°C .
- For wet chemical analysis of harvested plant part particularly field crops, After washing samples dry it in shade and after drying, put it in oven at 60 °C for 24 hours or till constant weight of samples.
- Fine grind the samples by using willey mill

Water sampling

Irrigation water management and quality may affect the crop growth and soil properties. Water pollution is becoming a serious issue for public health and toxic metal contamination in agricultural produce. Analysis of irrigation water is very important to save the soil and public health.
Water sampling require following accessories;

- New glass or plastic bottles (500 ml to 1000 ml)
- Cotton or nylon ropes
- Water table recorder
- Permanent markers

Water sample should be collected in the bottle after rinsing the same water. Sample from the tube well may be collected directly from discharge point after 10-20 minutes running out. Sampling of shallow ponds, lakes reservoir, canal and river may be done from mid-point the water body.
If there is delay in submission of water sample to the laboratory or testing, water sample must be protected from bacterial growth either by addition of 2-3 drops of toluene or by refrigeration at 4 °C temperature.

Work Sheet

Date:…….

Instructor Signature

3. Analysis of soil samples

Exercise1

Determination of pH & EC of soil

Determination of Soil pH

Objectives:

1. To know whether the soil is acidic, neutral or alkaline in nature
2. To know the degree of acidity or alkalinity of soil
3. To know the potency of toxic substances present in soil
4. To know the microbial environment of the soil, which further indicate the mineralization of organic matter and immobilization of available plant nutrients.

Principle:

The degree of acidity or alkalinity can be represented by its intensity. The intensity is represented by the amount of ions (g ions l^{-1}). The substances that give H^+ ions in its aqueous solution are called acids where as those who give OH^- ions are called bases. In an aqueous solution, the product of H^+ and OH^- ions is constant at constant temperature (i.e. 10^{-14}). Therefore, the intensity of acidity or alkalinity of a solution can be determined on the basis of its H^+ ions concentration. The pH is defined as the negative logarithm to the base 10 of H^+ ions activity (concentration) expressed in g ions (moles) l^{-1} of solution and expressed by the equation: $pH= -\log_{10} [H^+]$ or $pH= \log_{10} 1/[H^+]$. This concentration is practically measured by the electric potential produced between a glass and reference electrode.

Apparatus and Reagents:

1. pH meter with glass and reference electrode
2. beaker
3. buffer solutions(4.0,7.0 and 9.2 pH): Buffer are the solution which have exact pH and resist to change pH.

Procedure: (1:2 Soil water solution)

1. Weigh 20 g soil sample on electronic balance in a 100 ml beaker.
2. Add 40 ml distilled water into the beaker (Soil water ratio should be 1:2),
3. Stirrer it for 5 minutes
4. Switch on the pH meter and let it be warm up for 10-15 minute.
5. Calibrate the pH meter as per following manufacture guideline Dip the electrodes in sample solution and note the reading.

Soil pH Rating:

pH	Soil Reaction
<5.0	Strongly acidic
5.0-6.5	Moderately to slightly acidic
6.5-7.5	Neutral
7.5-8.5	Moderately alkaline

>8.5 Strongly alkaline

Result:
The pH of the given soil sample is……and soil is categorized as acidic/alkaline/neutral.

Soil Electrical Conductivity (EC)

The electrical conductivity represents the total soluble salts (Ca, Mg, K etc) present in a soil solution. It obeys Ohm,s law and EC measures ionic transport in soil and water water solution between anode and cathode.

Since the EC depends on the number of ions in the solution, it is important to know the soil/water ratio used. The EC of a soil is conventionally based on the measurement of the EC in the soil solution extract from a saturated soil paste, as it has been found that the ratio of the soil solution in saturated soil paste is approximately two-three times higher than that at field capacity.

Saturation soil paste method of EC is for the alkaline soil, although it is time consuming and cumbersome process and requires 400 to 500 g of soil sample. Whereas, neutral and acid soils, generally 1:2 soil water suspension methods used, which is very quick method.

Apparatus/Equipments

Electrical conductivity meter

Glassware

Beakers (100 ml),

Reagent

0.01M Potassium chloride standard solution: Weight 0.7456 g KCl and dissolve in a distilled water and make the volumetric flask volume to one litre. This solution gives an electrical conductivity of 1411.8 x 10^{-3} or 1.412 dSm^{-1} at 25°C. Standardize EC meter with the help of 0.01 M KCl before taking reading on instrument.

Procedure

1. Take 40 g soil into 250 ml Erlenmeyer flask, add 80 ml of distilled water,
2. Shake the flask on reciprocating shaker for one hour.
3. Filter through Whatman No.1 filter paper. The filtrate is ready for measurement of conductivity.
4. Wash the conductivity electrode with distilled water and rinse with standard KCl solution.
5. Standardize the EC meter by using 0.01 M KCl solution.
6. Pour some KCl solution into a 25 ml beaker and dip the electrode in the solution. Adjust the conductivity meter to read 1.412 $mScm^{-1}$, corrected to 25^0 C.
7. Wash the electrode and dip it in the soil extract.
8. Record the reading of EC from display and also note down the temperature, reading should

be corrected at 25°C.

Table 2: General interpretation of EC values

S. No.	Soil	EC (mS/cm)	Total salt content (%)	Crop reaction
1.	Salt free	0-2	<0.15	Negligible effect of salinity
2.	Slightly saline	4-8	0.15-0.35	Crop can grow with proper management practice
3.	Moderately saline	8-15	0.35-0.65	Grow salt tolerant crops with proper management practice
4.	Highly saline	>15	>0.65	Grow salt tolerant crops with proper management practice, leaching of salt by good quality irrigation water

Result:
The EC of the given soil sample is…….dS/m, and soil is categorized as……...

Work Sheet

Date:…….

Instructor Signature

Exercise 2

To determine Organic Matter and Organic Carbon Analysis from the Soil Sample

Amount of organic matter present in soil directly reflect the fertility status of the soils. Mostly organic carbon content in soils is used for determination of organic matter content in soil, which is also indirectly represents capacity mineralizable nitrogen in soils.

The following three methods are often used in determination of organic carbon content if soils

1. Total Organic Carbon (Weight loss method or ignition/dry combustion)
2. Volumetric methods proposed by Walkley and Black methods (1934)

Dry combustion methods

This method is very rapid and don't require any chemicals for the analysis. It provides total carbon content in soil. The principal is based on loss of weight on ignition.

Apparatus:

- 2 mm sieve;
- beaker;
- Wash bottle
- Silica /plutonium crucibles
- Hot air oven;
- Muffle Furnace.

Procedure:

1. Weigh 1 to 5 g of soil in plutonium crucibles (Amount of soil sample for analysis depends upon size of crucibles)
2. Place the crucibles + soil in a drying oven and set the temperature at 105 °C for 4 to 6 hours. Remove it from oven and place in a desiccators or dry place.
3. After the cooling of the crucible+ soil take the weight (W_1) it will remove the adsorbed water molecules from the soil.
4. Place the crucible with soil into a muffle furnace, and bring the temperature to 400 °C for 4 hours. Remove the crucible from the muffle furnace, cool in a dry atmosphere, and weigh it (W_2)

Calculation:

The percentage of Organic Matter is given by:

$$\%\ of\ Organic\ matter\ (loss\ on\ ignition) = ((W_1 - W_2) \div W_1) \times 100$$

where:

W_1 :weight of crucible + soil at 105 °C;

W_2 :weight of crucible + soil at 400 °C.

The percent of organic C is given by: % OM × 0.58.

If soil has high amount of $CaCO_3$, treat the soil sulphurous acid before the start of analysis.

The weight loss methods are susceptible to errors caused by the presence of salts having carbonate, water and hydroxyls.

Organic carbon by wet digestion

Principle: An excess of 1 N $K_2Cr_2O_7$ solution is added into the sample in presence of H_2SO_4thereby Chromic acid is formed, carbon present in organic matter is oxidized into carbon di oxide and water in presence of chromic acid. The untreated chromic acid is back titrated with 0.5 N ferrous ammonium sulphate using diphenyl amine and ortho phosphoric as an indicator. The amount of organic carbon in the soil is calculated from the amount of chromic acid used in oxidation of organic matter.

$$K_2\,Cr_2O_7 + H_2SO_4 \leftrightarrow H_2Cr_2O_7 + K_2SO_4$$

$$2H_2Cr_2O_7 + 3C + 6H_2SO_4 \;\leftrightarrow\; 2Cr_2\,(SO_4)_3 \;+\; 3CO_2 + 8H_2O$$

The method uses the heat of dilution for accelerating the above reaction but it does not provide complete oxidation of organic compounds. The fraction of organic matter oxidize depends on the nature of organic compounds & soil type. A range of 46 to 92 % oxidation has been reported by several scientists in various soils of India. Therefore, a correction factor of 1.3 is generally accepted for determination of total organic carbon.

Volumetric method

Apparatus

- Conical flask (capacity 500 ml);
- Pipettes (10 20 ml);
- Burette (capacity 50 ml).

Reagents:

- Ortho-Phosphoric acid (85 %).
- Sulphuric acid
- 1 M $K_2Cr_2O_7$: Dissolve 49.04 g of $K_2Cr_2O_7$ in water and make the volumetric flask to 1 litre.
- 0.5M Ferrous ammonium sulphate solution: Dissolve 196.1 g of $FeSO_4(NH_4)_2.6H_2O$ in 800 ml of water and add 20 ml of concentrated H_2SO_4 and make the volume up to 1 litre.
- Diphenylamine indicator: Dissolve 0.5 g of reagent-grade diphenylamine in 20 ml of water and add 100 ml of concentrated H_2SO_4.

Procedure:

1. Weigh 1.0 g of the processed soil sample (passed through 0.5 mm sieve) in a 500-ml conical flask.
2. Add 10 ml of 1 M $K_2Cr_2O_7$ solution and 20 ml of concentrated H_2SO_4.
3. Mix thoroughly and place it in dark or hot air oven to allow the reaction to complete for 30 minutes.
4. After that add 200 ml of distilled water and 10 ml of H_3PO_4 in a flask.
5. Add 1 ml of diphenylamine indicator.
6. Titrate the solution with standard 0.5M $FeSO_4(NH_4)_2.6H_2O$ solution to a brilliant green colour.
7. Simultaneously run a blank without sample.

Calculation:

The percentage of organic C is given by:

$$= \frac{10\ (B - S)\ \times 0.003}{B} \times \frac{100}{Wt.of\ Soil}$$

For conversion of % Organic carbon into Organic carbon (g kg^{-1}) multiply 10.
where:
B: Ferrous ammonium sulphate consumed (ml) for titration of blank;
S: Ferrous ammonium sulphate consumed (ml) for titration of soil sample;
0.003 = weight of C (1 000 ml 0.1667M $K_2Cr_2O_7$ = 3 g C.
Thus, 1 ml of 1 M $K_2Cr_2O_7$: 0.003 g C
Organic C recovery is estimated to be about 77 %.
Total organic carbon= % OC x 1.3
OM (%)= total organic carbon × 1.724 (organic matter contains 58 % organic C, hence 100/58 = 1.724).

Work Sheet

Date:…….

Instructor Signature

Exercise 3

Objective: To determine the mineralizable nitrogen from the soil

The Subbiah and Asija, 1956 was proposed a method for the determination of mineralizable nitrogen from the soil. It is indicator of available nitrogen. In this method $KMnO_4$ is used as a oxidizing agent and KMNO4 hydrolyze the organic matter present in the soil. The hydrolysis enhanced at alkaline pH hence NaOH added in the solution. During the process NH3 librated from the soil is condensed and trapped in boric acid or sulphuric acid, which is titrated against the standard sulphuric acid. This method is widely used for determination of nitrogen because rapidity and reproducibility of the results.

Apparatus/ Equipments:

- Nitrogen distillation unit
- Conical flasks
- Pipettes
- Burettes

Reagents:

1. 0.32 % $KMnO_4$: Weigh 3.2 g of $KMnO_4$ and dissolve in water and make the volume up to 1 litre.
2. 2.5 % Sodium hydroxide (NaOH): Dissolve 25 g of sodium hydroxide pellets in water and make the volume up to 1 litre.
3. 2 % boric acid: Dissolve 20 g of boric acid powder in warm water by stirring and dilute to 1 litre.
4. Mixed indicator: Weigh accurately 0.066 g of methyl red and 0.099 g of bromocresol green and dissolve it in 100 ml of ethyl alcohol.
5. Thereafter add 20 ml of this mixed indicator to each litre of 2 % boric acid solution.
6. 0.1M potassium hydrogen phthalate: Dissolve 20.42 g of the salt in water and dilute to 1 litre. This is a primary standard and does not require standardization.
7. 0.001M H_2SO_4: First prepare 0.1 N H_2SO_4 solution and after that dilute it for 0.001 M H_2SO_4.
8. 0.1M Sodium hydroxide: Dissolve 4 g of NaOH in 100 ml of distilled water. Standardize against potassium hydrogen phthalate.

Procedure:

1. Weigh 20 g of soil sample in Kjeldahl tube (250 ml).
2. Moisten the soil with about 10 ml of distilled water, wash down the soil, if any, adhering to the neck of the flask.
3. Add 100 ml of 0.32 % $KMnO_4$ solution.
4. If bumping and frothing in samples happening at the time of heating add a few glass beads or broken pieces of glass rod and 2–3 ml of paraffin liquid. It will prevent bumping and frothing.

5. Measure exact 20 ml of 2 % boric acid containing mixed indicator in a 250 ml conical flask and place it under the receiver tube.
6. Dip the receiver tube properly in the boric acid.
7. Run tap-water through the condenser.
8. Add 100 ml of 2.5 % NaOH solution, and immediately attach to the rubber stopper fitted in the alkali trap.
9. Switch the heaters on and continue distillation until about 100 ml of distillate is collected.
10. First, remove the conical flask containing distillate and then switch off the heaters to avoid back suction.
11. The automated kejdhal assembly are popular for the determination nitrogen. Just follow the user instructions for the nitrogen determination other regents preparation are remain same.
12. Titrate the distillate against 0.001M H_2SO_4 in a burette until a pink colour starts to appear.
13. Simultaneously run a blank without soil.

Calculation:

S: volume of 0.001M H_2SO_4 used in titration against ammonia absorbed in boric acid;
B : volume of 0.001M sulphuric acid used in blank titration.
Volume of acid used to neutralize ammonia in the sample = (*S*– *B)*ml

$$\% \text{ Nitrogen} = \frac{(S-B)\times 0.028}{Weight\ of\ soil\ sample} \times 100$$

where:
1 ml of 1M H_2SO_4 = 28 g N; thus, 1 ml of 0.001M sulphuric acid = 0.028 mg N.

Precautions:

- All the joints of the Kjeldahl apparatus should be checked and waxed properly in order to prevent any leakage and loss of ammonia.
- Hot Kjeldahl flasks should not be removed from the assembly.
- Read MSDS of sulphuric acid before using it.
- As procedure require handling of several chemicals during the analysis hence adopt guideline of wet chemistry lab.

Note: Devarda's alloy, is a reducing agent (alloy of aluminium, copper and zinc) used for the determination of nitrates after their reduction to ammonia under alkaline conditions.

Alloy: It is a mixture of metals.

Reference:
Subbiah, B.V. and G..L. Asija (1965) A rapid procedure for the estimation of available nitrogenin soils. Curr. Sci. 25: 259-60.

Work Sheet

Date:…….

Instructor Signature

Exercise 4

Estimate the available phosphorus in soil

The three major steps involved in analysis of available phosphorus are given below

1. Extraction of soil
2. Colour development of extractant
3. Determination of intensity of colour by use of spectrophotometer

Several extractants are being used by the researchers for phosphorus estimation in soil,

SN	Name of Methods	Acceptability
1	Mehlich No. 1 (0.5 N HCl + 0.025 M H_2SO_4)	Sandy acid soils, low CEC
2	Bray P1 (0.03 N NH_4F + 0.025 M HCl)	Acid soils moderate in CEC
3	Morgan (Wolf) 0.73 M NaOAC+7.4 HOAC at pH 4.8	All soils
4	Olsen (0.5 M $NaHCO_3$) at pH 8.5	Alkaline Soils
5	AB-DTPA (2 M $NaHCO_3$+ 0.005 M DTPA) at pH 7.6	Alkaline Soils

Two most commonly used method i.e. Brays P1 (acid soil) and Olsen (neutral to alkaline soil) are used for the available phosphorus determination.

In these methods, specific blue colour formed and the colour intensity is measured by the use of instrument spectrophotometer. Now days, spectrophometer detects all the wavelength however, for above two procedure require detection of wavelength in visible region (300-800 nanometer).
It is based on the Lambert's and Beer's law which stated that when a monochromate beam of light pass through a medium , the intensity of transmitted beam decreases exponentially with arithmetic increase in the thickness of medium or path length/ concentration.

Bray's Method No. 1:

Apparatus/glassware:

- Spectrophotometer;
- Mechanical shaker
- Different capacity pipettes (5, 10 and 20 ml);
- beakers/flasks (25, 50, 100 and 500 ml).
- measuring cylinder
- Filter paper
- Volumetric flask (25 ml)

Reagents:

- Bray's Extractant No. 1 (0.03M NH_4F in 0.025M HCl): Dissolve 2.22 g of NH_4F in 200 ml of distilled water, filter, and add to the filtrate about 1.75 litres of water and mix 4 ml of concentrated HCl into, and then make the volume up to 2 litres with distilled water.
- Reagent A: Weigh 12g ammonium molybdate and dissolve it in 250 ml of distilled water. Thereafter, weigh precisely 0.2908 g antimony potassium tartrate and dissolve in 100 ml

distilled water. Add these two solutions into 2 litre volumetric flask and add 1000 ml of 2.5 M H_2SO_4, mix thoroughly, and make up to 2000 ml volume.

- Reagent B: dissolve 1.056 g ascorbic acid in 200 ml reagent A and mix. Prepare daily as required. This does not keep for more than 12 hours at room temperature.
- 2.5 M Sulfuric acid : Take 140 ml of concentrated H_2SO_4 and dilute it to 1 L.
- Prepare fresh dilute solution every working day.

Procedure:

1. Weigh 5 g of soil sample in 150 ml conical flask
2. Add 50 ml of the Bray's Extractant No. 1 into soil
3. Shake for 5 minutes on rotary shaker and filter with whatmen filter paper 1.
4. The ratio of soil: extracting reagent must be 1:10.
5. Filter the solution immediately after the shaking
6. Transfer the 5 ml aliquot to 25 ml volumetric flask
7. Add 4 ml of reagent B
8. Make the volume 25 ml by using distilled water
9. Prepare blank as above using 5 ml extracting solution in place of soil extract
10. Standard curve: measures 0, 1, 2, 3, 4 ml of standard 2 mg L^{-1} P solution in 25 ml volumetric flask. Add 5 ml extracting solution and 0.5 to 1 ml 2.5 M H_2SO_4. Add 4 ml reagent B. Prepare the final volume with the help of distilled water. The P concentration of these solutions will be 0, 0.08,0.16,0.24 and 0.32 mg L^{-1} or ppm, respectively.
11. After 10 minutes (solution should be bluish purple), read P concentration at 882 nm after calibrating spectrophotometer with standards. The colour is stable for 24 hours and is not affected by the colour in the filtrate due to organic P.

Calculation:

where:

- ✓ weight of the soil: 5 g;
- ✓ volume of the extract: 50 ml;
- ✓ volume of the extract taken for estimation : 5 ml;
- ✓ Weight of 1 ha of soil : 2.24 x 10^6 kg.

$$Avilable\ P\ (kg/ha) = \frac{R\ \times Volume\ of\ extract}{Volume\ of\ aliquot} \times \frac{2.24 \times 10^6}{Weight\ of\ soil\ \times 10^6}$$

Where R= mg P in the aliquot (obtained from standard curve)

Olsen's method (Olsen et al. 1954)

Sodium bicarbonate solution extracts some exchangeable or surface-adsorbed P, calcium phosphates and other phosphates. Olsen's method is widely adopted for the determination of available P in neutral, saline and alkaline soils (7 to 9.5). Calcium carbonate is precipitated, resulting in the dissolution of P from calcium phosphate.

Determination of extracted P is based on principle that in an acid molybdate solution containing orthophosphate ions, forms that can be reduced by ascorbic acid to a molybdenum blue colour. The Bundelkhand region soils are commonly neutral to high in pH. Hence the Olsen method is more appropriate for phosphorus determination.

Apparatus/glassware:

- Electronic balance
- Spectrophotometer;
- Mechanical shaker

Glassware

- pipettes
- beakers/flasks (25, 50, 100 and 500 ml).
- measuring cylinder
- Filter paper
- Volumetric flask (25 ml)

Reagents:

- ✓ Sodium Bicarbonate extractant (0.5 M $NaHCO_3$): weigh 42 g of sodium bicarbonate and dissolve it in 1 litre of distilled water and adjust the pH to 8.5 by addition of dilute NaOH or HCl. After adjusting pH at 8.5 make the final volume of volumetric flask by using distilled water.
- ✓ Activated P-free carbon/charcoal: If charcoal is not activated then it required washing; wash it 2 to3 times with dilute 1 N HCl followed by distilled water.
- ✓ Reagent A: weigh 12g ammonium molybdate and dissolve it in 250 ml of distilled H_2O and weigh 0.2908 g antimony potassium tartrate and dissolve in 100 ml beaker. Add these two solutions to 1000 ml of 2.5 M H_2SO_4, mix thoroughly, and make up to 2000 ml.
- ✓ Reagent B: Ascorbic acid (1.056 g) dissolves in 200 ml reagent A and mixes it. Prepare daily reagent as required.
- ✓ Standard stock solution: Weigh precisely 0.439 g potassium dihydrogen orthophosphate (KH_2PO_4) A.R. grade and dissolve it in 500 ml distilled water. Add 25 ml of 7 N H_2SO_4 and adjust the final volume of volumetric flask by using distilled water. This gives 100 ppm stock solution. For this, prepare 2 ppm working solution is made by 2 ml from stock solution in 100 ml of volumetric flask and adjust final volume by distilled water.

Procedure:

1. Weigh 2.5 g soil in 150 ml conical flask
2. Add 50 ml of the bicarbonate extractant in conical flask containing soil
3. Add pinch amount of activated charcoal in flask.
4. Shake for 30 minutes on the mechanical shaker, and filter by using whatmen filter paper.
5. The ratio of soil: extracting reagent must be 1:20.
6. Transfer the 5 ml aliquot to 25 ml volumetric flask
7. Add 1 ml of 2.5 M H_2SO_4 to lower the pH 5.0 (as blue colour develops at this pH therefore it is very important step)
8. Add 4 ml of reagent B
9. Make the volume 25 ml by using distilled water

10. Prepare blank as above using 10 ml extracting solution in place of soil extract
11. Standard curve: Prepare series of standard solution as per the intensity of colour in samples. Prepare 0, 0.08,0.16,0.24 and 0.32 mg L^{-1} or ppm solution, by measuring 0, 1, 2,3, 4 ml of standard 2 mg L^{-1} P solution in 25 ml volumetric flask. Add 5 ml extracting solution (Sodium bi carbonate) and 0.5 to 1 ml 2.5 M H_2SO_4. Add 4 ml reagent B. Prepare the final volume with the help of distilled water. After 10 minutes (solution should be bluish purple), read P concentration at 882 nm after calibrating spectrophotometer with standards. The colour is stable for 24 hours and is not affected by the colour in the filtrate due to organic P.

Calculation:

$$Avilable\ P\ (kg/ha) = \frac{R\ \times Volume\ of\ extract}{Volume\ of\ aliquot} \times \frac{2.24 \times 10^6}{Weight\ of\ soil\ \times 10^6}$$

Where R= mg P in the aliquot (obtained from standard curve)

Always prepare fresh standard solution for every batch of analysis.

Work Sheet

Date:…….

Instructor Signature

Exercise 5

Objective: Estimation of available Potassium (K) from soils

The neutral ammonium acetate is being most commonly used as an extractant for the exchange the available potassium present in soil. This K is present in exchangeable site and available for the plant. The determination procedure is two step process in first step extract the K from soil and second stem determine concentration with the help of Flame photometer.

Flame photometer: is based on the emission principle. The liquid sample is subjected to burn on flame and the atom absorbs energy and excite from lower to higher state. When they returned back the lose energy and detected by detector. This energy is proportional to the K present in solution.

Equipments/Apparatus:

- ✓ Flame photometer
- ✓ Electronic balance
- ✓ Mechanical Shaker
- ✓ Funnel
- ✓ Filter paper

Reagents:

- ✓ 1.0 N $NH_4C_2H_3O_2$ solution: Weigh exactly 77 g of ammonium acetate and dissolve in 1 litre of water. Adjust the pH 7.0, if required either add ammonium hydroxide or acetic acid as per the need to bring pH 7.0.
- ✓ Standard potassium stock solution: weigh 1.908 g of pure KCl and dissolve it in 1 litre of distilled water. It will give 1000 ppm standard K solution. Further pre 100 ppm solution from 1000 ppm solution by using N1 x V1=N2xV2 formula.
- ✓ Working potassium standard solutions: Take 0, 5, 10, 15 and 20 ml of the 100 ppm K stock solution and dilute each volume separately to 100 ml with the molar ammonium acetate solution. These solutions contain 0, 5, 10, 15 and 20 ppm, respectively.

Procedure:

- ✓ Weigh 5 g soil in 150 ml of Erlenmeyer flask
- ✓ Add 25 ml of 1 N ammonium acetate and shake it for 5 minutes, the ratio of soil: extracting solution should be always 1:5.
- ✓ Filter immediate after shaking of solution
- ✓ Prepare blank without soil simultaneously
- ✓ Calibrate the flame photometer by the standard and after that determine the concentration of K in aliquot by flame photometer

Calculation:

$$Avilable\ K\ (kg/ha) = \frac{R\ \times Volume\ of\ extract\ \times\ 2.24 \times 10^6}{Weight\ of\ soil\ \times 10^6}$$

R concentration read from standard curve

Work Sheet

Date:…….

Instructor Signature

Exercise 6

Objective: To determine the available Sulphur (Turbidimetric method) in soil

The most common methods of sulphur estimation in soil are 0.15 % $CaCl_2$ extractable S, mono-calcium phosphate solution, morgans extract and Olsen' extract soluble S. Among these methods 0.15 % $CaCl_2$ extractable S is widely used for the sulphur determination as it has advantage of rapid and relatively easy determination procedure as compared to others.

Similar two other the it also two step process first extract the sulphur from the soil and second develop the turbidity and intensity of turbidity is measured by spectrophotometer.

Apparatus/Glassware

- ✓ Spectrophotometer
- ✓ Mechanical shaker
- ✓ Electronic balance

Glassware

- ✓ measuring cylinder
- ✓ Conical flask
- ✓ pipettes
- ✓ Filter paper
- ✓ Volumetric flask
- ✓ Funnel

Reagents:

- 0.15 % $CaCl_2$ Solution: Weigh 1.5 gram of $CaCl_2$ and dissolve it in distilled water preferably double distilled water and make the volume 1 litre.
- 0.25 % Gum acacia solution: Weigh 0.25 gram of Gum acacia and dissolve it in 100 ml of distilled water preferably double distilled water
- Barium chloride: ($BaCl_2$) Pass AR-grade $BaCl_2$ salt through a 1-mm sieve and store for use.
- Standard stock solution (1000 mg S/litre): Dissolve 5.434 g of oven-dried AR-grade potassium sulphate in 1 litre of water.
- Standard working solution (100 mg S/litre): Measure exactly 10 ml of the stock solution and dilute to 100 ml.

Procedure:

- ✓ Weigh 5 g of soil sample in a 100-ml conical flask.
- ✓ Add 25 ml of the 015 % $CaCl_2$ extracting solution and shake for 30 minutes hour mechanical shaker. Filter through No. 42 filter paper.
- ✓ Put 10 ml of the clear filtrate in a 25-ml volumetric flask.
- ✓ Add 1 g of barium chloride crystal pass through sieve and shake manually for 1 minute.

- ✓ Add 1 ml of gum acacia-acetic acid solution. Make the volume up to 25 ml and shake it 3 to 4 times.
- ✓ Measure the turbidity intensity at 440 nm (blue filter) on spectrophotometer after 30 minutes.
- ✓ Simultaneously run a blank side solution (fallow all the steps except soil).

Preparation of standard curve:

- Put 0, 1.0, 2.0, 3.0, and 5.0 ml of the working standard solution (100 mg S/litre) into a series of 25-ml volumetric flasks in order to obtain 4.0, 8.0, 12.0 and 20 ppm of S.
- Proceed to develop turbidity as described above for sample aliquots.
- Read the turbidity intensity and prepare the curve by plotting readings against S concentrations.

Calculations

$$\text{Available sulphur } (SO_4 - \text{S}) \text{in soil } \left(\frac{\text{mg}}{\text{kg}}\right) = Reading\ of\ S\ from\ standard\ curve\ (ppm)\ x\ df$$

Where:

Df: dilution factor
first dilution weight of soil 5 g and extracting agent 25 ml and it will be 25/5.
Second dilution 10 ml aliquot from extract and final value prepared 25 ml and it will be 25/10.

Work Sheet

Date:…….

Instructor Signature

Exercise 7

Objective: Determination of available micronutrient cations

Ethylene diamine tetra acetic acid (EDTA) titration method and Diethylene triamine penta acetic acid DTPA extractable followed by determination from AAS are commonly used for estimation of available micronutrient in soil. The ETDA method is very time consuming and tedious procedure. DTPA extractable method is simple and require less time, hence commonly used for determination of available micronutrient cations of the soil. Diethylenetriamine penta acetic acid (DTPA) is proposed by Lindsay and Norvell, 1978. DTPA is a chelating agent that combines with free metal ions in the solution to form soluble complexes of elements.

The TEA (Triethonolamine) is used as buffer because it burns neatly during estimation of micronutrients.

DTPA Regent:

DTPA 0.005M, 0.01M $CaCl_2.2H_2O$ and 0.1M TEA extractant:

- ✓ Weigh 1.967 g of DTPA and measure 13.3 ml of TEA in measuring cylinder, dissolve both in 400 ml of distilled water.
- ✓ Put 1.47 g of $CaCl_2.2H_2O$ in distilled water and dissolve it
- ✓ Put both the above solution in 1 litre volumetric flask and shake it thoroughly.
- ✓ Adjust the pH to 7.3 by using 1M HCl or NaOH before making the volume.

Standards

- Zn: 1 g of pure zinc dissolved in 50 ml of HNO_3 (1:1) and diluted to 1000 ml gives 1000 ppm Zn stock solution. Further prepare 0, 0.5,1.0 and 1.5 ppm Zn working solution by diluting the stock solution.
- Fe: 1 g of pure iron wire is dissolved in 50 ml of HNO_3 (1:1) and diluted to 1 000 ml to obtain 1 mg/ml of standard Fe. Further prepare 0, 2.5, 5.0, 7.5 and 10 ppm Fe working solution by diluting the stock solution.
- Mn: 1 g each of pure Mn in 50 ml of HNO_3 (1:1) and making the volume up to 1 000 ml. It will give 1000 ppm Mn solutions. Further prepare 0, 2.5, 5.0, 7.5 and 10 ppm Mn working solution by diluting the stock solution.
- Cu: 1 g each of pure Cu in 50 ml of HNO_3 (1:1) and making the volume up to 1 000 ml. It will give 1000 ppm Cu solutions. Further prepare 0, 0.5,1.0, 1.5 and 2.0 ppm Zn working solution by diluting the stock solution.

Procedure:

- ✓ Take 10 g of soil in 100 ml conical flask,

- ✓ Add 20 ml DTPA reagent
- ✓ Put flask on mechanical shaker for two hours.
- ✓ Filter the solution by using d (whatman No. 40.42) and
- ✓ Determine the concentration of Zn, Cu, Fe & Mn the help of atomic absorption photometer.

Calculation:

$$Avilable\ micronutrinet\ (Zn, Cu, Fe\ \&\ Mn\ (mg/kg) = R\ \times d.f.$$

Where, R concentration read from standard curve/or equipment directly provide the reading ; d.f.: dilution factor (it will be 2)

Work Sheet

Date:…….

Instructor Signature

Exercise 8

Determination of soil Exchangeable Sodium Percentage

Determination of soil ESP

Exchangeable sodium in soil varies from a small portion to a large portion of the exchange capacity depending on the sodicity of the soil. Likewise water soluble sodium varies from trace amount to a large amount depending on the salinity level of the soil. The degree of sodicity of a soil is normally assessed by exchangeable sodium percentage or ESP which is defined as follows:

$$ESP = (Exch.\ Na/CEC) \times 100$$

Both exch. Na and CEC i.e. cation exchange capacity are expressed in c mol (+)/ kg of soil or meq/ 100 gm of soil. The salinity level of soil is assessed by the electrical conductivity of the saturation extract (ECe) of the soil, which is expressed in dS/ m or m. mhos/ cm. A sodic soil contains an exchangeable sodium (ESP >15), a saline soil contains an excess amount of soluble salts (ECe>4 dS/ m) and a saline sodic soil contains both salt and sodium in excess amount (ESP > 15, ECe> 4 dS/ m).

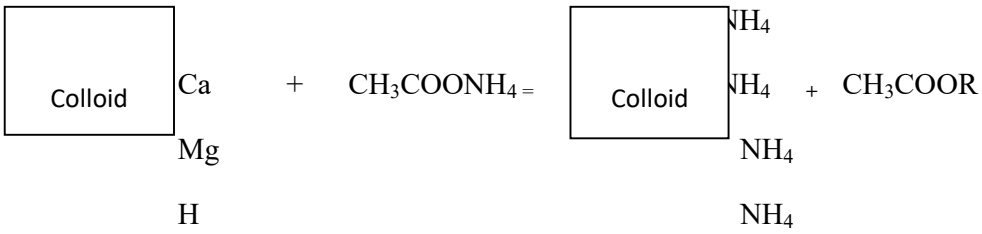
Methods of determination:

Exchangeable plus water-soluble sodium may be extracted by number of extractants like l N NH_4OAc, $BaCl_2$ buffered at a of 7.0 to 8.2 and Na may be determined by Flame photometric determination, Atomic absorption determination and Gravimetric determination. Among these, extraction by NH_4OAc and determination by flame photometer is commonly used procedure for determination of exchangeable plus soluble sodium. Water soluble sodium is determined separately in saturation extract by flame photometer. The exchangeable sodium is then determined by subtracting the results of water-soluble Na from the results obtained for exchangeable plus water soluble sodium.

Principle:

In this method water soluble and the exchangeable sodium of the soil is extracted by shaking the soil with neutral normal ammonium acetate in 1:5 soil: solution ratio. During equilibrium, ammonium ions exchange with the exchangeable sodium ions along with other exchangeable cations. After equilibrium the suspension is filtered through Whatmanno.l filter paper. The sodium content of the filtrate is determined with a flame photometer.

The reaction is stated below:



Where, R = Exchangeable cations like Na, Ca, Mg, Na etc.

Since ammonium acetate extracts both exchangeable and soluble sodium, water soluble sodium of saturation extract is determined by flame photometer and exchangeable sodium content is determined by subtracting the amount found in water soluble sodium from the amount found in NH_4OAc extracted sodium.

Materials Required:

Apparatus and Glassware

Balance, flame photometer, pH meter. Conical flask, funnels, beakers, pipette, volumetric flask, Whatman no.l filter paper, spatula, Buchner, suction flask, vacuum pump.

Reagents:

(1) Normal neutral ammonium acetate solution: 77.09 gm of ammonium acetate is dissolved in water and the volume is made up to one liter. Adjust the pH 7.0 with the help of pH meter by using chemical ammonia solution or acetic acid.

(2) Standard sodium solution:

(a) Stock solution: 2.542 gm of pure AR grade NaCl (dried at 110˚C for 1 hour) is dissolved in distilled water and the volume is made up to one liter. This solution contains 1000 ppm Na. It is treated as stock solution of Na.

(b) 100 ppm standard Na: It is preparedby diluting 100 ml of 1000 ppm stock solution to one liter with the extracting solution (neutral normal ammonium acetate).

Preparation of standard curve: 0, 5,10,15,20 and 25 ml of 100 ppm solution is taken into 100 ml volumetric flasks and the volume is brought up to the mark with extracting solution (i.e. neutral normal ammonium acetate). The solutions contain 0, 5,10,15,20 and 25 ppm Na respectively.

After inserting the Na filter and regulating the appropriate gas air pressure, the flame photometer is set up at 0 for blank (NH_4OAc) and at 100 for 25 ppm of Na solution alternatively till both values are obtained without any adjustment, then working standard solutions are atomized intermittently and the meter readings are recorded. These meter readings are plotted against the sodium contents the points are connected with a straight line.

Procedure:

5 gm of soil sample is taken in a 150 ml conical flask and 25 ml of the ammonium acetate extractant is added to it. The contents of the flask are shaken for five minutes and the suspension is filtered through Whatman no.1 filter paper. The filtrate is atomized in the flame photometer in which 0 and 100 have been set with blank and 40 ppm Na solutions respectively and the reading is noted. The sodium (Na) content of the filtrate is determined from the standard curve and the Na content is calculated. This is exchangeable plus soluble sodium.

The soluble sodium is determined from saturation extract. A saturated soil paste is prepared using 100 to 150 gm soil by adding water to the soil and stirring with spatula.

The saturated soil paste is transferred on a Whatman No. 42 filter paper placed in a Buchner funnel fitted on a suction flask which in turn is connected with a vacuum pump. The vacuum extraction is terminated when air begging to pass through filter paper. If the filtrate is turbid, it is refiltered. The clear extract is atomized in the Flame photometer to determine soluble Na.

Observation and calculations:

A. For soluble & exchangeable sodium:

a.	Weight of the soil	:	5 gm
b.	Volume of the neutral $N NH_4OAc$	:	25 ml
c.	Let the readings of the flame photometer for the test solution	:	-X-…… ppm
d.	Let the concentration at X readingsread from the standard curve	:	-Y-…… ppm
e.	Dilution factor	:	25/5= 5 times
f.	Soluble plus exchangeable Na in soil	:	D X 5=……..ppm
g.	Soluble plus exchangeable Na for 15 cm depth	:	=f X 2.24=…….kg/ha

B. For soluble sodium:

h.	Weight of the soil	:	150 gm
	Volume of water	:	……..ml
i.	Let the reading of the flame Photometer for the test solution	:	=X=……..ppm
j.	Let the concentration at X reading read from standard curve	:	=Y=……..ppm
k.	Dilution factor = *i*/h	:	……..times
l.	Soluble sodium in soil = k × l	:	……ppm
m.	Soluble Na for 15 cm soil Depth = m × 2.24	:	…….kg/ha
n.	Exchangeable sodium for 15 cm Soil depth = g – n	:	……kg/ha
o.	Exchangeable sodium in mg/100 gm soil = (o × 10^6)/(2.24 × 10^6 10)= o/(2.24×10)	:	…….mg/100g Soil
p.	Exchangeable sodium of soil Inmeq/100 gm soil = p/23		……..meq/100g Soil
q.	If CEC of soil is z meq/100 gm soil Then ESP = (q/z) × 100		

Work Sheet

Date:…….

Instructor Signature

Calculation of Sodium Absorption Ratio:

It is a secondary parameter, it can be derived from following equation after the determination of sodium (determination procedure same as exchangeable K), calcium and Magnesium.

The SAR may be calculated as follows:

$$SAR = \frac{Na^{+}}{\sqrt{0.5\ (Ca^{2+}) + (Mg^{2+})}}$$

Where, $[Na^{+}]$, $[Ca^{2+}]$ and $[Mg^{2+}]$ are the concentrations in *c*mol (+)/ kg of the sodium, calcium and magnesium ions in the soil solution.

4. Analysis of Plant Samples

Determination of N, P, K, Ca, Mg, S and micronutrient in plant samples

Exercise 1: Determination of total nitrogen in plant samples

Total N: Total nitrogen is determined by modified kjaldhal method in which the organic nitrogen is changed into ammonium by acid hydrolysis with H_2SO_4, together with reagents to raise temperature and to hasten the rate of decomposition as catalysts. For distillation of ammonium, the solution is made alkaline. The distilled ammonium is received in boric acid and is titrated with a standard acid.

Reagents:

1. 4 % Boric acid (H_3BO_3) – indicator solution: Weigh 40 gram of H_3BO_3 in a 1 litre volumetric flask add distilled water in it and swirl the flask until the $H_3BO_3$is dissolved. Add 20 ml of mixed indicator solution (dissolving 0.099 g of bromcresol green and 0.066 g of methyl red in 100 ml of ethanol). Add 0.1 NaOH continually until the solution assumes a reddish purple tint (pH 5.0) and make solution to 1 litre by addition of water.
2. Salt mixture: The ratio of salt mixture (100:10:1). Hence take dry 50 g of potassium sulphate , 10 g of CuSO4 and 1 g of selenium and mix all the chemical thoroughly.
3. NaOH: 0.8 kg NaOH dissolve in a water beaker containing approx. 1 litre of water cool it and transfer the solution in 2 litre volumetric flask and make the solution 2 litre by addition of distilled water.
4. H_2SO_4 concentrated
5. H_2SO_4 or HCl, 0.05 N

Apparatus

1. Micro kjaldhal digestion unit
2. Micro kjaldhal glass tubes, 800 ml
3. Micro kjaldhal distillation unit

Procedure:

A. Digestion
   - ✓ Weigh 0.2 g tissue sample or plant (grain or straw) thoroughly grinded sample in a kjaldhal tube
   - ✓ Add 1 g of salt mixture
   - ✓ Add 5 ml of concentrated H_2SO_4
   - ✓ Digest the sample. Regulate heating so that the H_2SO_4 condense about one third of the way up to neck of glass tube. The digestion is stopped when clear light green colour solution appears in glass tube.
   - ✓ Allow the flask to cool
   - ✓ Add 100 ml of water (slowly and with shaking). Then cool the tube and transfer it distillation assembly.

B. Distillation
   - ✓ To determine the ammonium N liberated by digestion, place a 125 ml Erlenmeyer flask containing 20 ml of H_3BO_3 indicator solution under the condenser of the

distillation apparatus so that the end of the condenser is below the surface of the H_3BO_3.

- ✓ Pore 25 ml of 40 % NaOH into glass tube (containing digest plant material) and put it immediately into distillation assembly.
- ✓ Switch on the assembly and set temperature and distillation time or watch flask containing boric acid when it volume reached 150 ml take out the flask.
- ✓ Determine the ammonium-N in the distillate by titration with standard 0.05 N sulfuric acid. The colour change at the end point is from green to grey.
- ✓ A blank must be run and titration carried to the same end point.

Calculation:

Total Nitrogen

$$\% \text{ N} = \frac{(T - B) \times N \times 0.014}{S} \times 100$$

Where, T= sample titration, ml of standard acid

B: blank titration, ml of standard acid

N= normality of standard acid

S= oven dry weight of sample in g

Work Sheet

Date:…….

Instructor Signature

Exercise 2

Wet digestion for estimation of P, K, Ca, Mg, S, and micronutrients cations

The di acid (HNO_3 and $HClO_4$ (9:4)) or tri acid (HNO_3, H_2SO_4 and $HClO_4$) in the ratio of 9:4:1 are commonly used for sample digestion.

Digestion Procedure

- ✓ Take one gram of fine grinded plant samples in a 100 ml conical flask.
- ✓ Add 10 ml of di acid or tri acid in a flask and gently swirl the flask
- ✓ Place the flask on hot plate and heat initially 60-70 °C and then raise the temperature 150 °C.
- ✓ Initially brownish red fumes will liberate from the samples, and thereafter white fumes will release in constant rate.
- ✓ Heat the flask until volume reduces 2-3 ml and become colourless.
- ✓ Cool the flask and add 20-30 ml distilled water
- ✓ Filter it and by several washing of flask
- ✓ Transfer the solution to 100 ml volumetric flask and make final volume. This solution will be used for P,K,S & micronutrient estimation.

  Note: All the acid work must be carried out in fume hood or just below exhaust fan with all the safety measure

Exercise 3

Estimation of phosphorus concentration in plants

Vanadium phospho-molybdate method is used for the determination of total phosphorus in plant samples.

The digestion process converts phosphorus present in plant samples to orthophosphates. The digested sample is used for P estimation.

$$Orthophosphate + \text{molybdate and vanadate (Solution)} \rightarrow \text{yellow} - \text{coloured vanado} - \text{molybdo} - \text{phosphoric heteropoly complex (Formed)}$$

The intensity of yellow colour read on the visible range at 660 nm on the spectrophotometer. The intensity of colour is directly proportional of the P concentration in plant.

Apparatus

- Spectrophotometer
- Digestion block
- Weighing balance
- Hot plate
- Spectrophotometer;

Reagents:

Ammonium molybdate – ammonium vanadate in HNO_3 (vanado-molybdate): Dissolve 22.5 g of $(NH_4)_6MO_7O_2.4H_2O$ in 400 ml of distilled water. Dissolve 1.25 g of ammonium vanadate in 300 ml of boiling distilled water. Add the vanadate solution to the molybdate solution and cool to room temperature. Add 250 ml of concentrated HNO_3 and dilute to 1 litre.

Standard phosphate solution: Dissolve 0.4387 g of analytical-grade KH_2PO_4 and dilute to 1 litre. This solution contains 100 ppm P stock solution.

Procedure:

1. Weigh 1 g of plant samples in 150 ml conical flask
2. Add 10 ml of di acid (HNO_3 & $HClO_4$ in 9:4 ratio).
3. Put the volumetric flask on hot plate and digest the plant samples at 100 °C.
4. When 2-3 ml of residue remain in flask and fumes will stop coming out from the flask.
5. Transfer the solution in 100 ml volumetric flask by repeated washing of conical flask.
6. Pipette the 5 ml of digested aliquot into 50 ml of volumetric flask
7. Add 10 ml of 10 ml of vanadomolybdate reagent.
8. Make the volume of 50 ml and shake the flask.
9. Leave the flask for 30 minutes in order to colour development.
10. Run blank simultaneously.
11. Prepare standard solution from the 100 ppm standard stock solution, put 0, 0.5, 1.0, 1.5, 2.0 & 2.5 in 50 ml volumetric flask and add 10 ml of vanadomolybdate reagent to each flask and make up the volume.

12. Keep the flask for 30 minutes in order to colour development.
13. Determine the absorbance/concentration from spectrophotometer at 420 nm.
14. Prepare standard curve for the determination of phosphorus concentration.

Calculation:

$$\% P = \mathrm{C} \times \mathrm{df} \times 100$$

Where, C: concentration of P (ppm) as read from the standard curve;

df = dilution factor will 1000 (100 × 10 = 1 000), as calculated below:

• 1 g of plant sample in 100 ml of volumetric flask (100/1= 100 times);
• 5 ml of digested aliquot to 50 ml volumetric flask (50/5= 10 times).

Exercise 4
Estimation of potassium concentration in plants

The plant samples were digested with the di acid on hot plate as earlier described in section of wet digestion. Thereafter, flame photometer used for the determination of K concentration in digested solution.

Apparatus:

- Flame photometer
- Weighing Balance

Regent only for the Standard:

- ✓ Standard 1000 ppm potassium solution: Weighing accurately 1.908 g of pure KCl and dissolve it in 1 litre volumetric flask containing distilled water and make the final volume shake the solution. It will be 1000 ppm K stock solution. Further make 100 ppm K solution for the preparation of series of standards.
- ✓ Take 0, 10, 20, 30 and 40 ml of the 100 ppm stock solution and dilute each volume separately to 100 ml with the molar ammonium acetate solution. These solutions contain 0, 10, 20, 30 and 40 ppm, respectively.

Procedure:

- ✓ Calibrate the flame photometer by adopting the user manual guideline.
- ✓ Take the reading of digested solution on flame photometer.

Calculation:

$$\% K = \mathrm{C} \times \mathrm{df} \times 100$$

Where, C: concentration of K (ppm) as read from the standard curve or directly given by the instrument.
df = dilution factor will 100 times as 1 g of plant samples in 100 ml of volumetric flask.

Work Sheet

Date:…….

Instructor Signature

Exercise 5
Determination of Sulphur concentration in plant samples.

Only di acid is used of the digestion of plant samples for the determination of the sulphur. A H2SO4 contains S. After , the digestion turbidity developed in samples and measure the intensity of turbidity on specctrophotmeter.

Apparatus:

- Spectrophotometer;
- Volumetric flasks.
- Hot plate for digestion

Regents

- 0.25 % Gum acacia solution: Weigh 0.25 gram of Gum acacia and dissolve it in 100 ml of distilled water preferably double distilled water
- Barium chloride: ($BaCl_2$) Pass AR-grade $BaCl_2$ salt through a 1-mm sieve and store for use.
- Standard stock solution (1000 mg S/litre): Dissolve 5.434 g of oven-dried AR-grade potassium sulphate in 1 litre of water.
- Standard working solution (100 mg S/litre): Measure exactly 10 ml of the stock solution and dilute to 100 ml.

Procedure:

- Digest 1 g of plant sample in di-acid (as described in Wet digestion for estimation of P, K, Ca, Mg, S, and micronutrients cations section) and make the volume up to 100 ml.

1. Take10 ml of the aliquot in of 100-ml volumetric flask.
2. Add 1 g of sieved $BaCl_2$ and shake for 1 minute.
3. Add 1 ml of gum acacia acetic – acid solution, make the volume up to the mark and shake for 1 minute.
4. Run a blank sample.
5. Keep the solution for 30 minutes after that measure the turbidity form spectrophotometer at 440 nm.
6. Prepare series of concentration from 100 ppm S working solution.
7. Read the S content in the sample from the standard curve against the similar absorbance as noted for the sample.

The relevant calculation is:
S content (%) in 1 g of sample = C (concentration from standard curve) × dilution factor x 100

Work Sheet

Date:…….

Instructor Signature

Exercise 6
Estimation of micronutrient cations concentration in plants

The digestion of plant samples for micronutrient determination will be remaining same as described in wet digestion section. Micronutrient cations (Zn, Fe,Mn & Cu)) can be determined by the help of Atomic Absorption Spectrophometer (AAS).

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Standards

- Zn: 1 g of pure zinc dissolved in 50 ml of HNO_3 (1:1) and diluted to 1000 ml gives 1000 ppm Zn stock solution. Further prepare 0, 0.5,1.0 and 1.5 ppm Zn working solution by diluting the stock solution.
- Fe: 1 g of pure iron wire is dissolved in 50 ml of HNO_3 (1:1) and diluted to 1 000 ml to obtain 1 mg/ml of standard Fe. Further prepare 0, 2.5, 5.0, 7.5 and 10 ppm Fe working solution by diluting the stock solution.
- Mn: 1 g each of pure Mn in 50 ml of HNO_3 (1:1) and making the volume up to 1 000 ml. It will give 1000 ppm Mn solutions. Further prepare 0, 2.5, 5.0, 7.5 and 10 ppm Mn working solution by diluting the stock solution.
- Cu: 1 g each of pure Cu in 50 ml of HNO_3 (1:1) and making the volume up to 1 000 ml. It will give 1000 ppm Cu solutions. Further prepare 0, 0.5,1.0, 1.5 and 2.0 ppm Zn working solution by diluting the stock solution.

The multi element standards are also available in the market. That can also used as a standard.

Procedure:

- Calibrate the instrument as per the user manual guideline.
- Prepare the serial dilution of each micronutrient cations as described above.
- Concentration of each metal determine from the digested solution by using AAS.

The relevant example calculation, which is valid for all micronutrients, is:
Micronutrient content (mg/kg) in 1 g of sample = $C \times df$
where:

- ✓ C = concentration of micronutrient (mg/kg) as read from the standard curve;
- ✓ df = dilution factor, which is 100 × 10 = 1 000, as calculated below:
  1 g of sample made to 100 ml (100 times);
  10 ml of sample solution made to 100 ml (10 times).

Exercise 7
Determination of Calcium & Magnesium concentration in plant samples

Titration of calcium plus magnesium with EDTA in presence of Erichromme black T indicator.

Reagents:

1. Erichromme black T indicator: Dissolve 0.2 g of erichromme black T indicator in 50 ml of methanol containing 2 g of hydroxylamine hydrochloride. Store in dark.
2. Buffer solution: Dissolve 67.5 g of ammonium chloride (NH_4Cl) in about 200 ml of water and add 570 ml of ammonium hydroxide (NH_4OH). Dilute the solution with distilled water and adjust to pH 10 by adding NaOH or HCl. Make the final volume of 1 litre after the pH adjustment.
3. Potassium cyanide solution 2 %: Dissolve 2 g of potassium cyanide in 100 ml of distilled water

Procedure

- Pipette a 5 ml aliquot of the plant extract, into a 125 ml Erlenmeyer flask and make to about 50 ml with distilled water.
- Add 5 ml of the buffer solution to make the solution to pH 10.
- Add 1 ml of potassium cyanide
- Add 8 drops of eriochrome black T indicator and titrate with EDTA solution to the blue end point.

Calculation:
For 5 ml aliquot and 1.0 g sample:
ml for Mg = (ml for Ca + Mg)- (ml for Ca)

$$\% \, Mg = ml \; for \; Mg \; \times 0.025 \times \frac{24.32 \; \times 50 \; \times 100}{2 \; \times 5 \; \times 1000}$$

% Mg= ml for Mg x 0.304

Where 24.32/2= equivalent weight of Mg
0.025: N of EDTA
50/5: ml ash extract/ml aliquot

Calcium :EDTA method

Apparatus: Magnetic stirrer

Reagents:

1. Murexide indicator: mix 40 g of powdered potassium sulfate and 0.2 g of murexide powder indicator

2. Potassium hydroxide, 10 %. Dissolve 50 g of potassium hydroxide in distilled water and make to 500 ml
3. EDTA solution, 0.025N. Dissolve 4.650 g of the analytical grade disodium salt of EDTA in distilled water and dilute to 1 L in a volumetric flask

Procedure:

1. Pipette a 5 ml aliquot of the plant tissue ash extract into a 125 ml Erlenmeyer flask
2. Add distilled water to bring the volume to about 50 ml
3. Adjust the pH of 12 with potassium hydroxide, 2 ml should be enough
4. Add 50 mg of murexide powder and mix.
5. Titrate with EDTA to the violet endpoint.

Calculation:

For 5 ml aliquot and 1.00 g sample:

$$\% Ca = ml\ for\ Ca\ \times 0.025 \times \frac{40\ \times 50\ \times 100}{2\ \times 5\ \times 1000}$$

% Ca= ml for Ca x 0.5

Where 40/2= equivalent weight of Mg
0.025: N of EDTA
50/5: ml ash extract/ml aliquot

Work Sheet

Date:…….

Instructor Signature

5.Analysis of irrigation water

Criteria of irrigation water quality analysis

Guideline for interpretation of irrigation water quality established by the World Food and Agriculture Organization (FAO)

S.N.	Parameters	Suitable	Moderate Suitable	Not Suitable
1	EC (mSm-1)	25.0	25-75	>75.0
2	SAR	<10.0	10-18	>18.0
3	Adjusted SAR	3.0	3.0-9.0	>9.0
4	RSC (me/lt)	<1.25	1.25-2.50	>2.50
5	HCO3 (me/lt)	<1.50	1.5-8.5	>8.5
6	NO3-N (me/lt)	<5.0	5.0-30.0	>30.0
7	Boron (mg/lt)	<0.75	0.75-2.0	>2.0
8	Chloride (me/lt)	<4.0	4.0-10.0	>10.0
9	Fluoride (me/lt)	<1.0	1.0-15.0	>15.0
10	pH	6.5-8.4	0-5	>9.5

Source: FAO (1994)

Exercise 1

Determination of pH of irrigation water

The pH is a indicator of the soil reaction. It is expressed as the negative logarithm of the hydrogen ion (H^+) activity in the soil solution. It is the represent the neutral, acidic, and sodic soils on the basis of H^+ ions in soil solution. It is an indicator of soil fertility and well as quality of irrigation water. Since quality of irrigation water plays a major role in the development/reclamation of problematic soil. The determination process of pH is very simple and quick given below.

Apparatus/Glassware

• pH meter with a range of 0-14 pH
• Beaker

Reagent

• Buffer solutions of pH 4, 7 and 9.2

Procedure

1. Calibrate the pH meter before start the analysis by following manufacture guideline.
2. The pH meter is calibrated by using either 4.0 and 7.0 pH buffer solution or 7.0 and 9.2 pH buffer solution as per the pH of your soils.
3. After calibration of pH meter start the analysis
4. Take about 50 ml water sample in 100 ml beaker.
5. Immerse the electrodes in the water sample
6. Take the reading for pH.
7. Wash the electrodes with distilled water and dry them by soaking adhere water by tissue paper after each reading.

Result: pH of Soil.....

Exercise 2

Determination of Electrical conductivity of irrigation water

Electrical conductivity (EC) represents the total salt present in irrigation water. EC is considered as the most important parameter for determining soluble salt contain in irrigation water. Ideal value of EC in irrigation water should be less than 0.75 dSm^{-1} and widely used between 0.75-2.25 dSm^{-1} (Richards, 1954).

Apparatus

• EC meter

• Beakers (100 ml)

Reagents

Standard 0.01 M KCl solution: Dissolve 0.7465 g of KCl in 100 ml of double distilled water in a volumetric flask. The EC of this solution will be 1.41 dSm^{-1} at 25°C

Procedure

1. Take about 50 ml water sample in 100ml beaker
2. Calibrate the Conductivity Meter by following manufacturer guideline against the standard 0.01 M KCl solution and determine the cell constant.
3. Immerse the conductivity bridge or electrode in water sample.
4. Take the reading for EC.
5. Wash the conductivity bridge with distilled water and dry them by soaking the adhere water by tissue paper after each reading.

Work Sheet

Date:…….

Instructor Signature

Exercise 3

Determination of Calcium and Magnesium

The most common method for estimation of Ca+Mg is by versenate (EDTA) titration proposed by Cheng and Bray 1951. In case of soils, generally exchangeable calcium and magnesium are estimated. Total plant Ca is determined by acid digestion. Once extracted, either as exchangeable, soluble or total, further estimation by EDTA method is same.

Apparatus:

- Porcelain dish
- Volumetric flasks
- Burette
- Pipette
- Glass rod

Reagents

- Standard EDTA (0.01N) solution: Dissolve 2 g disodium salt of ethylene diamine tetra acetic acid (EDTA) in distilled water and dilute to 1 litre. Standardise it against standard calcium solution
- Ammonium chloride-ammonium hydroxide buffer (pH 10): Take 67.50 g ammonium chloride dissolved in 570 ml of concentrated ammonium hydroxide and adjust the pH 10.0 before making final volume with distilled water.
- Eriochrome black T (EBT) indicator: Dissolve 0.2g of EBT in 50 ml methanol.
- Hydroxylamine hydrochloride: Dissolve 5 g of hydroxylamine hydrochloride in 100 ml with distilled water.
- Calcon indicator/Muroxide: Dissolve 0.2 g calcon in 50 ml ethanol/methanol
- Sodium hydroxide solution (10%): Dissolve 10g NaOH in distilled water and make up volume to 100 ml.

Procedure

Titration for Ca and Mg

1. Take 5 ml of the water sample in a porcelain dish.
2. Dilute by addition of distilled water upto 25 ml
3. Add 1 ml. of ammonium chloride-hydroxide buffer
4. 3 to 4 drops of eriochrome black T (EBT) indicator.
5. Titrated with the standard versenate EDTA solution.

The change in colour from wine red to bright blue or bluish green will indicate the end point (compilation of the reaction).

Titration for Ca:

1. Take 5 ml of the water sample in a porcelain dish.
2. Dilute to about 25 ml with distilled water.
3. Add 1ml of 10% NaOH solution to raise the pH of the solution to 12.
4. Add 5 drops of calcon/muroxide indicator

5. Titrate with standard EDTA solution until the colour changes from pink to blue.
6. Run one blank solution simultaneously by adopting all the steps except water sample

Calculation

From the volume of 0.01N EDTA (standardized against 0.01N CaCl2) solution required for titration, the concentration of Ca+Mg is directly obtained in me/litre as follows:

$$Ca + Mg\ (g/litre) = \frac{(\text{ml versenate(EDTA)usedin sample} - \text{ml ETDA used in blank}) \times \text{normality of EDTA x1000}}{ml\ aliquot\ taken}$$

$$Ca + Mg\ (g/litre) = \frac{\text{Ca Mg in me/litre} \times \text{equivalent wt.}}{1000}$$

$$Ca + Mg\ (g/litre) = \frac{\text{Ca Mg in me/litre} \times 32.196}{1000}$$

For Ca –

$$Ca\ (me/litre) = \frac{\text{ml versenate(EDTA) used} \times \text{normality of EDTA x1000}}{ml\ aliquot\ taken}$$

For Mg –

Mg (me/litre) = [(Ca+Mg) – Ca] (in me/litre)

Work Sheet

Date:…….

Instructor Signature

Exercise 4

Determination of carbonates (CO_3) and bicarbonates (HCO_3) in irrigation water

The estimation is based on simple acidimetric titration using different indicators which work in alkaline pH range (above 8.2) or in acidic pH range (below 6.0).

Apparatus

- ❖ Porcelain dish
- ❖ Burette
- ❖ Pipette
- ❖ Glass rod

Reagents

- Phenolphthalein indicator: 0.25% solution in 60% ethyl alcohol: Take 0.25 g of phenolphthalein and dissolve it into 100 ml of 60 % ethyl alcohol.
- Methyl orange indicator: 0.5% solution in 95% alcohol (weigh 0.5 g of methyl orange powder and dissolve it into 100 ml of 95 % alcohol.
- Standard sulphuric acid (0.01M): Take 1.4 mL of concentrated H_2SO_4 with the help of automatic pipette and dilute to one litre distilled water.

Procedure

1. Take 5 ml of the water sample in a porcelain dish.
2. Dilute with distilled water to about 25 ml.
3. Add few drops (2 to 4) of phenolphthalein indicator, if pink colour appears then titrate the solution with 0.01M sulphuric acid until the colour just disappears. This burette reading (volume consumed in neutralization) is designated as Y. (The process indicates conversion of alkali carbonate to bicarbonate and refers as half neutralization stage).
4. Add 1 to 2 drops of methyl orange indicator in a colourless solution (or to the original sample of water if there was no colour with phenolphthalein).
5. Titrate the solution with 0.01M sulphuric acid until colour changes from brisk stirring to the methyl orange end point (yellow) and the final reading (volume used) is designated as Z.
6. Run one blank solution simultaneously by adopting all the steps except water sample

Calculation

Carbonates (me/litre) = 2(Volume of H_2SO_4) x Molarity of H_2SO_4 x1000/ ml of aliquot

= 2Y x 0.01x1000/5

= 2Y x 2 = 4Y

Carbonates (g/litre) = 4Y x Eq. Wt of carbonates (30)/1000

= 0.12Y

Note:

The volume of acid used for half-neutralization of carbonate is Y, hence for full neutralization it has been assumed as 2Y.

Bicarbonates (me/litre) = (Z-2Y) x molarity of H_2SO_4x1000/ml of aliquot

= (Z-2Y) x0.01x1000/5

= (Z-2Y) x2

Where carbonate is absent: Z x 2

Work Sheet

Date:…….

Instructor Signature

Exercise-5

Determination of Sodium in irrigation water

Small amount of sodium is generally present even in the best quality of irrigation water. The concentration of sodium may be quite high in saline water with EC greater than 1 dS/m and containing relatively less amount of Ca and Mg. Obviously, its estimation is of interest when the water sample tests saline (i.e. having EC above 1.0 dS/m at 25^0C).

Equipments/Glassware

- Flame photometer
- Volumetric flasks
- Beakers

Reagents

• NaCl (AR grade)

Procedure

1. Preparation of standard curve:
   - Take 2.5413 g of NaCl (AR), dissolve in water to make to the volume to 1 litre and this will give a solution of 1000 ppm Na. Further, from this solution take 10 ml and dilute to 100 ml volumetric flask to obtain 100 ppm Na as stock solution.
   - Take 0, 5, 10, 15 and 20 ml of stock solution in 100 ml volumetric flask and make up the volume. It would give 0, 5, 10, 15 and 20 ppm Na.
   - Calibrate the instrument as per user manual guideline, Feed the standards on the flame photometer one by one to obtain a standard curve taking absorbance on Y-axis and respective concentrations of Na on X axis.

Procedure

1. Water samples are fed on the flame photometer and absorbance is recorded for each sample.
2. Concentration of Na is observed against each absorbance which is in ppm Na.

Calculation

Content of Na in mg/litre of water =Ax1000/1000=A

Where,

A = concentration of sodium (ppm) derived from the standard curve

Work Sheet

Date:…….

Instructor Signature

Exercise 6

Determination of Chloride

The chloride concentration in water generally very low, however, brackish water and saline water have appreciable amount of chloride. The Mohr's titration is generally used for the determination of chloride when the electrical conductivity of water is more than 1 dSm^{-1}.

Chloride present in water samples is titrate against standard silver nitrate

It depends upon the formation of a sparingly soluble brick-red silver chromate (Ag_2CrO_4) precipitate at the end point when the sample is titrated against standard silver nitrate ($AgNO_3$) solution in the presence of potassium chromate (K_2CrO_4) as indicator. Initially the Cl ions are precipitated as AgCl and dark brick-red precipitate of Ag_2CrO_4 starts just after the precipitation of AgCl is over.

Apparatus

- Beakers
- porcelain dish
- Burette
- Glass rod

Reagents

- Potassium chromate (K_2CrO_4) indicator (5%) solution: Dissolve 5 g of K_2CrO_4 in about 75 ml distilled water and dilute to 100 ml.
- Standard silver nitrate solution (0.05M): Dissolve 8.494 g of silver nitrate ($AgNO_3$) in distilled water and make the volume to one litre. Standardize it against standard NaCl solution and keep in amber coloured bottle away from light.

Procedure:

1. Take 5 ml of the sample in a 100 ml beaker or a porcelain dish and diluted to about 25 ml with distilled water.
2. Add 5-6 drops of K_2CrO_4 indicator (making it dark yellow), and titrate against the standard $AgNO_3$ solution with continuous stirring till the first brick-red tinge appears.
3. Run a blank to avoid error due to any impurity in chemicals.

Calculation

Cl mg/litre of water= Xx1.775x1000/ml of sample

Where,
ml of water sample taken = 5
X = ml of 0.05M $AgNO_3$ consumed in titration
1.775 = factor representing mg of Cl in aliquot/sample as calculated below:
ml of 1M $AgNO_3$ = 1 me of Cl
1 ml of 0.05M $AgNO_3$ = 0.05 me of Cl = 35.5 x 0.05
= 1.775 mg of Cl (in aliquot).

Work Sheet

Date:…….

Instructor Signature

Annexure 1:

Essential Nutrients for plant growth

Macro nutrients		Micro Nutrients
Carbon	Primary nutrients	Zinc
Hydrogen	Nitrogen	Iron
Oxygen	Phosphorus	Manganese
	Potassium	Copper
	Secondary nutrients	Molybdenum
	Calcium	Boron
	Magnesium	Chlorine
	Sulphur	Nickel`
		Cobalt

Critical limit of Macronutrients in Soil

Element	Extractant Used/Standard Method	Critical limit in Soil		
		Low	Medium	High
Organic Carbon (%)	Walkley and Black, 1934	<0.45	0.45-0.75	>0.75
Available Nitrogen (kg/ha)	Alkaline potassium per manganate (Subbiah and Asija, 1956)	<272	272-544	>544
Available Phosphorus (kg/ha)	0.5 M NaHCO3 (Olsen et al 1954)	<12.4	12.4-22.4	>22.4
Exchangeable Potassium (kg/ha)	1 N Ammonium Acetate	<113	113-280	>280
Sulphur (ppm)	0.15% CaCl2	<10	10-20	>20

Critical Limit of Micronutrients in Soil

Element	Extractant Used	Critical limit in Soil (mg/kg)		
		Low	Medium	High
Zinc	DTPA	<0.6	0.6-1.2	>1.2
Iron	DTPA	< 2.5	2.5-4.5	> 4.5
Manganese	DTPA	<2.0`	2.0-4.0	>4.0
Copper	DTPA	<0.2	0.2-0.4	>0.4
Boron	Hot Water	<0.5	0.5-1.0	> 1.0
Molybdenum	Ammonium oxalate		0.2	

Approximate concentration of nutrients in mature leaf of various crops plants

Nutrients	Deficient	Sufficient	Toxic
N (%)		1-5	
P (%)		0.1-0.4	
K (%)		1-5	
Ca (%)		0.2-1	
Mg (%)		0.1-0.4	
S (%)		0.1-0.4	
Fe (mg/kg)	< 50	100-500	>500
Mn (mg/kg)	15-25	20-300	300-500
Zn (mg/kg)	10-20	27-150	100-400
Cu (mg/kg)	2-5	5-30	200-100
B (mg/kg)	5-30	10-20	50-200
Mo (mg/kg)	0.03-0.15	0.1-2.0	>100
Cl (mg/kg)	<100	100-500	500-1000
Ni (mg/kg)	<0.1		

Sources: Jones et al (1991); Tisdale et al (1997)

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