Soil Fertility and Fertilizer Use

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Preface

Practical is the most important device to check the reality of happenings. Many times ill define procedure and methods incur great error to subject as well as implementers. To avoid such situation, a well written manual can ease the task and also cultivate likeness for the work. Agriculture education is truly practical base, where any deficiency has a cause. A right diagnosis can only provide the remedy and corrective measures. It is necessary to have right knowledge of various symptoms, deficiencies, condition associated with soil and plant. On the basis of such information one can formulate the remedies.

In this manual, "Soil fertility and fertilizer use", we have tried to incorporate the entire necessary element required to examine soil fertility and there after deficiencies correction.

We believe this unique manual will be helpful to beginners, researchers, soil analysts, quality control and extension workers.

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Chapter 1

Soil Sampling and Processing

The main focus of this manual is to present an easy-to-use methodology for soil sampling and its analysis. To start the analysis proper knowledge of sampling is very much essential.

Soil Sampling

The process of obtaining soil for analysis i.e. soil sampling, is often ignored or poorly considered. A good sampling plan should provide a measure of the average fertility level of a field its variability. If a sample is not representative of the field or is incorrectly taken, the resulting analytical data would be meaningless, or at best, difficult to interpret. The error in field sampling is generally much greater than that of chemical analysis. Therefore, obtaining a representative soil sample from a field is the most important step for making a meaningful soil analysis.

A soil sample should be composed of several sub-samples representing a seemingly uniform area or field with similar cropping and management history. There is no universally accepted numbers of sub-samples for different field situations.

However, the following points can serve as guidelines:

A. Composite Sampling

Sampling areas are traversed in a *zig-zag* pattern to provide a uniform distribution of sampling sites. In general eight sub-samples are taken per hectare (ha) in a diagonal pattern for obtaining one composite sample or 5 to 25 borings or sub-samples per composite sample with sample units varying from 2 to 8 ha.

Fewer sub-samples are needed where little or no fertilizer has been used. Correspondingly, more sub-samples are needed where fertility is variable due to hand broadcasting of fertilizers and/or with cropping-livestock systems. Banding of fertilizer poses serious problems for reliable sampling. Thus the number of sub-samples taken should be realistic considering the particular field situation.

B. Time of Sampling

Soil samples can be taken any time if soil conditions permit. However, sampling after fertilization or amendment application should be avoided. It is important to collect the sample at regular intervals year after year for comparing analysis.

C. Depth of Sampling

For most purposes, soil sampling is done to a depth of about 20-cm. Depth wise soil samples can be taken where there is a concern to know sub soil nutrient status or for profile study.

D. Sampling Tools

There are two important requirements of a sampling: first, that a uniform slice should be taken from the surface to the depth of insertion of the tool and second that the same volume of soil should be obtained in each sub-sample.

Auger generally meets these requirements. In areas where the topsoil is dry, e.g., during summer, the topsoil sampling can be done by digging with spade. It is impossible to sample dry top soils with an auger. Soil samples for micronutrient analysis should be taken by using a stainless steel auger.

2. Processing

Soil samples must be put in plastic bags. After tagging and marking soil samples should be transported to the laboratory in cardboard boxes. All information about samples is recorded and each sample is given a laboratory number.

Samples are put in a freezer for minimizing microbial activity. Samples are air-dried at room temperature. Dried samples are cleaned off for stones and plant residues. Samples are ground in a stainless steel soil grinder and passed through a 2-mm sieve. Sieved soils are collected and stored in plastic bottles.

Soil Analysis

ORGANIC CARBON

Walkley and Black method

Reagents

1. **1*N* Potassium dichromate**: Dissolve 49.04 g of AR grade $K_2Cr_2O_7$ in about 500 ml of distilled water and make the volume to one litre.
2. **Conc. Sulphuric acid**
3. **0.5*N* Ferrous ammonium sulphate**: Dissolve 196 g of ferrous ammonium sulphate in distilled water, add 20 ml of conc. H_2SO_4 and make volume to one litre. The ferrous ammonium sulphate should be from a fresh lot and light green in colour. Yellowing of the salt indicates its oxidation.
4. **Diphenylamine indicator**: Dissolve 0.5 g of the dye in a mixture of 20 ml of distilled water and 100 ml of conc. H_2SO_4.
5. **Orthophosphoric acid (85%) or sodium fluoride.**

Procedure

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

7. Titrate the contents until green colour starts appearing.

8. If the titre value is <6, repeat taking 0.2 to 0.5 g of soil sample.

Note:

1. Use of NaF along with H_3PO_4 gives a sharper end point.

2. High chloride content, as in case of saline soils, interferes in the estimation. It can be prevented by adding Ag_2SO_4 @ 1.25 per cent to the conc. sulphuric acid.

Calculation

$$\text{Organic carbon (\%) in soil} = \frac{10(B-S)}{B} \times 0.003 \times \frac{100}{\text{wt. of sample (g)}}$$

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

NITRATE-NITROGEN

Instruments

Colorimeter or Spectrophotometer or Autoanalyser

Reagents

1. **Extraction solution**: Mix 20 ml 0f 0.5 M copper sulphate solution and 100 ml of 6% silver sulphate solution dilute to 1 litre with distilled water. Mix well.

2. **$Ca(OH)_2$ (AR grade)**

3. **$MgCO_3$ (AR grade)**

4. **Phenoldisulphonic acid**: Weigh 25 g of AR grade phenol (white crystals) or 25 ml of pure liquid phenol in a round-bottom or Kjeldahl flask. Add 150 ml of NO_3 – free conc. H_2SO_4 while shaking the flask. Add 75 ml of fuming sulphuric acid and mix well. Place the flask in boiling water, dipping it up to the height of the contents in the flask. Heat for 2 hours, cool and store the resultant phenoldisulphonic acid in a glass-stoppered bottle. In case fuming sulphuric acid is not available; the same amount of conc. sulphuric acid may be added and boiling done for 6 hours. This reagent is also available commercially.

5. **Dilute ammonia solution**: Conc. ammonia solution (sp. gravity 0.91) diluted with equal amount of distilled water.

6. **Standard nitrate solution**: Accurately weigh 0.7215 g of AR grade, oven-dried and cooled KNO_3, add a little water to dissolve and make the volume to 1 litre. This stock solution contains 100 mg L^{-1} nitrate-nitrogen. From this, prepare a working solution containing 10 mg L^{-1} NO_3-N.

Procedure

1. Weigh 5-10 g of soil sample in a 100 ml conical flask.
2. Add 25 ml of the extracting solution and shake for 10 minutes.
3. Add a pinch (about 0.2 g) of $Ca(OH)_2$ and shake for 5 minutes.
4. Add about 0.5 g of $MgCO_3$ and again shake for 10 minutes.

5. Allow to settle for 5 minutes and filter through Whatman no. 42 filter paper, discarding first few ml.
6. Transfer 10 or 25 ml of the clear and colourless filtrate to a 50 ml porcelain/silica or 100 ml beaker. Work in NO_3-free atmosphere.
7. Evaporate the contents to dryness on a water bath. Do not heat beyond dryness. Cool at room temperature.
8. Rapidly add 3 ml of phenoldisulphonic acid, covering the entire residue by rotating the dish/beaker.
9. After 10 minutes, add 10 ml of distilled water and stir with glass rod.
10. Cool and transfer the contents quantitatively to a 100 ml volumetric flask.
11. Add diluted (1:1) ammonia solution slowly, while mixing until the solution becomes alkaline. This is indicated by the development of yellow colour due to the presence of nitrate.
12. Add another 2 ml of diluted ammonia solution and make the volume to 100 ml with distilled water.
13. Measure the intensity of the yellow colour at 470 nm (blue filter).

Preparation of standard curve

1. Transfer 2, 5, 10, 15, 20 and 25 ml of 10 mg L^{-1} NO_3 – N solution into dishes/beakers and evaporate to dryness.
2. Run a blank without aliquot.
3. Develop yellow colour as described above and read the colour intensity under identical manner.
4. Draw a standard curve by plotting the concentrations of NO_3 – N against readings.

Calculation

$$NO_3\ N\ (mg\ kg^{1)} = \mu g\ of\ NO_3\ \ N\ in\ test\ solution\ X\ \frac{25}{volume\ of\ aliquot\ (ml)}$$

$$X\frac{1}{wt.\ of\ soil\ (g)}$$

AMMONIACAL NITROGEN
(Colorimetric Method)

Instruments

1. Colorimeter or Spectrophotometer
2. Mechanical shaker

Reagents

1. 10% Sodium chloride (acidified): Dissolve 100 g of NaCl per litre and adjust the pH at 2.5 with HCl.
2. Nessler's reagent: Dissolve 45.5 g of mercuric iodide and 35.0 g of potassium iodide in small amount of water. Wash it into a one litre volumetric flask. Add 112g of KOH and bring the volume to about 800 ml. Mix well, cool and make up the volume. Allow to stand for a few days and decant the supernatant liquid into an amber coloured bottle.
3. 10% Sodium tartrate: Dissolve 100 g $Na_2C_4H_4O_6.2H_2O$ in one litre of water.
4. Standard NH_4Cl solution: Dissolve 1.337 g of pure NH_4Cl in water and make to one litre to get 0.025 m. e. NH_4^+ ml^{-1}. Dilute suitable volume of it 25 times to get a working solution containing 0.001 m. e. of NH_4^+ ml^{-1}.

Procedure

1. Weigh 100 g of fresh soil sample in a 500 ml conical flask. Determine moisture content separately by drying at 150 ^oC in an oven.
2. Add 200 ml of acidified NaCl solution and shake for 30 minutes.
3. Fit an 11 cm size Whatman no. 42 filter paper circle in a Buchner funnel, moisten and seat it firmly by a mild suction through electric suction pump or a simple glass-made suction device fitted to a water tap.
4. Pour the contents into the Buchner funnel to filter.

5. Add 25 ml of the NaCl solution in the conical flask to wash and rinse out and transfer to the funnel.
6. Add 8 to9 portions of 25 ml of NaCl solution each and collect the entire leachate.
7. Transfer the leachate into a 500 ml volumetric flask and make up the volume.
8. Take 50 ml aliquot into a 100 ml volumetric flask and add 2 ml each of the tartrate and acidified NaCl solutions.
9. Add a pinch of gum acacia as a protective colloid and bring the volume to about 93 ml.
10. Add 5 ml of Nessler's reagent with rapid mixing.
11. Make the volume to 100 ml.
12. Measure the intensity of the orange colour developed, after 25 minutes at 410 nm (blue filter).

Preparation of standard curve for NH_4 – N

1. Place 3 to 30 ml portions of the working standard solution containing 0.001 m. e. of NH_4^+ ml^{-1} in a series of 100 ml volumetric flasks.
2. Proceed for colour development as in the case of sample aliquots.
3. Measure the colour intensity and draw a standard curve by plotting colorimeter readings against the NH_4^+ concentrations.

Calculation

4. Add about 0.5 g of $MgCO_3$ and again shake for 10 minutes.

NH_4^+ (m. e. / 100 g soil or c mol kg^{-1} soil) on dry weight basis

$$= \frac{\text{R X Volume of extracant used}}{\text{Volume of aliquot taken}} \text{ X } \frac{100}{(100\text{-M})}$$

where, R stands for the NH4+ in me in the final volume i.e. 100 ml as read from standard curve, and M stands for moisture percentage in soil sample. The results may then be expressed on fresh weight basis also.

MINERALISABLE NITROGEN
(SUBBIAH AND ASIJA, 1956)

Instruments

Nitrogen distillation unit, preferably with six regulated heating elements.

Reagents

1. **0.32% $KMnO_4$**: Dissolve 3.2 g of $KMnO_4$ in water and make to one litre.
2. **2.5% NaOH**: Dissolve 25 g of sodium hydroxide pellets in water and make to one litre.
3. **2% Boric acid**: Dissolve 20 g of boric acid powder in warm water by stirring, and dilute to one litre.
4. **Mixed indicator**: Dissolve 0.666 g of methyl red and 0.999 g of bromocresol green in 100 ml of ethyl alcohol. Add 20 ml of this mixed indicator to each litre of 2% boric acid solution. Adjust the pH to 4.5 with dilute NaOH.
5. **0.1N Potassium hydrogen phthalate**: Dissolve 20.422 g of the salt in water and dilute to one litre. This is a primary standard and does not require standardization.
6. **0.1N NaOH**: Dissolve 4 g of NaOH in water and dilute to one litre. Standardize it against 0.1*N* potassium hydrogen phthalate solution.
7. **0.1N H_2SO_4**: Prepare approximately 0.1N H_2SO_4 by adding 2.8 ml of conc. H_2SO_4 to about 990 ml of distilled water. From this, prepare 0.02*N* H_2SO_4 by diluting a suitable volume five times with distilled water. Standardize it against 0.1N NaOH solution.

Procedure

1. Weigh 20 g of soil sample in an 800 ml Kjeldahl flask.
2. Moisten the soil with about 10 ml of distilled water, wash down the soil adhering to the neck of flask, if any.

3. Add 100 ml of 0.32% $KMnO_4$ solution.
4. Add few glass beads or broken pieces of glass rod.
5. Add 2-3 ml of paraffin liquid, avoiding contact with upper part of the neck of flask.
6. Measure 20 ml of 2% boric acid containing mixed indicator in a 250 ml conical flask and place it under the receiver tube. Dip the receiver tube end in the boric acid.
7. Run tap water in 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 heater 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 heater to avoid back suction.
11. Titrate the distillate against 0.02N H_2SO_4 taken in burette until pink colour starts appearing.
12. Run a blank without soil with each set of five samples.
13. Carefully remove the Kjeldahl flask after cooling and drain the contents in the sink.
14. Collect the glass beads for reuse.

Caution

1. Check all the joints of the Kjeldahl apparatus to prevent any leakage and loss of ammonia.
2. Hot Kjeldahl flasks should neither be washed with cold water immediately nor allowed to cool for long to avoid deposits at the bottom which are difficult to remove.

3. In case frothing takes place and reach the trap and passes through to the boric acid, such samples should be discarded and fresh distillation done after thorough washing of the system.

4. Opening ammonia bottles in the laboratory should be strictly prohibited while distillation is on. The titration should be carried out in an ammonia-free atmosphere.

5. In case the titration is not to be carried out immediately, the distillate should be stored in ammonia-free cupboards after tightly stoppering the flasks.

Calculation

Available (mineralisable)

$$\text{N in soil (kg ha}^{-1}) = \frac{(S-B) \times 0.00028}{20} \times 10^6 \times 2.24$$

$$= (S - B) \times 31.36$$

$$= (S - B) \times 31.36$$

where, S and B stand for the titre values of sample and blank, respectively.

The factor 0.00028 is arrived at by considering the following simple equation:

$$2NH_4OH + H_2SO_4 = (NH_4)_2SO_4 + 2H_2O$$

or 98 g of H_2SO_4 (or 1L of 2N H_2SO_4)

or 1 ml of 0.02N H_2SO_4 = 0.00028 g N

AVAILABLE PHOSPHORUS

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

Instruments

1. Mechanical shaker
2. Colorimeter or Spectrophotometer

Reagents

1. Bray's P-1 extractant: Dissolve 1.110 g of AR grade ammonium fluoride in one litre of 0.025N HCl.
2. 1.5% Dickman and Bray's reagent: Dissolve 15 g of AR grade ammonium molybdate in 300 ml of warm water, cool and add exact 350 ml of 10N HCl. Make the volume to one litre.
3. 40% $SnCl_2$ stock solution: Weigh 10 g pure stannous chloride in a 100 ml glass beaker. Add 25 ml of conc. HCl and dissolve by heating. Cool, transfer to an amber coloured bottle and store in dark after adding a piece of small piece of Zn metal (AR grade) to prevent oxidation. From this, prepare a dilute $SnCl_2$ solution (0.5 ml diluted to 66 ml) immediately before use.
4. Standard stock solution: weigh 0.439 g of AR grade KH_2PO_4 dried in oven at 60^{o}C for 1 hour in a one litre beaker, add about 500 ml of distilled water and dissolve. Add 25 ml of approx. 7N H_2SO_4 and make the volume to one litre. This is 100 mg P L^{-1} solution.
5. Standard working solution: Dilute a suitable volume of 100 mg P L^{-1} solution 50 times to get 2 mg P L^{-1} solution.

Procedure

1. Weigh 5 g of soil sample in a 150 ml conical flask.
2. Add 50 ml of Bray's P-1 extractant and shake for 5 minutes.
3. Filter through Whatman no.1 filter paper quickly so as to collect the filtrate within 10 minutes.

4. Transfer 5 ml aliquot into a 25 ml volumetric flask.
5. Add 5 ml of ammonium molybdate solution shake a little and dilute to about 22 ml.
6. Add 1 ml of diluted $SnCl_2$ (0.5 ml diluted to 66 ml), mix by shaking a little, and make up the volume.
7. Run a blank without soil under identical conditions.
8. Measure the intensity of the blue colour developed, using 660 nm wavelengths (red filter).

Preparation of Standard Curve for P

1. Take a series of 25 ml volumetric flasks.
2. Pipette out 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of 2 mg L^{-1} P solution.
3. Add 5 ml of the extractant (Bray's).
4. Add ammonium molybdate as for Bray's method and proceed to develop blue colour as described earlier.
5. Measure blue colour intensity and draw a standard curve by plotting concentrations of P in μg against absorbance readings.
6. If a straight line is obtained, find out a factor for each reading.

Calculation

$$\textbf{Available P (kg ha}^{-1}\textbf{)} = \frac{Q \times V \times 2.24 \times 10^6}{A \times S \times 10^6} = \frac{Q \times V \times 2.24}{A \times S}$$

where, Q = quantity of P in μg read on X-axis against a sample reading,
V = volume of extracting reagent used (ml),
A = volume of aliquot used for colour development (ml), and
S = weigh of soil sample (g)

Thus, Bray's P (kg ha^{-1}) = Q x 4.48

AVAILABLE PHOSPHORUS
(ASCORBIC ACID METHOD)

Reagents

1. **Molybdate-tartrate solution**: Dissolve 12 g of ammonium molybdate in about 250 ml of distilled water to get solution 'A'. Prepare solution 'B' by dissolving 0.291 g of antimony potassium tartrate in 100 ml of distilled water. Prepare one litre of 5*N* H_2SO_4 and add solutions 'A' and 'B' to it. Mix thoroughly and make the volume to 2 litres with distilled water.
2. **Ascorbic acid solution**: Dissolve 1.056 g of ascorbic acid in 200 ml of the molybdate-tartrate solution and mix well. Prepare it fresh as and when required.
3. **p-nitrophenol indicator**: Dissolve 0.5 g of p-nitrophenol in 100 ml of distilled water.
4. **5N H_2SO_4**: Carefully dilute 140 ml of conc. H_4SO_4 to 1L with distilled water (H_4SO_4 to be slowly added to water) to get approx. 5*N* H_2SO_4.

Procedure

1. Pipette 5 ml of the Olsen's reagent into a 25 ml volumetric flask and add 2-3 drops of p-nitrophenol indicator. It develops yellow colour.
2. Add known quantity of 5N H_2SO_4 drop by drop to acidify the Olsen's reagent to pH 5.0 at which the yellow colour will disappear. Note the volume of 5N H_2SO_4 used.
3. Transfer a 5 ml aliquot of the Olsen's extract of soil to a 25 ml volumetric flask and add the required quantity of 5N H_2SO_4 to bring it to pH 5.0.
4. Dilute to 20 ml with distilled water.
5. Add 4 ml of the ascorbic acid solution, make the volume to 25 ml and shake well.
6. Wait for 10 minutes and then measure the colour intensity at 730-840 nm.

7. Run a blank with the extracting solution (without soil).

8. Prepare standard curve as described for Dickman and Bray's method.

Note:

1. All glassware to be used for P determination should be cleaned with chromic acid, followed by thorough washing with water to minimize contamination.
2. In case of delay in measuring colour intensity, cover the samples, store properly and add $SnCl_2$ just before measurements.

Colour intensity measurement should be carried out exactly 10 minutes after developing blue colour.

AVAILABLE POTASSIUM

Ammonium Acetate Method of K Determination (Hanway and Heidel, 1952)

Instruments

1. Flame photometer
2. Mechanical shaker
3. pH meter

Reagents

1. 1N Ammonium acetate: Dissolve 77.08 g of ammonium acetate in about 500 ml of distilled water and make the volume to one litre. Adjust the pH to 7.0 with glacial acetic or ammonia solution. This reagent may also be prepared by taking 800 ml of distilled water and adding to it 57 ml of glacial acetic acid and 68 ml of ammonia solution (sp. gr. 0.91), followed by dilution to 1 litre and adjusting pH at 7.0 after cooling.
2. Standard K solution: Prepare 1000 mg L^{-1} K solution by dissolving 1.908 g of AR grade KCl salt (dried in oven at 70 ^{o}C for two hours) per litre solution. Dilute suitable volumes of this solution to get 100 ml of working standards containing 5, 10, 15, 20, 25, 30 and 40 mg K L^{-1}. The working standards should be prepared in the medium of extraction (ammonium acetate in this case).

Procedure

1. Weigh 5 g of soil sample in 100mml conical flask.
2. Add 25 ml of the neutral 1N ammonium acetate solution and shake for 5 minutes.
3. Filter through Whatman no. 1 filter paper.
4. Measure K concentration in the filtrate using flame photometer.

Preparation of Standard Curve for K

Record the flame photometer readings for each of the working standards of K after adjusting blank to zero. Draw a standard curve by plotting the readings against K concentrations.

Calculation

$$\textbf{Available K (kg ha}^{-1}\textbf{)} = \textbf{C x } \frac{25}{5} \textbf{ x 2.24 = C x 11.2}$$

where, C stands for the concentration (mg L^{-1}) of potassium in the sample filtrate obtained on X-axis, against the reading.

Note:

1. The filtrate should be clear in order to avoid choking of capillary tube of the flame photometer, which occurs quite often.
2. During the operation of flame photometer, a constant air pressure and steady flow of gas (LPG) and air combination is absolutely necessary for precise estimation. An appropriate combination is achieved by fixing the air pressure around 0.75 kg cm^{-2} and then adjusting the gas supply so as to get a non-luminous blue flame.
3. Fans and coolers should be switched-off so that flame is not affected.
4. Ammonia bottle should be cooled before opening.
5. Potassium standards should be prepared fresh after every 2-3 weeks.

AVAILABLE SULPHUR

CaCl2 – Extractable S

(Williams and Steinbergs, 1969)

Instruments

1. Colorimeter or Spectrophotometer
2. Mechanical shaker

Reagents

1. **0.15% Calcium chloride solution**: Dissolve 1.5 g of calcium chloride dehydrate ($CaCl_2$. $2H_2O$) in about 500 ml of distilled water and make the volume to 1 litre.
2. **Barium chloride crystals**: Grind $BaCl_2$ (AR grade) crystals to pass through a 30-mesh sieve and retain on a 60-mesh sieve, and store in a clean bottle.
3. **Standard S solution**: Dissolve 0.5434 g of oven-dried AR grade K_2SO_4 in distilled water and dilute to 1 L. This contains 100 $\mu g\ S\ ml^{-1}$.
4. **Gum acacia solution**: Dissolve 0.25 g of gum acacia in distilled water and dilute to 100 ml.

Preparation of Standard Curve

1. Take 0.25, 0.50, 1.0, 2.5 and 5.0 ml of standard S solution in 25 ml volumetric flasks, and add 10 ml of extracting solution (i.e. 0.15% $CaCl_2$ solution) to each flask. For blank take 10 ml of extracting solution in a 25 ml volumetric flask.
2. Add 1 g of $BaCl_2$ crystals to each flask and swirl to dissolve the crystals.
3. Add 1 ml of 0.25% gum acacia solution, make up the volume with distilled water and shake well manually.
4. Within 5-30 minutes of development of turbidity (white colour), read the absorbance at 340 nm on a spectrophotometer, or on a colorimeter using blue filter.
5. Draw a curve taking S concentrations on X-axis and absorbance readings on Y-axis.

Procedure

1. Weigh 10 g of air-dry soil in a 150 ml conical flask and add 50 ml of 0.15% $CaCl_2$ solution.
2. Shake for 30 minutes on a rotary shaker and filter through Whatman no. 42 filter paper.
3. Take 10 ml of the clean filtrate in a 25 ml volumetric flask, follow the steps given for standard curve to develop the turbidity and record the absorbance.

Calculation

$$\textbf{Available S in soil (mg kg}^{-1}\textbf{)} = \textbf{R x} \frac{\textbf{50}}{\textbf{10}} \textbf{ x} \frac{\textbf{1}}{\textbf{10}}$$

where, R stands for S content in μg as read on X-axis

Note:

In case, the available S content of the soil is very low, a 20 ml aliquot (instead of 10 ml) should be taken to develop the turbidity.

PHOSPHATE EXTRACTABLE SULPHUR
(AN ALTERNATIVE METHOD)

Instruments

1. Colorimeter or Spectrophotometer or Autoanalyser
2. Mechanical shaker

Reagents

1. **Mono-calcium phosphate extracting solution (500 mg P L^{-1})**: Dissolve 2.035 g of $Ca(H_2PO_4)_2.H_2O$ per litre.
2. **Gum acacia-acetic acid solution**: Dissolve 5 g of chemically pure gum acacia powder in 500 ml of hot water and filter in hot condition through Whatman no. 42 filter paper. Cool and dilute to one litre with dilute acetic acid.
3. **Barium chloride**: Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
4. **Standard stock solution (2000 mg S L^{-1})**: Dissolve 1.089 g of oven-dried AR grade potassium sulphate per 100 ml.
5. **Working standard solution (10 mg S L^{-1})**: Measure exactly 2.5 ml of the stock solution and dilute to 500 ml.
6. **Barium sulphate seed suspension**: Dissolve 18 g of AR grade $BaCl_2$ in 44 ml of hot water and add 0.5 ml of the standard stock solution (given above). Heat the contents to boiling and then cool quickly. Add 4 ml of gum acacia-acetic acid solution to it. Prepare a fresh seed suspension for each estimation every day.
7. **Dilute nitric acid (approx. 25%)**: Dilute 250 ml of AR grade conc. HNO_3 to one litre.
8. **Acetic-phosphoric acid**: Mix 900 ml of AR grade glacial acetic acid with 300 ml of H_3PO_4 (AR grade).

Procedure

1. Weigh 20 ml of soil sample in a 250 ml conical flask.
2. Add 100 ml of the monocalcium phosphate extracting solution (500 mg P L^{-1}) and shake for one hour. Filter through Whatman no. 42 filter paper.
3. Measure 10 ml of the clear filtrate into a 25 ml volumetric flask.
4. Add 10 ml of 25% HNO_3 and 2 ml of acetic-phosphoric acid. Dilute to about 22 ml, stopper the flask and shake well.
5. Shake the $BaSO_4$ seed suspension and then add 0.5 ml of it and 0.2 g of $BaCl_2$ crystals. Stopper the flask, invert three times and keep.
6. After 10 minutes, invert 10 times and keep. After another 5 minutes, invert 5 times.
7. Allow to stand for 15 minutes and then add 1 ml of gum acacia-acetic acid solution.
8. Make the volume, invert three times and keep aside for 90 minutes.
9. Invert 10 times and measure the turbidity at 440 nm (blue filter).
10. Run a blank side by side.

Preparation of Standard Curve for S

1. Place 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ml portions of the working standard solution (10 mg SL^{-1}) into a series of 25 ml volumetric flasks to obtain 25, 50, 75, 100, 125 and 150 µg S.
2. Proceed to develop turbidity as described above for sample aliquots.
3. Read the turbidity and prepare the curve by plotting readings against sulphur concentrations (in µg in the final volume of 25 ml).

Calculation

$$\text{Available S in the soil (mg kg-1)} = \frac{\mathbf{R \times 100}}{\mathbf{10 \times 20}} = \frac{\mathbf{R}}{\mathbf{2}}$$

Where, R stands for the quantity of S in μg as obtained on X-axis against a reading.

MORE RAPID METHOD
(Chesnin and Yien, 1950)

Reagents

1. **0.25% Gum acacia solution**: Dissolve 0.25 g of gum acacia in 100 ml of distilled water.
2. **$BaCl_2$ crystals**: Grind AR grade $BaCl_2$ in an agate mortar to pass the crystals through a 30-mesh sieve, but retained on a 60-mesh sieve.

Procedure

1. Transfer 10 ml of the aliquot to a 25 ml volumetric flask.
2. Add 1.0 g of the sieved $BaCl_2$ crystals and shake for 1 minute.
3. Add 1 ml of the gum acacia solution, make the volume to mark and shake for 1 minute.
4. Measure the turbidity, 25 to 30 minutes after the precipitation, using a blue filter (440 nm).
5. Run a blank and prepare standard curve for calculation under identical manner.

ESTIMATION OF AVAILABLE MICRONUTRIENTS

DTPA-CaCl2-TEA Extraction method for Zn, Cu, Fe and Mn

The DTPA micronutrient soil test by Lindsay and Norvell (1969) is useful for the extraction of all the micronutrient cations Zn, Cu, Fe, and Mn, especially for their determination by atomic absorption spectrophotometer. The chemical composition of DTPA extractant is 0.005M DTPA-0.01 M $CaCl_2.2H_2O$-0.1 M triethanolamine. Its pH is adjusted to 7.3. The theoretical basis for the DTPA extraction is the equilibrium of elements in the soil with the chelating agent. The pH 7.3 enables DTPA to extract Fe and other metals. For the triethanolamine, pH is kept close to 7.3.

Determination of available zinc

Instruments

1. Atomic absorption spectrophotometer (AAS)

2. Mechanical shaker

Reagents

1. **Dilute HCl**: Dilute AR grade HCl 5 times with double distilled water (DDW).

2. **DTPA extractant**: Dissolve 1.967 g of AR grade diethyline-triamine-penta acetic acid (DTPA) and 1.470 g of $Cacl_2.2H_2O$ (AR grade) in about 25 ml of double distilled water (DDW) by adding 13.3 ml of triethanolamine (TEA), followed by 100 ml more of DDW. Transfer the solution to one litre volumetric flask giving 4 to 5 washings. Just before making up the volume, adjust pH to 7.3 with dilute HCl. This reagent has 0.005M DTPA, 0.1*M* TEA and 0.01 M $CaCl_2.2H_2O$.

3. **Standard stock solution 'A' (1000mg Zn L^{-1})**: Weigh exactly 1.0 g of pure Zn metal (AR grade) and dissolve it in minimum volume (about 10 ml) of dil. HCl (1:1) and make the volume to one litre.

4. **Standard solution 'B'**: Dilute 5 ml of solution A to 100 ml to get solution B containing 50 mg Zn L^{-1}.

5. **Standard working solutions**: Dilute 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 ml portions of solution B to 50 ml to get working standards containing 0.5, 1.0, 1.5, 2.0, 2.5 and 5.0 mg Zn L^{-1}. The working standards should be prepared in the medium of the extracting solution after every few days as these cannot be preserved for long.

Procedure

1. Weigh 10 g soil sample in 100 ml conical flask.
2. Add 20 ml of the DTPA extractant and shake for 2 hours on a mechanical shaker.
3. Filter through Whatman no. 42 filter paper, discarding first few drops. For quick filtration, Whatman no. 1 filter paper can also be used if the filtrate is clear.
4. Use the filtrate for Zn measurement on AAS.
5. Feed the standard working solutions and prepare a standard curve by plotting AAS readings against Zn concentrations.

Calculation

$$\textbf{Available (DTPA-extractable) Zn in soil (mg kg}^{-1}\textbf{)} = \frac{\textbf{A x 20}}{\textbf{10}} = \textbf{A x 2}$$

where, A stands for the Zn concentration in aliquot as read from X-axis of standard curve against the sample reading.

Determination of Available Copper

Available copper can be determined in the DTPA extract similar to Zn, using AAS. For this, the standard stock solution can be prepared as given below:

Accurately weigh 1.0 g AR grade copper metal wire or turning and dissolve it in 50 ml of diluted HNO_3 (1:1 with DDW) and finally make the volume to one litre. This is solution 'A' containing 1000 mg Cu L^{-1}. Prepare solution 'B' containing 50 mg Cu L^{-1} by diluting appropriate volume of solution 'A'. Finally prepare working standards containing 0.25, 0.50, 1.0, 1.5, 2.0 and 2.5 mg Cu L^{-1} from solution 'B'.

Determination of Available Iron

Iron in the DTPA extract can also be determined with the help of AAS exactly in the same manner as Zn and Cu described above. However, the working standard solutions of Fe should be prepared for higher concentrations, as the DTPA-extractable Fe content of soil is generally more than both Zn and Cu. Thus, the Fe standards may be prepared as given below:

Prepare standard stock solution (solution 'A') by dissolving exact 1.0 g of AR grade Fe metal in about 50 ml of 1:1 diluted HNO_3 and dilute the contents to one litre with DDW. Prepare solution 'B' by diluting 50 ml of solution 'A' to 500 ml to get 100 mg Fe L^{-1}. Finally prepare working standard solutions containing 1.0, 2.0, 3.0, 5.0 and 10.0 mg Fe L^{-1} by diluting appropriate volume of solution 'B' with the medium of extraction (DTPA in this case).

Determination of Available Manganese

DTPA-extractable Mn is also determined following the same technique as adopted for Zn, Cu and Fe. For this, prepare the standard solutions as follows:

Weigh 1.583 g of AR grade MnO_3 or 1.0 g of pure Mn metal and dissolve it in 50 ml of diluted HNO_3 (AR grade). Make the volume to 1 litre with DDW to get solution 'A' having a Mn concentration of 1000 mg L^{-1}. From solution 'A' dilute 25 ml to 250 ml with DDW to get solution 'B' having 100 mg Mn L^{-1}. Finally prepare working solutions of 0.5, 1.0, 2.0, 2.5 and 5.0 mg Mn L^{-1} concentrations by diluting 0.5, 1.0, 2.0, 2.5 and 5.0 ml portions of solution 'B' to 100 ml.

Determination of Available Boron

Boron exist in organic as well as in inorganic forms in soil, and most of the B-compounds that have high availability are water soluble. Boron deficiency is encountered in calcareous and acid soil, whereas toxicity occurs in salt-affected soils as well as in soils irrigated with high B water. Among the methods proposed from time to time for B determination, the most widely used one was that of Berger and Truog (1939), which involved refluxing of soil with hot water. The method was, however, time consuming and also required special reflux apparatus. Subsequently, Gupta (1967) developed a rapid and easy method wherein the soil could be extracted by boiling with water directly on a hot plate. Use of azomethine-H (John *et al.*, 1975) in place of carmine or curcumin has further simplified the determination of hot-water soluble B. Azomethine-H forms a stable coloured complex with H3BO3 at pH 5.1 in

aqueous media, which retains proportional absorbance-concentration properties for several hours independent of the presence of a variety of salts.

Hot-water Soluble Boron (Gupta, 1967)

Instruments

1. Colorimeter or Spectrophotometer
2. Hot plate
3. Refrigerator

Reagents

1. **Buffer solution**: Dissolve 250 mg of ammonium acetate and 15 g of EDTA (disodium salt) in 400 ml of distilled water, slowly add 125 ml of glacial acetic acid and mix thoroughly.
2. **Azomethine-H reagent**: Dissolve 0.45 g of azomethine-H in 100 ml of 1% L-[1]ascorbic acid solution. Store in polypropylene bottle in a refrigerator. Prepare fresh solution every week.
3. **Boron standard solution**: Dissolve 0.114 g of AR grade boric acid (H_3BO_3) in distilled water and make the volume to 1000 ml. Each ml of this solution contains 20 μg of B. Dilute 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40 and 50 ml of this stock solution to 100 ml with distilled water to have a solution concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 μg B ml^{-1}, respectively.
4. **Activated charcoal**

Procedure

1. Weigh 20 g of air-dry (20-mesh) soil sample in 250 ml quartz or other boron-free conical flask and add 40 ml of distilled water.
2. Add 0.5 g of activated charcoal and boil for 5 minutes on a hot plate, filter immediately through Whatman no. 42 filter paper.

3. Cool the contents to room temperature and transfer 1 ml aliquot of blank (filtrate obtained after boiling of distilled water and activated charcoal), diluted B standard or sample filtrate into 10 or 15 ml polypropylene tubes.
4. Add 2 ml of buffer solution and mix.
5. Add 2 ml of azomethine-H reagent, mix and after 30 minutes read the absorbance at 420 nm on a spectrophotometer.
6. Prepare a standard curve plotting B concentrations (0 to 10 μg B ml^{-1}) on X-axis and absorbance on Y-axis.
7. Refer the absorbance readings of sample aliquots to the standard curve to obtain B content of aliquots.

Calculation

Available B (mg kg^{-1} or ppm) in soil = A x 2

where, A is the B content (μg) obtained from standard curve.

Precautions

1. As far as possible, avoid the use of borosilicate glass in determinations and storage of reagents.
2. Don't store azomethine-H reagent for long. Prepare a fresh solution every week.
3. In order to eliminate interferences due to Al in high-Al acid soils, use tetrasodium salt of EDTA instead of disodium salt for preparation of buffer solution.

Determination of Available Molybdenum

Instruments

1. Colorimeter or Spectrophotometer
2. Hot plate
3. Refrigerator
4. Water bath

Reagents

1. 50% Potassium iodide solution: Dissolve 50 g of KI in 100 ml of double distilled water (DDW).
2. 50% Ascorbic acid solution: Dissolve 50 g of ascorbic acid in 100 ml of DDW.
3. 10% Sodium hydroxide solution: Dissolve 10 g of NaOH in 100 ml of DDW.
4. 10% Thiourea solution: Dissolve 10 g Thiourea in 100 ml of DDW and filter. Prepare fresh solution before use.
5. Toluene-3, 4-dithiol solution (commonly called dithiol): Weigh 1.0 g of AR grade melted dithiol (51 ^{o}C) in a 250 ml glass beaker. Add 100 ml of the 10% NaOH solution and warm the contents up to 51 ^{o}C with frequent stirring for 15 minutes. Add 1.8 ml of thioglycolic acid and store in a refrigerator.
6. 10% Tartaric acid: Dissolve 10 g in 100 ml of DDW.
7. Iso-amyl acetate
8. Ethyl alcohol
9. Ferrous ammonium sulphate solution: Dissolve 63 g of the salt in about 500 ml of DDW and then make the volume to one litre.
10. Extracting reagent: Dissolve 24.9 g of AR grade ammonium oxalate and 12.6 g oxalic acid in water and make the volume to one litre. Adjust the pH at 3.3 with dil. HCl / dil. ammonia solution.
11. Standard stock solution (100 μg Mo ml^{-1}): Dissolve 0.150 g of AR grade MoO_3 in 100 ml of 0.1N NaOH, make slightly acidic with dil. HCl and make the volume to 1L.
12. Working standard solution (1 μg Mo ml^{-1}): Dilute 10 ml of the stock solution of 1L.

Procedure

1. Weigh 25 g of air-dry soil sample in 500 ml conical flask.
2. Add 250 ml of the extracting solution and shake for 10 hours.
3. Filter through Whatman no. 50 filter paper. Transfer 200 ml of the clear filtrate in a 250 ml glass beaker and evaporate to dryness on a water bath.
4. Heat the contents in the beaker at 500 °C in a furnace for 5 hours to destroy organic matter and oxalates. Keep overnight.
5. Digest the contents with 5 ml of HNO_3-$HClO_4$ mixture (4:1), then with 10 ml of 4*N* H_2SO_4 and H_2O_2, each time bringing to dryness.
6. Add 10 ml of 0.1N HCl and filter. Wash the filter paper with additional 10 ml of 0.1N HCl and DDW until the volume of the filtrate becomes 100 ml.
7. Run a blank side by side (without soil).
8. Take 50 ml of filtrate in 250 ml separatory funnels and add 0.25 ml of the solution and 20 ml of DDW, and shake vigorously.
9. Add excess of KI solution and clear the liberated iodine by adding ascorbic acid drop by drop while shaking vigorously.
10. Add one ml of tartaric acid and 2 ml of Thiourea solution and shake vigorously.
11. Add 5 drops of dithiol solution and allow the mixture to stand for 30 minutes.
12. Add 10 ml of iso-amyl acetate and separate out the contents (green colour) in colorimeter tubes / cuvettes.
13. Read the colour intensity at 680 nm (filter paper).

Preparation of Standard Curve for Mo

1. Measure0, 2, 5, 15 and 20 ml of the working standard Mo solution containing 1 µg Mo ml^{-1} in a series of 250 ml separatory funnels.
2. Proceed for colour development as described above for sample aliquots.
3. Read the colour intensity and prepare the standard curve by plotting Mo concentrations against readings.

Calculation

$$\textbf{Available Mo in soil (µg g}^{-1}\textbf{)} = A \times \frac{250}{200} \times \frac{100}{50} \times \frac{1}{25} = \frac{A}{10}$$

where, A stands for Mo concentration in µg as obtained on X-axis against a sample reading.

DETERMINATION OF LIME REQUIREMENT OF SOIL

Instrument

pH meter

Reagent

1. Extractant buffer solution: Dissolve 1.8 g of nitrophenol, 2.5 ml of triethalnolamine, 3.0 g of potassium chromate, 2.0 of calcium acetate and 53.1 g of calcium chloride dehydrate in one litre of distilled water and adjust the pH to 7.5 using dilute NaOH.
2. pH buffer solutions: solutions of pH 4.0, 7.0 and 9.2, as described under pH determination.

Procedure

1. Weigh 5 g of air-dry soil sample in 50 ml beaker.
2. Add 5 ml of distilled water and 10 ml of the extractant buffer solution.
3. Stir continuously for 10 minutes or intermittently for 20 minutes with a glass rod.
4. Measure the pH of the soil-buffer suspension on a pH meter after standardizing with known pH buffer solutions.
5. Against the measured pH, find out the amount of lime required to bring the soil to a desired level (e.g. 6.0, 6.4 or 6.8) as given in Table. Make necessary correction to get the value of agricultural lime based on purity percentage.

Table - Lime requirement for different pH targets

Measured pH of soil-buffer suspension	Lime requirement in tonnes ha^{-1} of pure $CaCO_3$ for achieving different soil pH targets		
	pH 6.0	pH 6.4	pH6.8
6.7	2.43	2.92	3.40
6.6	3.40	4.13	4.62
6.5	4.37	5.35	6.07
6.4	5.59	6.56	7.53
6.3	6.56	7.78	8.99
6.2	7.53	8.99	10.21
6.1	8.50	10.21	11.66
6.0	9.48	11.42	13.12
5.9	10.69	12.64	14.58
5.8	11.66	13.85	15.79
5.7	12.64	15.07	17.25
5.6	13.61	16.28	18.71
5.5	14.58	17.50	20.17
5.4	15.79	18.71	21.63
5.3	16.77	19.93	22.84
5.2	17.98	20.90	24.30
5.1	18.95	22.11	25.76
5.0	19.93	23.33	27.22
4.9	20.99	24.54	28.67
4.8	22.11	25.76	30.13

Chapter 3

Plant Sampling

Accumulation of nutrient elements in plant tissues indicates the accessibility of the concerned elements from the soil to the plant. Although different plant species and even different varieties of the same species may vary in their nutrient requirements, composition of a part or of the whole plant may well be adopted as a guideline to support the soil-test results for factual assessment of soil's nutrient supplying power. Also, the concentration of certain heavy metals in plants might be a result of their solubility in soil and may lead to polluting effects. To a certain extent, there exist a good relationship between the concentration of an element in the plant and the total biomass of the plant. A correct balance of nutrients in the plant tissues is closely associated with the maximum yield, except in the cases of luxury consumption of nutrients like potassium.

Plant analysis as an aid to soil testing has its limitations too. These, among other things, include the risk of sampling error as well as the chances of wrong interpretations of the test results for making recommendation. Significant error in the plant analysis may arise due to wrong sampling alone, especially when the plant analysis aims at diagnosis of nutrient deficiency in standing crops. Selecting the right plant part, stage of growth and time of sampling are very crucial in plant analysis.

For a meaningful plant analysis, utmost care should be exercised in plant sampling. Whole plant analysis is done for working out the total nutrient uptake, which is usually carried out on the aboveground (shoot) material. Analysis of roots may either be taken up separately or individual plant parts like leaf, petioles etc. are sampled for the purpose of diagnosing nutrient deficiency in perennial fruit trees. Thus, depending on the purpose of analysis, plant sampling needs to be planned.

Procedure for Plant Sampling

1. For analysis of seasonal crop plants, pick up few representative plants at random from each plot. Remove the shoot (aerial part) with the help of a sharp stainless steel knife or blade or scissors for whole shoot analysis or the desired part for analysis of specific plant parts.
2. If the roots are to be included, uproot the whole plant carefully from wet soil,

retaining even the fine/active roots. Gently dip the plant roots in water several times to remove the adhering soil as far as possible.

3. Wash the plant parts with water several times.
4. Wash the samples thoroughly with about 0.2% detergent solution to remove the waxy/greasy coating on the leaf surface, which is often present.
5. Wash with 0.1*N* HCl followed by thorough washing with plenty of water. Give final rinse with distilled water.
6. Rinse with double distilled water, especially if micronutrient analysis is to be carried out.
7. Soak dry with good quality tissue paper.
8. Air dry the samples on a perfectly clean surface at room temperature for at least 2-3 days in a dust-free atmosphere away from any kind of contaminants.
9. Place the samples in an electric oven and dry at 60°C for 48 hours.
10. Grind the samples in an electric stainless steel mill using 0.5 mm sieve. Clean the cup and blades of the grinding mill before each sample.

Place back the samples in oven and dry again for few hours more for constant weight. Store in well-stoppered plastic or glass bottles or paper bags for analysis.

Plant Analysis

NITROGEN

Instruments/Apparatus

1. Infrared Digestion system (or Digestion Chamber having a fume exhaust system, if gas burners are used).
2. Automatic distillation-cum-titration system or distillation system (programmable or ordinary).

Reagents

1. **0.1N H_2SO_4** : Dilute suitable volume of conc. H_2SO_4 to get approximately 0.1*N* H_2SO_4 and standardize it against 0.1N NaOH.
2. **Boric acid (4%) with mixed indicator:** Prepare 4% aqueous solution of boric acid, add mixed indicator and adjust pH at 4.5.
3. **Digestion accelerator mixture:** Mix 20 parts of anhydrous Na_2SO_4 or K_2SO_4 with 1 part of $CuSO_4.5H_2O$ or commercially available tablets composed of K_2SO_4 and $CuSO_4.5H_2O$.

Procedure for Digestion on Gas Burner

1. Weigh 0.5 to 1.0 g of finely-ground, dried, 0.5 mm sieved plant sample, wrap in a circle of Whatman no. 42 filter paper and drop into 800 ml capacity Kjeldahl flask.
2. Add 20 g of the digestion accelerator mixture and 35 ml of conc. H_2SO_4.
3. Place the flask on the burner at about 45° angle in a fume chamber and heat at low flame for approx. 30 minutes to avoid too much frothing.
4. Raise the flame gradually, keeping it below the level of liquid inside in order to prevent ammonia loss and excessive volatilization of H_2SO_4. Continue heating

until yellow or dark colour totally disappears and does not re-appear on keeping away from the flame.

5. Remove the flask and keep aside to cool. After the contents have cooled, add cautiously about 300 ml of ammonia-free distilled water. Swirl a little to mix and again cool at room temperature or under tap water.
6. Run a blank (without sample) under identical conditions.
7. Take 25 ml of 4% boric acid containing mixed indicator in a 250 ml conical flask and place it under the ammonia-receiving tube of the distillation assembly.
8. Add a few glass beads or pieces of glass rod and about 3-4 ml of paraffin liquid to the previously diluted and cooled sample.
9. Add 100 ml of 40% NaOH solution to the sample and immediately attach to the alkali trap of the distillation unit.
10. Continue distillation for about 30-40 minutes collecting about 100 ml of distillate and then remove the conical flask before switching off the heater, to prevent back suction.
11. Titrate the distillate against 0.1N H_2SO_4 until a purple colour just starts appearing.

Procedure for Digestion of Sample on Digestion Block

1. Weight the sample and wrap in filter paper as described above and place in digestion tube. Add one tablet of the digestion accelerator or mixture and 6 ml of conc. H_2SO_4.
2. Set the digestion system to attain a temperature of about 385^{o}C and then attach the digestion tube to the heating unit as per the instruction given in the operation manual.
3. Run the tap water with desired flow rate for safe disposal of fumes.

4. Allow the digestion to continue till completion (no black or brown colour), which takes about 60 to 75 minutes.
5. Switch-off the system.
6. Remove the rack of sample tubes along with the exhaust system from the heating unit. Do not stop water flow as the fumes continue coming for some more time.
7. Set the distillation unit to perform various steps, *viz.* dilution, addition of alkali, steam generation, titration *etc*.
8. Keep boric acid in conical flask as described earlier and run the distillation for 150 seconds.
9. Use the automatic titration system if available, or do it manually.

NOTE:-

1. Once the samples are digested, the subsequent steps *i.e.* distillation and titration can also be followed manually.
2. Utmost care should be exercised during dilution of H_2SO_4 digest and addition of 40% NaOH, if done manually.

PLANT DIGESTION FOR ANALYSIS

For nutrients other than N, the plant material can be digested in a diacid mixture or a triacid mixture or dry ashed and dissolved in acid. Diacid digestion is normally recommended for plant analysis since H_2SO_4 of triacid mixture can contribute some micronutrients and heavy metals. Wet digestion is normally not used for the estimation of B and Mo. Dry ashing can be used for sample preparation for the determination of Na, K, Ca, Mg, Cu, Fe, Mn, Zn, B and Mo in plant tissue and is the preferred technique for B and Mo particularly.

TRI ACID DIGESTION

The triacid digestion is recommended only when P and K are to be estimated. Sulphur cannot be estimated from triacid extract. Calcium will also be under estimated. Triacid digestion is carried out a mixture of HNO_3:H_2SO_4:$HClO_4$ in the ratio of 9:1:4.

The triacid digestion is used for the determination of P, K, Ca, Mg, S, Fe, Mn, Zn and Cu. It must be followed for the determination of Ca and Mg. It is carried out using 9:4 mixture of HNO_3:$HClO_4$. If the sample is high in fats/oil, pre-digestion using 25 ml HNO_3 per gram sample is recommended to avoid explosion.

Instruments

1. Water bath
2. Hot plate

Reagent

1. **Tri-acid mixture**: Mix AR grade conc. HNO_3, H_2SO_4 and $HClO_4$ in 9:1:4 ratio and cool.

Procedure

1. Transfer 0.5 to 1.0 g of dried and processed plant sample to a 100 or 150 ml conical flask. Add 5 ml of conc. HNO_3.
2. Keep a glass funnel on the flask, place it on a water bath and heat at 100^{o}C for about 30 minutes.
3. Shift the flask to a hot plate and heat at 180-200^{o}C. Measure temperature in a flask containing glycerol kept on a hot plate.

4. Continue boiling until near to dryness, but not drying completely. Cool and add 5 ml of the tri-acid mixture.
5. Heat at 180-200°C until the dense white fumes are evolved. Continue digestion until the mixture is largely volatilized.
6. If the contents are still brown, cool a little and add 3-4 ml of the tri-acid mixture and continue the digestion as described above. This is rare as there is still sufficient $HClO_4$ to oxidize the charred material.
7. Remove the flasks when only moist, clear and white contents are left. The entire quantity of $HClO_4$ has volatilized by this stage.
8. Cool and add about 50 ml of distilled water (or double distilled water if micronutrients are to be analysed).
9. Filter into 100 ml flask, giving washings to make the volume to 100 ml.
10. Use the filtrate for analysis.

DI-ACID DIGESTION

Instrument

1. Hot plate

Reagents

1. Conc. HNO_3 (AR grade)
2. 60 per cent $HClO_4$ (AR grade)
3. Approx. 2N HCl (AR grade)

Procedure

1. Weigh 0.5 to 1.0 g of dried and processed plant samples in a 100 or 150 ml conical flask.
2. Add 10 ml of conc. HNO_3, place a funnel on the flask and keep for about 6-8 hours or overnight at a covered place/chamber for pre-digestion.
3. After pre-digestion when the solid sample is no more visible, add 10 ml of conc. HNO_3 and 2-3 ml of $HClO_4$.
4. Keep on a hot plate in acid-proof digestion chamber having fume exhaust system and heat at about 100°C for first one hour and then raise the temperature to about 200°C.
5. Continue digestion until the contents become colourless and only white dense fumes appear.
6. Reduce the acid contents to about 2-3 ml by continuing heating at the same temperature. Do not allow to dry up.
7. Remove the flasks from hot plate, cool and add about 30 ml of distilled water.
8. Filter through Whatman no.42 filter paper into a 100 ml volumetric flask.

Give 3-4 washings of 10-15 ml portions of distilled water and make the volume to100ml.

PHOSPHORUS

Instrument

1. Spectrophotometer or Colorimeter

Reagents

1. **Vanadate-molybdate reagent**: Prepare solution 'A' by dissolving 25 g of ammonium molybdate in about 400 ml of warm water. Prepare solution 'B' separately by dissolving 1.25 g of ammonium metavanadate in about 300 ml of boiling, cool it and add 250 ml of conc. HNO3. Cool again at room temperature. Now add solution 'A' to solution 'B' and dilute to one litre.
2. **Standard P solution** : Prepare solution containing 100 mg P L^{-1} by dissolving 0.439 g of dried KH_2PO_4 in water, acidifying with 25 ml of 7N H_2SO_4 and making the volume to 1 L. Prepare a working standard containing 50 mg P L^{-1} from it.

Procedure

1. Transfer a suitable volume, not more than 30 ml of aliquot to a 50 ml volumetric flask so that it contains 0.05 to 1.0 mg of P and the acid equivalent is between 0.6 and 1.6*N* in the final volume of 50 ml.
2. Add 10 ml of the vanadate-molybdate solution and dilute to 50 ml with water.
3. Mix well and read the absorbance after 10 minutes using 420 nm wavelength (blue filter).
4. Run a blank (without P) simultaneously.
5. Take 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5 ml of the 50 mg P L^{-1} solution in 50 ml volumetric flasks and develop colour in identical manner.
6. Prepare standard curve by plotting P concentrations on X-axis and per cent transmission/colorimeter readings on Y-axis.

Calculation

$$\text{P content of sample (\%)} = \frac{\mathbf{A}}{\mathbf{100V}}$$

where, A stands for P concentration in μg as read against the sample reading on the standard curve, and V stands for volume of aliquot taken (ml)for colour development out of the 100 ml acid digest made from 1 g sample.

POTASSIUM

Potassium in the acid-digest of plant samples can be determined using flame photometer exactly in the manner as described for available K in soil. Depending on the concentration of K in the plant sample, the digest can be used either directly or after dilution for flame photometric determination. For example wheat grain digest can be taken up directly while straw samples are diluted generally 10 times for this purpose. Alternatively, use AAS which provides better precision.

CALCIUM AND MAGNESIUM

These elements can be determined in the di-acid digest of plant sample either using AAS or versenate titration method. Calcium can be estimated on a flame photometer also, though the sensitivity is less than that for K and Na.

SULPHUR

Since dry ashing leads to volatilization of S present in the organic combination, and the wet oxidation based on tri-acid mixture includes H_2SO_4, both of these methods cannot be used for S determination in plant samples. Therefore, HNO_3-$HClO_4$ digest of plant is conveniently used. Turbidimetric method as described for soil sulphur or the barium chromate colorimetric method (Palasker *et al*. 1981) can be used for determination of S in the digest.

IRON, ZINC, MANGANESE AND COPPER

These four metals can be determined in the di-acid digest of plant tissues using AAS as in case of soil analysis. Dithizone method (Shaw and Dean 1952) for Zn and Cu, orthophenanthroline method for Fe and Na-paraperiodate method for Mn can be used as alternative colorimetric methods.

BORON

For B determination in plant tissue, follow the steps given below:

1. Proceed for dry ashing as described earlier, with 0.5 g sample.
2. Extract the dry ash with 10 ml of 0.1N HCl.
3. Filter or centrifuge the suspension to a clear state.
4. Pipette 1 ml of clear aliquot and proceed for B determination by azomethine-H method as described earlier.

MOLYBDENUM

For the determination of Mo in plant tissues, ashing and fusion of the sample is done as given below:

1. Take a suitable sample (1 to 10 g) in a platinum dish.
2. Perform dry ashing in an electric furnace at 500^{o}C.
3. Fuse the ash with 2 g of anhydrous Na_2CO_3 at 1000^{o}C in the electric furnace, ensuring complete contact of the entire ash with the flux.
4. Cool the dish and drop the cake into a 250 ml beaker by inverting the dish.
5. If the cake does not detach, place the dish in beaker and disintegrate the cake in 100 ml water containing 2% ethanol by volume, with the help of a policeman.
6. Measure the suspension in a cylinder and filter through Whatman no. 42 filter paper in a 250 ml beaker.
7. Measure the undiluted clear filtrate and use for Mo determination as described earlier.
8. Calculate on the basis of the former volume.

Appendices

Harvest index (HI)

Harvest index was calculated by using the following formula and expressed in per cent.

$$HI = \frac{\text{Seed yield (kg/ha)}}{\text{Biological yield (kg/ha)}} \times 100$$

Nutrient uptake (kg ha^{-1})

Nutrient uptake by grain and straw of rice crop was calculated in kg ha^{-1} in relation to dry matter production ha^{-1} by using following formula

$$\text{Uptake (kg ha}^{-1}) = \frac{\text{Nutrient (\%) in grain / straw} \times \text{grain / straw yield(kg ha}^{-1})}{100}$$

Agronomic efficiency

Agronomic efficiency is defined as the economic production obtained per unit of nutrient applied. It can be calculated with the help of following equation:

$$\text{Agronomic efficiency (kg/kg)} = \frac{\text{Grain yield of fertilized crop in kg - grain yield of unfertilized crop in kg}}{\text{Quantity of fertilizer applied in kg}}$$

Physiological efficiency

Physiological efficiency is defined as the biological production obtained per unit of nutrient absorbed. Sometimes, it is also known as biological efficiency or efficiency ratio. It can be calculated with the help of the following equation:

$$\text{Physiological efficiency (kg/kg)} = \frac{\text{Total dry matter yield of fertilized crop in kg} - \text{Total dry matter yield of unfertilized crop in kg}}{\text{Nutrient uptake by fertilized crop in kg} - \text{Nutrient uptake by unfertilized crop in kg}}$$

Apparent recovery

The apparent recovery efficiency is defined as the quantity of nutrient absorbed per unit of nutrient applied. It can be calculated with the help of following expression:

$$\text{Apparent recovery efficiency (kg/kg)} = \frac{\text{Nutrient uptake by fertilized crop} - \text{Nutrient uptake by unfertilized crop}}{\text{Quantity of fertilizer applied}}$$

Molecular and equivalent weight of some important compounds

S. No.	Compound	Formula	Molecular weight (g)	Equivalent weight (g)
1.	Ammonium acetate	CH_3COONH_4	77.08	77.08
2.	Ammonium chloride	NH_4Cl	53.49	53.49
3.	Ammonium fluoride	NH_4F	37.04	37.04
4.	Ammonium nitrate	NH_4NO_3	80.04	80.04
5.	Barium acetate	$(CH_3COO)2Ba$	255.43	127.72
6.	Barium chloride	$BaCl_2.2H_2O$	244.28	122.14
7.	Boric acid	H_3BO_3	61.83	20.61
8.	Calcium acetate	$(CH_3COO)_2Ca$	158.0	79.0
9.	Calcium carbonate	$CaCO_3$	100.09	50.05
10.	Calcium chloride	$CaCl_2 2H_2O$	147.02	73.51
11.	Calcium hydroxide	$Ca(OH)_2$	74.0	87.0
12.	Calcium nitrate	$Ca(NO_3)_2$	164.0	82.0
13.	Calcium sulphate	$CaSO_4.2H_2O$	172.17	86.08
14.	Ferrous ammonium sulphate	$(NH_4)_2SO_4.FeSO_4.6H_2O$	329.13	329.13
15.	Magnesium chloride	$MgCl_2.6H_2O$	203.30	101.65
16.	Magnesium nitrate	$Mg\ (NO_3)_2\ 6H_2O$	256.41	128.20
17.	Potassium chloride	KCl	74.55	74.55
18.	Potassium dichromate	$K_2Cr_2O_7$	294.19	49.40
19.	Potassium hydroxide	KOH	56.10	56.10
20.	Potassium permanganate	KM_NO_4	158.03	31.60
21.	Potassium nitrate	KNO_3	101.10	101.10
22.	Potassium sulphate	K_2SO_4	174.27	87.13
23.	Potassium hydrogen phthalate	$COOHC_6H_4COOK$	204.22	204.22
24.	Oxalic acid	$C_2H_2O_4.2H_2O$	126.0	68.0
25.	Silver nitrate	$AgNO_3$	169.87	169.87
26.	Sodium acetate	CH_3COONa	82.04	82.04
27.	Sodium bicarbonate	$NaHCO_3$	106.0	53.0
28.	Sodium carbonate	Na_2CO_3	58.45	58.45
29.	Sodium chloride	NaCl	40.0	40.0
30.	Sodium hydroxide	NaOH	84.99	84.99
31.	Sodium nitrate	$NaNO_3$	134.00	67.00
32.	Sodium oxalate	$Na_2C_2O_4$	134.0	
33.	Sodium sulphate	Na_2SO_4	142.04	71.02
34.	Sodium thiosulphate	$Na_2S_2O_{3.}5H_2O$	248.18	248.18

Percent concentration, specific gravity, normality and amount needed for making 1N solution of some commonly used liquid reagent

S. No.	Reagent	Concentration (% w/w)	Specific gravity at 20°C	Normality (approximate)	Quantity (ml) required for 1 litre of 1N
1.	Acetic acid glacial	99.0	1.06	17.5	58
2.	Ammonium hydroxide	28.3	0.91	15.0	67
3.	Hydrochloric acid	39.0	1.19	11.8	78
4.	Nitric acid	71.0	1.42	15.6	62
5.	Phosphoric acid	96.0	1.84	36.0	28

Rating chart for soil test values of primary nutrients

S. No	Nutrient	Low	Medium	High
1.	Organic carbon (%)	<0.5	0.50-0.75	>0.75
2.	Alkaline $KMnO_4$-N ($kgha^{-1}$)	<280	281-560	>560
3.	Olsen's P ($kg\ ha^{-1}$)	<10	11-25	>25
4.	Ammonium acetate-K ($kg\ ha^{-1}$)	<120	121-280	>280

Some important conversion factors

$N \times 1.286 = NH_4$

$N \times 4.43 = NO_3$

Organic carbon × 1.724= organic matter

$P \times 2.29 = P_2O_5$

$K \times 1.20 = K_2O$

$Ca \times 1.40 = CaO$

$Ca \times 1.85 = Ca(OH)_2$

P × 2.29	P_2O_5
P_2O_5 × 0.436	P
K × 1.205	K_2O
K_2O × 0.830	K
Mg × 1.1658	MgO
MgO × 0.603	Mg
Ca × 1.399	CaO
CaO × 0.715	Ca
Na × 2.5	NaCl

Procedure for preparing 1000 mg L^{-1} standard solutions

S. No.	Element	Procedure for making 1L of standard solution
1.	Aluminum	Dissolve 10000g of Al metal is 25 ml of concentrated HCl and few drops of concentrated HNO_3
2.	Calcium	Dissolve 2.497 g of $CaCO_3$ (dried at 150°C) in 25 ml of 1N HCl
3.	Cadmium	Dissolve 1.0 g of concentrated metal (99.99%) in 50 ml of 1+1HCl
4.	Cobalt	Dissolve 1.0 g of Co metal (99.99%) in minimum of 6N HNO_3 or dissolve 2.630 g of $CaSO_4$ (dried at 250-300°C for 6-8 hours) in about 100 ml of double distilled water and 1 ml of concentrated H_2SO_4 (AR grade)
5.	Chromium	Dissolve 1.0 g of chromium metal (99.99%) in 50 ml of concentrated HCl or Dissolve 2.828 g of $K_2Cr_2O_7$ in double distilled water
6.	Copper	Dissolve 1.0 g of Cu metal (99.99%) in 50 ml of 1+1 HNO_3
7.	Iron	Dissolve 1.0 g of Fe metal (99.99%) in 100 ml of 3.5N H_2SO_4
8.	Potassium	Dissolve 1.906 of dried KCl in double distilled water
9.	Magnesium	Dissolve 1.0 g of Mg metal ribbon or turning (99.99%) in 50 ml of 5N HCl
10.	Manganese	Dissolve 1.0 of Mn metal wire or foil (99.99%) in 50 ml of 6N HNO_3 or dissolve 3.076 g of manganous sulphate monohydrate ($MnSO_4.H_2O$) in 200 ml of double distilled water and add 1.5 ml of concentrated HNO_3 or dissolve 1.582 g manganese dioxide (MNO_2) is minimum quantity of concentrated HCl evaporate to dryness, dissolve residue in double distilled water.
11.	Molybdenum	Dissolve 1.5003 g of molybdenum trioxide (MoO_3) in 10 ml of concentration
12.	Nickel	Dissolve 1.0 g of Ni metal (99.99%) in 50 ml of 1+1 HNO_3
13.	Lead	Dissolve 1.0 g Pb metal (99.99%) in 20 ml of 6N HNO_3 or 1.598 g of $Pb(NO_3)_2$ in double distilled water and add 20 ml of concentrated HNO_3
14.	Selenium	Dissolve 1.0 g of Se metal (99.99%) in 20 ml of aqua-regia (15 ml concentrated HCl+5 ml concentrated HNO_3)
15.	Silicon	Fuse 4.278 g of silicon dioxide (SiO_2) with 20 g of sodium carbonate in a platinum crucible and dissolve in distilled water
16.	Zinc	Dissolve 1.0 g of Zn metal (99.99%) in 50 ml of 1+1 HCl or Dissolve 4.549 g of zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] in double distilled water.

Some indicator plants and nutrient deficiency

S. No.	Nutrients	Indicator plants
1	Nitrogen	Maize, Cereal (small grains), Mustard, Apple, Citrus
2	Phosphorus	Maize, Barley, Lettuce, Tomato
3	Potassium	Potato, Lucerne, Beans, Tobacco, Cucurbits, Cotton, Tomato, Maize
4	Calcium	Lucerne, other legume crops
5	Magnesium	Potato, Cauliflower
6	Sulphur	Lucerne, Raya
7	Iron	Sorghum, Barley, Citrus, Peach
8	Zinc	Maize, Onion, Citrus, Peach
9	Manganese	Apple, Cherry, Citrus, Maize, Oats, Pea, Radish, Wheat
10	Cupper	Apple, Citrus, Barley, Maize, Lettuce, Oats, Onion, Tobacco, Tomato
11	Boron	Lucerne, Turnip, Cauliflower, Apple, Peach
12	Chlorine	Lettuce
13	Molybdenum	Cauliflower, Brassica spp., Citrus, Legume, Oats, Spinach.

General sufficiency or optimum range of micro and micronutrient element in plant

Nutrients	Sufficiency or optimum range
Macronutrients (%)	
N	2.0-5.0
P	0.2-0.5
K	1.0-5.0
Ca	0.1-1.0
Mg	0.1-0.4
S	0.1-0.3
Micronutrients (µg/g)	
Zn	20-100
Fe	50-250
Mn	20-300
Cu	5-20
B	10-100
Mo	0.1-0.5
Cl	2000-20000

Concentration

1%	10 000 ppm =10 g/l
0.1%	1000 ppm=1000 mg/l
0.01%	100 ppm=100 mg/l
0.001%	10 ppm =10 mg/l
0.0001%	1 ppm = 1 mg/l

Elements and its atomic weight and valence

Element	**Symbol**	**Atomic weight**	**Valence**
Aluminum	Al	26.970	3
Boron	B	10.820	3
Bromine	Br	79.916	1,3,5,7
Cadmium	Cd	112.410	2
Calcium	Ca	40.080	2
Carbon	C	12.010	2,4
Chlorine	Cl	35.456	1,3,5,7
Cobalt	Co	58.940	2,3
Copper	Cu	63.570	1,2
Fluorine	F	19.000	1
Hydrogen	H	1.008	1
Iodine	I	126.920	1,3,5,7
Iron	Fe	55.850	2,3
Lead	Pb	207.210	2,4
Magnesium	Mg	24.320	2
Manganese	Mn	54.940	2,3
Mercury	Hg	200.610	1,2
Molybdenum	Mo	95.950	3,4,6
Nickel	Ni	58.690	2,3
Nitrogen	N	14.008	3,5
Oxygen	O	16.000	2
Phosphorus	P	30.980	3,5
Potassium	K	39.096	1
Selenium	Se	78.960	3
Silicon	Si	28.060	4
Sodium	Na	22.997	1
Sulphur	S	32.060	2,4,6
Zinc	Zn	65.380	2

Nutrient and its function and deficiency symptoms in plant

S. No	Element	Symbol	Essentiality established		Function	Deficiency symptoms
			By	Year		
1	Hydrogen	H	Since time immemorial			Water has been known as essential requirement for all life
2	Oxygen	O	Since time immemorial			
3	Carbon	C	Pristlay and others	1800		Establish the dependence of plant life on atmospheric CO_2
4	Nitrogen	N	Theodore de Saussure	1804	Vital for nucleic acid, chlorophyll and cytochromes synthesis	Leaf chlorosis, first in older leaves
5	Phosphorus	P	C. Sprengel	1839	Constituent of nucleic acid and phospholipids; co-enzymes NADP and ATP	Leaf necrosis, dark or blue green colouration of leaf
6	Potassium	K	C. Sprengel	1839	Required in carbohydrate metabolism and protein synthesis	Leaf chlorosis followed by necrosis at the tip of margin of older leaves, deficiency results in leaf curling and shortening of internodes
7	Calcium	Ca	C. Sprengel	1839	Constituent of cell wall, required for mitotic activities, activate phospholipase and ATP - ase etc.	Meristematic portions are affected and die, root growth stunted, marginal chlorosis in younger leaves
8	Magnesium	Mg	C. Sprengel	1839	Part of chlorophyll molecule, required by enzymes involved in carbohydrate metabolism and nucleic acid synthesis	Chlorosis between the veins of leaves which may turn brown or necrotic in severe deficiency, symptoms first appear in older leaves
9	Sulphur	S	Sachs Knop	1857	Constituent of amino acid (cysteine, cystine, methionine) and vitamins, needed for activity of many enzymes	Chlorosis first in younger leaves

Nutrient and its function and deficiency symptoms in plant

S. No	Element	Symbol	Essentiality established		Function	Deficiency symptoms
			By	Year		
10	Iron	Fe	D. Gris	1844	Needed for chlorophyll synthesis, constituent of many enzymes, active in biological oxidation	Interveinal chlorosis, first in younger leaves
11	Manganese	Mn	J.S. McHargue	1922	Activator of enzymes in respiration and metabolism, also activate many other enzymes	Interveinal chlorotic and necrotic spot on leaves
12	Boron	B	K. Warington	1923	Involved in carbohydrate transport in plants	Death of shoot tips, root stunted, leaves process a thick coppery texture and curl, and become brittle
13	Zinc	Zn	A. L. Sommer and C. L. Lipman	1926	Required in auxin and protein synthesis, affects activity of many enzymes	Interveinal chlorosis followed by necrosis in older leaves, leaves small, short internodes, plant show rosette appearance
14	Copper	Cu	A.L. Sommer and others	1931	Activator of enzymes	Young leaves become necrotic at tips
15	Molybdenum	Mo	D. I. Arnon and P. R. Stout	1939	Essential in N_2 fixation and nitrate assimilation, constituent of nitrate reductase	Chlorotic interveinal mottling of the basal leaves followed by marginal necrosis
16	Chlorine	Cl	T. C. Broyer and others	1954	Involved in primary photosynthetic and cyclic reaction photophosphorylation	Wilted appearance of foliage, stuffy roots with lateral branching
17	Nickel	Ni	P. H. Brown, R. M. Welch and E. E. Cary	1987	Associated with nitrogen metabolism, ensures optimum hydrogenase activity in free-living *Rhizobia*, and facilitates transport of nutrients to seeds	Reduction in dry matter, decrease in amino acid content and accumulation of nitrates

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