4. BASIC PRINCIPLES AND METHODOLOGIES INVOLVED IN SOIL ANALYSIS FOR ACID SOILS

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Processing of Soil Samples

Dry the collected sample under shade. Crush the soil clods lightly with wooden pestle and mortar. Sieve using a stainless steel 2 mm sieve and discard plant residues, gravel and other foreign matter retained on the sieve. If the gravel content is substantial, it has to be recorded. For certain types of analysis (*e.g.* organic carbon) it is necessary to grind the soil further so as to pass it through 0.5mm sieve.

Procedures for Soil Analysis

1. Soil pH

1.1 Principle

The pH of 1:2.5 soil water suspension is estimated using a pH meter. It is a measure of hydrogen ion activity of the soil water system and indicates whether the soil is acidic, neutral or alkaline in reaction.

1.2. Reagents

- *Standard buffer solutions:*

Prepare buffer solutions of pH 4.0, 7.0, 9.2 using commercially available buffer tablets. Dissolve the respective tablets in freshly prepared distilled water and make up the volume to 100 mL. It is necessary to prepare fresh buffer solutions after every few days as these solutions do not keep for long.

1.3. Procedure

Calibrate the pH meter using buffer solutions. The pH of soil is determined in 1:2.5 soil water suspension. Take 10 g sample of soil sifted through 2 mm sieve in a 50 or 100 mL beaker. Add 25 mL of distilled water, stir well for about 5 minutes and keep for half an hour. Stir well again and take the reading using the pH meter.

2. Electrical Conductivity

2.1 Principle

Electrical conductivity in soil water system is a measure concentration of soluble salts and extent of salinity in the soil and is measured using a conductivity meter

2.2 Reagent

- *0.01N Potassium chloride solution:*

Dry a small quantity of AR grade potassium chloride at 60^0C for 2 hours in a hot air oven. Weigh 0.7456 g of it and dissolve in freshly prepared distilled water and make to one litre. This solution gives an electrical conductivity of 1.41 dS m^{-1} at $25^\circ C$.

2.3 Procedure

The clear supernatant of 1:2.5 soil water suspension prepared for pH measurement can be used for estimation of EC. Calibrate the conductivity meter using 0.01N KCl solution prepared and determine the cell constant. Determine the conductivity of the supernatant liquid.

3. Organic Carbon in Soil

Soil organic matter has been defined as the organic fraction of soil, including plant, animal and microbial residues, fresh and at all stages of decomposition and the relatively resistant soil humus. However soil organic matter estimate includes only those organic materials that accompany soil particles through a 2 mm sieve. Carbon is the chief element present in soil organic matter, and forms 48-58% of the total weight. Therefore organic carbon determinations are often used as a basis for estimation of organic matter. For many years the *Van Bemmelen factor* of 1.724 was used based on the assumption that the organic matter contains 58% organic carbon.

3.1 Principle

Schollenberger (1927) first proposed that the organic matter in soil may be oxidised by treatment with a hot mixture of $K_2Cr_2O_7$ and H_2SO_4 according to the equation;

$$2\ Cr_2O_7^{2-} + 3\ C + 16\ H^+ = 4\ Cr^{3+} + 3\ CO_2 + 8\ H_2O$$

After the reaction, the un-reacted $Cr_2O_7^{2-}$ is titrated with standard Fe $(NH_4)_2SO_4.6H_2O$ and the $Cr_2O_7^{2-}$ reduced during the reaction with soil is assumed to be equivalent to the organic carbon present in the sample. Jackson (1958) recommended that o-phenanthroline can be used as an indicator in the $Cr_2O_7^{2-}$ titrations because the colour change (formation of the complex with Fe^{2+}) occurs at higher oxidation-reduction potential.

Dichromate methods that use heat of dilution or minimal heating do not give complete oxidation of organic compounds in soil although the most active forms of organic carbon are converted to CO_2. Walkley and Black (1934) found that on an average about 76% of the organic carbon was recovered by the heat of dilution procedure and they proposed that a correction factor of 1.32 be used to account for unrecovered organic carbon.

3.2 Walkley-Black Wet Digestion Method (Walkley, 1947)

3.2.1. Reagents

- *Potassium dichromate ($K_2Cr_2O_7$), 1N*

Dissolve 49.04 g of reagent grade $K_2Cr_2O_7$ (dried at 105°C) in water and dilute the solution to a volume of 1000 ml.

- *Ferrous ammonium sulphate hexahydrate solution, 0.5N*

Dissolve 197 g of reagent grade $Fe(NH_4)_2SO_4.6H_2O$ in water, add 15 ml of concentrated sulphuric acid, cool the solution and dilute it to a volume of 1000 ml. Standardize this reagent daily by titrating it against 10 ml of 1*N* potassium dichromate.

- *O-phenathroline-ferrous complex (ferroin), 0.025M*

Dissolve 14.85 g of o-phenanthroline monohydrate and 6.95 g of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) in water. Dilute the solution to a volume of 1000 ml. The o- phenathroline-ferrous complex is available under the name of Ferroin.

- *Sulphuric acid (H_2SO_4) concentrated*

If Cl^- is present in soil, add Ag_2SO_4 to the acid @15 g L^{-1}

3.2.2. Procedure

Grind the soil to pass through a 0.5 mm sieve avoiding iron or steel mortars. Transfer a weighed sample, containing 10-25 mg of organic carbon (approximately 0.5 to 1 g soil), but not in excess of 10 g soil, into a 500 ml wide mouth conical flask. Add 10 ml of 1*N* $K_2Cr_2O_7$ and swirl the flask gently to disperse the soil in the solution. Then rapidly add 20 ml of concentrated H_2SO_4. Immediately swirl the flask gently until the soil and the reagents are mixed, then more vigorously for a total of one minute. Allow the flask to stand on an asbestos sheet for about 30 minutes. Then add 200 ml of water to the flask. Add 3-4 drops of o-phenanthroline indicator and titrate the solution with 0.5*N* ferrous ammonium sulphate.

As the endpoint approaches, the solution takes on a greenish cast and then changes to a dark green colour. At this point add the ferrous ammonium sulphate drop by drop until the colour changes sharply from blue to red. Make a blank determination in the same manner, but without soil, to standardize the $Cr_2O_7^{2-}$.

3.2.3. Calculation

$$\text{Organic carbon (\%)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } Fe(NH_4)_2SO_4) \times 0.003 \times 100 \times 1.3}{\text{Weight of soil}}$$

$$\text{O C (\%)} = \frac{\{10 \times 1 - \text{Titre Value(mL)} \times \text{Normality of } Fe(NH_4)_2SO_4\} \times 0.003 \times 100 \times 1.3}{\text{Weight of soil}}$$

$$\text{O C (\%)} = \frac{\{10 \times 1 - \text{Titre Value(mL)} \times \text{Normality of } Fe(NH_4)_2SO_4\} \times 0.39}{\text{Weight of soil}}$$

4. Available Phosphorus

Determination of plant available P in soil has two distinct phases – first, the extraction of plant available pool of phosphorus, present in soil, and second the quantitative determination of the P in the extract. The choice of a colorimetric method for determining P depends on the P concentration in the solution, the concentration of interfering substances in the solution to be analysed and the particular acid system involved in the analytical procedure. The molybdenum blue method is the most sensitive and widely used one for soil extracts containing small amounts of P.

4.1 Principle

In an acid molybdate solution, the orthophosphate ions gets precipitated as phosphomolybdate complex forms that can be reduced by ascorbic acid, stannous chloride and other reducing agents. This reduced phosphomolybdate has blue colour. The intensity of the blue colour varies with the P concentration but is affected also by other factors such as acidity, arsenates, silicates and substances that influence the oxidation – reduction conditions of the system.

As the available pool of P varies depending on the pH of the soil, reagents used for extraction of this pool also are different.

4.2 Available P in acidic soils

Available P is commonly extracted using Bray No.1 (Bray and Kurtz, 1945), which consists of 0.03 *N* NH_4F and 0.025 *N* HCl. The combination of HCl and NH_4F is designed to remove easily acid soluble P forms, largely calcium phosphates, and a portion of the aluminium and iron phosphates. The NH_4F dissolves aluminium and iron phosphates by its complex ion formation with these metal ions in acid solution.

4.2.1 Reagents

- *Ammonium fluoride (NH_4F), 1N*

Dissolve 37 g of NH_4F in distilled water and dilute the solution to 1 litre. Store the solution in polyethylene bottle.

- *Hydrochloric acid (HCl), 0.5 N*

Dilute 20.2 mL of concentrated HCl to a volume of 500 mL with distilled water.

- Bray No.1

Add 15 mL of 1*N* ammonium fluoride (NH_4F) and 25 mL of 0.5*N* Hydrochloric acid (HCl) to 460 mL of distilled water. This solution can be kept in glass for more than one year.

- *Ammonium paramolybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)*

Dissolve 12 g of ammonium paramolybdate in 250 mL of distilled water. Dissolve 0.2908 g of potassium antimony tartarate ($KSbO.C_4H_4O_6$) in 100 mL of distilled water. Add these dissolved reagents to 1 litre of 5*N* sulphuric acid (H_2SO_4) (141 ml of concentrated H_2SO_4 diluted to 1 litre), mix thoroughly, and dilute with distilled water to 2 litre. Store in a Pyrex glass bottle in a dark and cool compartment ***(Reagent A)***.

- *Ascorbic acid*

Dissolve 1.056 g of ascorbic acid in 200 mL of Reagent A and mix. This ascorbic acid ***(Reagent B)*** should be prepared as required because it does not keep more than 24 hours.

- *Standard phosphate solution*

Dissolve 0.4393 g of oven-dry AR grade potassium dihydrogen phosphate (KH_2PO_4) in distilled water, and dilute the solution to 1 L. One millilitre of this solution contains 100 μg of P. From this solution, prepare a secondary standard of 2 $\mu g\ mL^{-1}$ by pipetting out 2 mL and diluting to 100 mL with distilled water.

4.2.2 Procedure

Extraction

Weigh out 5 g of soil to a 100 mL conical flask and add 50 mL of Bray No.1 reagent and shake for exactly 5 minutes. Filter through Whatman No.42 filter paper. To avoid interference of fluoride, 7.5 mL of 0.8 *M* boric acid (50 g of H_3BO_3 per litre) can be added to 5 mL of the extract if necessary. Estimate phosphorus in the extract by ascorbic acid method (Watanabe and Olsen, 1965).

Estimation by reduced molybdate blue colour method

Pipette out 5 mL of the extract into a 25 mL volumetric flask and dilute it to approximately 20 mL. Add 4 mL of Reagent B. Make up the volume with distilled water and shake the contents well. Read the intensity of colour after 10 minutes at 660 nm. The colour is stable for 24 hours and the maximum intensity develops within 10 minutes. The concentration of P in the sample is computed from the standard curve.

Preparation of standard curve

Prepare different concentrations of P taking 1, 2, 3, 4, 5 and 10 mL of 2 $\mu g\ mL^{-1}$ P solution in 25 mL volumetric flasks. Add 5 mL of the extracting reagent (Bray No.1) and develop colour as described above by adding reagent B. Plot the concentration *vs.* absorbance curve on a graph paper.

4.2.3 Calculation

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \mu\text{g P mL}^{-1}\text{ of the aliquot} \times \frac{50}{5} \times \frac{25}{5}$$

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for sample}}{\text{Slope of Std. curve}} \times 50$$

$$\text{Available P (kg ha}^{-1}\text{ soil)} = \text{Available P (mg kg}^{-1}\text{ soil)} \times 2.24$$

4.3 Available P in alkaline/calcareous soils

The phosphorus extracted from the soil with 0.5*M* sodium bicarbonate ($NaHCO_3$) at a nearly constant pH of 8.5. In calcareous, alkaline or neutral soil containing calcium phosphates, this extractant decreases the concentration of calcium in solution by causing precipitation of calcium as $CaCO_3$; as a result, the concentration of P in solution increases. In the presence of solid phase calcite, the concentration of calcium in the extracting solution at equilibrium is 6×10^{-7} *M* (Olsen *et al*, 1954).

4.3.1. Reagents

- *Sodium bicarbonate ($NaHCO_3$) solution, 0.5*

Dissolve 42 g of $NaHCO_3$ in distilled water to give 1litre of the solution. Adjust the pH of the solution to 8.5 with 1*M* sodium hydroxide (NaOH). Add mineral oil to avoid exposure of the solution to the air. Store the solution in a polyethylene container for periods more than one month, but check the pH of the solution each month.

- *Sulphuric acid (H_2SO_4) 5N*

Add 141 mL of concentrated H_2SO_4 to 800 mL of distilled water. Cool the solution, and dilute to 1000 mL with distilled water.

- *Ammonium paramolybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)*

Dissolve 12 g of ammonium paramolybdate in 250 mL of distilled water. Dissolve 0.2908 g of potassium antimony tartarate ($KSbO.C_4H_4O_6$) in 100 mL of distilled water. Add these dissolved reagents to 1 L of 5*N* sulphuric acid (H_2SO_4) (141 mL of concentrated H_2SO_4 diluted to 1 L), mix thoroughly, and dilute with distilled water to 2 L. Store in a pyrex glass bottle in a dark and cool compartment ***(Reagent A)***.

- *Ascorbic acid*

Dissolve 1.056 g of ascorbic acid in 200 mL of Reagent A and mix. This ascorbic acid ***(Reagent B)*** should be prepared as required because it does not keep more than 24 hours.

- *Standard phosphate solution*

Dissolve 0.4393 g of oven-dry AR grade potassium dihydrogen phosphate (KH_2PO_4) in distilled water, and dilute the solution to 1 L. One millilitre of this solution contains 100 µg of P. From this solution, prepare a secondary standard of 2 µg mL^{-1} by pipetting out 2 mL and diluting to 100 mL with distilled water.

4.3.2. Procedure

Extraction

Add 5 g of soil and 100 mL of extracting solution to a 250 mL conical flask. Shake the flask for 30 minutes. Filter the suspension through Whatman No. 42 filter paper. Add carbon black (activated charcoal) to obtain a clear filtrate if necessary.

Estimation by reduced molybdate blue colour method

Place a 5 mL aliquot of the extract in 25 mL volumetric flask and acidify with 5*N* H_2SO_4 to pH 5. This can be done by taking 5 mL of 0.5 *M* extracting solution ($NaHCO_3$) and determining the amount of acid required to bring the solution to pH 5 using *p*-nitrophenol indicator. Then add the required acid to all the unknown samples. Add distilled water to

20 mL, and then add 4 mL of reagent B. Make up the volume with distilled water and shake the contents well. Read the intensity of colour after 10 minutes at 660 nm. The concentration of P in the sample is computed from the standard curve.

Preparation of standard curve

Prepare different concentrations of P taking 1, 2, 3, 4, 5 and 10 mL of 2 $\mu g\ mL^{-1}$ P solution in 25 mL volumetric flasks. Add 5 mL of the extracting reagent (Bray No.1) and develop colour as described above by adding reagent B. Plot the concentration *vs.* absorbance curve on a graph paper.

4.2.3 Calculation

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \mu g\ P\ mL^{-1} \text{ of the aliquot} \times \frac{100}{5} \times \frac{25}{5}$$

$$\text{Available P (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for sample}}{\text{Slope of Std. curve}} \times 100$$

$$\text{Available P (kg ha}^{-1}\text{)} = \text{Available P (mg kg}^{-1}\text{ soil)} \times 2.24$$

5. Available Potassium

A relatively small portion of the total K in soils is exchangeable (approx. 1%). Exchangeable K generally ranges from <100 to 2000 $\mu g\ mL^{-1}$ or more when compared with total K values which is in the order of 1 to 2%. Water soluble K seldom exceeds a few parts per million except in the case of certain saline soils.

Exchangeable plus water soluble K contributes to the plant available pool of potassium in the soil. Hence, most soil test procedures to estimate plant available K involve extractants that replace a significant portion of the exchangeable K. In highly weathered soils, or soils where parent material contain little K- bearing minerals, the exchangeable K can be depleted by K removal and is replenished only by fertilizer application or return of K from plant residues.

5.1. Principle

By definition the exchangeable potassium is that, which is free to exchange with cations of salt solution added to soils. But the quantity exchanged from the soil depends on the nature of the replacing solution. Hence with reference to fertility evaluation, exchangeable K is defined more specifically as that which is extracted with neutral 1N ammonium acetate minus the water soluble K. In normal soils, as the water soluble K is so small, there is no appreciable error even if it is included and the water soluble plus exchangeable K represent the available pool. The removal of water soluble K before extraction with ammonium acetate is not recommended because as the salt content of soil solution is decreased, the adsorption of divalent cations in solution increases.

5.2. Reagents

- *Neutral 1N ammonium acetate solution (CH_3COONH_4)*

Dissolve 77.08 g of ammonium acetate in distilled water and made up to 1 L. Adjust the pH if necessary to 7 with acetic acid or ammonium hydroxide.

- *Standard K solution*

Dissolve 1.908 g of dried potassium chloride (KCl) AR grade in distilled water and made up to 1 L so as to get 1000 $\mu g\ mL^{-1}$ K solution.

5.3. Procedure

Extraction

Shake 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filter immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded. Potassium concentration in the extract is determined using flame photometer after necessary setting and calibration of the instrument.

Standard curve for potassium

Dilute measured aliquots from the standard solution using ammonium acetate solution to give concentrations of 5 to 20 $\mu g\ mL^{-1}$ K. After attaching the appropriate filter and adjusting the gas and air pressure, set reading in the flame photometer as zero for the blank (ammonium acetate) and at 100 for 20 $\mu g\ mL^{-1}$ K. The curve is obtained by plotting the readings against the different concentrations (5, 10, 15 and 20 $\mu g\ mL^{-1}$) of K. Fluctuation in gas and air pressure does not allow steady reading in the meter and must be taken care of.

5.4. Calculation

$$\text{Available K (mg kg}^{-1}\text{ soil)} = \mu g\ K\ mL^{-1} \text{ of the aliquot} \times \frac{25}{5}$$

$$\text{Available K (mg kg}^{-1}\text{ soil)} = \mu g\ K\ mL^{-1} \text{ of the aliquot} \times 5$$

Available K (kg ha^{-1}) = Available K (mg kg^{-1} soil) x 2.24

6. Available Calcium and Magnesium

As in the case of potassium, exchangeable plus water soluble calcium and magnesium contribute to the plant available pool. The neutral normal ammonium acetate extracts the pools of calcium and magnesium also along with potassium and sodium.

6.1. Principle

In the absence of excess of $CaCO_3$, the cations Ca^{2+}, Mg^{2+} and Na^+ along with K^+ appear to be completely exchangeable by neutral normal ammonium acetate.

6.2 Reagents

- Neutral normal ammonium acetate solution (CH_3COONH_4)

Dissolve 77.08 g of ammonium acetate in distilled water and made up to 1 L. Adjust the pH if necessary to 7 with acetic acid or ammonium hydroxide.

6. 3. Procedure

Extraction of available Calcium and Magnesium

Shake 5 g of soil with 25 mL of neutral normal ammonium acetate for 5 minutes and filter immediately through a dry Whatman No.42 filter paper. First few mL of the filtrate may be discarded.

Estimation of Calcium and Magnesium by Atomic Absorption Spectrophotometry

From the soil extract Ca and Mg can be estimated by Atomic Absorption Spectrophotometry (AAS). The chemical interference, resulting from the formation of stable compounds between Ca and Mg ions and the accompanying anions may reduce the absorption. This interference may be overcome by using a realising agent such as Lanthanum or Strontium.

6.4. Calculation

$$\text{Available Ca/Mg (mg kg}^{-1}\text{ soil)} = \mu\text{g Ca/Mg mL}^{-1}\text{ of the aliquot} \times \frac{25}{5}$$

$$\text{Available Ca/Mg (mg kg}^{-1}\text{ soil)} = \mu\text{g Ca/Mg mL}^{-1}\text{ of the aliquot} \times 5$$

7. Available Sulphur (By $CaCl_2$ Extraction)

7.1 Principle

Different reagents have been proposed for extracting plant available sulphur from the soil. These include water, salt solutions such as 0.15% $CaCl_2$, 500 ppm P as $Ca(H_2PO_4)_2$ or KH_2PO_4 and acidic solutions such as 0.5 *N* ammonium acetate plus 0.25 *N* acetic acid and Bray No. 1. Generally phosphate solutions extract more sulphate sulphur from soils than can be extracted with water or salt solutions because phosphate ions displace the adsorbed sulphate, which is known to be readily available to plants.

7.2 Reagents

- *0.15% $CaCl_2$ solution*

Dissolve 1.5 g of Calcium chloride dehydrate ($CaCl_2.2H_2O$) in about 500 mL of distilled water and make up the volume to 1L.

- *Gum acacia solution*

Dissolve 0.25 g of chemically pure gum acacia in hot water and filter the hot solution through Whatman No. 42 filter paper. Cool the filtrate and dilute to 100 mL.

- *Barium chloride ($BaCl_2$)*

Grind analytical grade $BaCl_2$ to pass through 1 mm sieve.

- *Concentrated standard sulphate solution (100 mg S L^{-1})*

Dissolve 0.5434 g oven dried AR potassium sulphate (K_2SO_4) in distilled water and make up to 1L.

7.3. Procedure

Extraction (Tabatabai, 1982)

Shake 10 g of air-dried processed soil with 50 mL of 0.15% $CaCl_2$ solution in a 250 mL conical flask for 30 minutes. Filter the extract through Whatman No. 42 filter paper and estimate the sulphate content by turbidimetric procedure.

Preparation of standard curve

1. Pipette out 0, 0.25, 0.5, 0.75, 1.0, 1.25, and 2.5 ml of standard sulphate solutions in separate 25 mL volumetric flasks and add 10ml of extracting solution (0.15% $CaCl_2$). Prepared fresh standards each time when a batch of sample is analysed.
2. Add 1 g of $BaCl_2$ crystals to each flask and swirl to dissolve.
3. Add 1 mL of 0.25% gum acacia solution, make up the volume with distilled water and shake well.
4. Within 5-30 minutes of development of turbidity, read the absorbance at 440 nm on a spectrophotometer.

5. Draw the standard curve with absorbance on Y axis and concentration on X axis.

Turbidimetric estimation of Sulphur (Massoumi and Cornfield, 1963)

1. Pipette out10 mL of the soil extract into a 25 mL volumetric flask.
2. Add 1 g of $BaCl_2$ crystals and swirl to dissolve.
3. Add 1 mL of 0.25% gum acacia solution, make up the volume with distilled water and shake well.
4. Within 5-30 minutes of development of turbidity, read the absorbance at 440 nm on a spectrophotometer.

7.4. Calculation

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument} \times \frac{25}{10} \times \frac{50}{10}$$

$$\text{Amount of sulphur (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for the sample}}{\text{Slope of Std. curve}} \times 12.5$$

8. Iron, Manganese, Zinc and Copper

8.1. Principle

The major categories of micronutrient extractants presently in use are dilute acids, and solutions containing chelating agents, such as DTPA or EDTA.

Dilute acids (0.025-0.1 *M*) have been used as micronutrient extractants for many years, primarily on acidic soils. Their applicability is confined to acidic soils because they generally are not sufficiently buffered to extract meaningful levels of micronutrients from calcareous soils. Acidic extractants do not have a particularly sound theoretical basis, but, due to their extensive use in field and laboratory studies, a well developed database exists relating acid extractable levels of micronutrients to crop response. The most commonly used dilute acids are: Mehlich – 1 (dilute double acid, 0.0125 M H_2SO_4 + 0.05 M HCl) and 0.1 M HCl (Sims and Johnson, 1991).

Among the chelating agents, DTPA is the most commonly used one. The DTPA soil test, developed for near neutral and calcareous soil by Lindsay and Norvell (1978) illustrates the evolution of a soil test extractant from theoretical principles. The extracting solution consists of 0.005 M DTPA and 0.01 M $CaCl_2.2H_2O$, buffered at pH 7.3 by 0.1 M triethanolamine (TEA). The DTPA extractant offered the most favourable combination of stability constants necessary to simultaneously extract four micronutrient cations (Fe, Mn, Cu and Zn). The buffered pH and presence of soluble Ca^{2+} prevent excessive dissolution of calcium carbonate avoiding the release of unavailable micronutrients occluded by this solid phase. At pH 7.3, 70-80% of the buffering capacity provided by TEA has been consumed. Therefore use of DTPA extractant on acidic soils, will result in neutralisation of remaining buffer capacity and unpredictable extraction pH.

8.2. Reagents

- *Hydrochloric acid (HCl), 0.1 N*

Add 8.1 mL of concentrated HCl (reagent grade) to approximately 900 mL of distilled water, mix cool to room temperature and make up to 1 L.

- *DTPA*

Prepare the extractant by dissolving 1.967 g of DTPA, 14.92 g of TEA and 1.47 g of $CaCl_2.2H_2O$ in 200 mL of distilled water and dilute to approximately 900 mL. Adjust the pH to 7.3 ± 0.05 with 1:1 HCl and make up the volume to 1 L.

8.3. Estimation of Fe, Mn, Zn, and Cu in Acid Soils (pH < 6.5)

8.3.1. Extraction and estimation

Shake 2 g of soil with 20 mL of 0.1 *M* HCl for 5 minutes. Filter through Whatman No. 42 filter paper. Collect the filtrate and estimate the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

8.3.2. Calculation

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \frac{\text{Concentration from the instrument} \times 20}{2}$$

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument} \times 10$$

8.4. Estimation of Fe, Mn, Zn, and Cu in near neutral to alkaline soils (pH > 6.5)

8.4.1. Extraction and estimation

Shake 10 g of soil with 20 mL of DTPA for 2 hours. Filter through Whatman No. 42 filter paper. Collect the filtrate and estimate the contents of Fe, Mn, Zn and Cu using an Atomic Absorption Spectrophotometer.

8.4.2. Calculation

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \frac{\text{Concentration from the instrument} \times 20}{10}$$

$$\text{Amount of micronutrient (mg kg}^{-1}\text{ soil)} = \text{Concentration from the instrument} \times 2$$

9. Available Boron (Hot-Water Soluble Boron (Gupta, 1967))

9.1. Principle

Although there are a variety of chemical tests for predicting crop response to boron, the hot water extraction procedure developed by Gupta (1967) is the easiest method

9.2. Reagents

- *Buffer solution*

Dissolve 250 g 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.

- *Azomethine-H reagent*

Dissolve 0.45 g of azomethine-H in 100 ml of 1% L-ascorbic acid solution. Store in polypropylene bottle in a refrigerator. Prepare fresh solution every week.

- *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 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 of B mL^{-1}, respectively.

- *Activated charcoal.*

9.3. Procedure

Extraction and Estimation

1. Weigh 20 g of air-dried processed soil in 250 mL quartz or other boron-free conical flask and add 40 mL 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, diluted boron standard, or sample solution into a 10 mL polypropylene tubes
4. Add 2 mL of buffer 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.

9.3.2. Calculation

$$\text{Amount of B in soil (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance reading}}{\text{Slope from curve}} \text{ x } \frac{40}{20}$$

$$\text{Amount of B in soil (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance reading}}{\text{Slope from curve}} \text{ x } 2$$

10. Estimation of Lime Requirement

10.1. Principle:

A buffer solution (pH7.5) is prepared by dissolving p-nitrophenol triethanolamine, potassium chromate, calcium acetate and calcium chloride in distilled water. The soil is equilibrated with buffer solution, and the pH of soil buffer suspension is determined by a pH meter. The drop in the pH of buffer on equilibration with the soil depends on the extractable acidity which forms the basis of estimation of lime requirement to bring the soil to a desired pH. The lime requirement is noted from a table giving pH of soil buffer suspension and the corresponding lime requirement to bring the soil to a pH of 6.0, 6.4 and 6.8

10.2. Reagents

Buffer solution: Dissove 1.8 g p- nitrophenol ,2.5 ml triethanolamine ,3.0 g potassium chromate, 2.0 g calcium acetate and 53.1 g calcium chloride dehydrate in distilled water and make the volume to 1 l. Adjust the pH of the buffer solution to 7.5 with sodium hydroxide.

10.3. Procedure:

Transfer 10 g air dry 2 mm soil sample having a pH of 6.3 or below into a 150 ml conical flask. Add 10 ml distilled water and 20 ml buffer solution. Stopper and shake the conical flask on a mechanical shaker at moderate speed for 10 minutes. Remove the conical flask from the shaker and pour the suspension immediately after shaking into a 100 ml beaker. Measure the pH of soil water buffer suspension by a pH meter. Note down the pH and record it as pHb. Refer to the table giving the requirement of pure $CaCO_3$ in t/acre at desired pH of 6.0, 6.4 and 6.8 against the pH of the soil water buffer i.e. pHb. Note down the lime requirement (LR) from the table Table.10.1.a. The amount may be converted in terms of locally available lime stone on the basis of % $CaCO_3$ equivalence using the following equation.

$$\text{Quantity of liming material} = \frac{\text{Quantity of calcium carbonate needed X100}}{\%\ CaCO_3\ \text{equivalence}}$$

Table10.1.a. Lime required to bring the soil to an indicated pH according to pH of the soil buffer suspension (Shoemaker *et al.* 1961).

pH of soil buffer	**Pure $CaCO_3$ in t/acre**		
	Indicated pH		
Suspension	**pH 6.0**	**pH 6.4**	**pH 6.8**
6.7	1.0	1.2	1.4
6.6	1.4	1.7	1.9
6.5	1.8	2.2	2.5
6.4	2.3	2.7	3.1
6.3	2.7	3.2	3.7
6.2	3.1	3.7	4.2
6.1	3.5	4.2	4.8
6.0	3.9	4.7	5.4
5.9	4.4	5.2	6.0
5.8	4.8	5.7	6.5
5.7	5.2	6.2	7.1
5.6	5.6	6.7	7.7
5.5	6.0	7.2	8.3
5.4	6.5	7.7	8.9
5.3	6.9	8.2	9.4
5.2	7.4	8.6	10.0
5.1	7.8	9.1	10.6
5.0	8.2	9.6	11.2
4.9	8.6	10.1	11.8
4.8	9.1	10.6	12.4

Multiply t/acre by 2.47 to obtain t/ha

11. Calcium Carbonate Equivalence (Neutralizing Value) of Liming Material

11.1. Principle:

A small dried ground lime stone sample passed through 0.26mm sieve is made to react with known volume of standard 1N HCl and the unreacted acid titrated back with standard 1N sodium hydroxide using phenolphthalein indicator. The milliequivalence of base neutralized by the acid in 100 g sample is estimated and is multiplied by the molecular weight of CaCO3 and the product is divided by the milliequivalence of base present in one mole of CaCO3 to obtain the percentage $CaCO_3$ equivalence

11.2. Reagents

- *10N HCl*

Dilute 218.2 mL of AR grade HCl (Sp.gravity-1.18, minimum assay 35.4%) to 250 mL with distilled water.

- *1N HCl*

Dilute 25 mL of 10 N HCl to 250 mL with distilled water. Standardize the reagent against 1N Na_2CO_3 using methyl orange indicator.

- *0.1% Methyl Orange*

Dissolve 0.1 g of methyl orange in distilled water and make up the volume to 100 mL.

- *1% Phenolphthalein indicator*

Dissolve 1 g of phenolphthalein in 100 ml of 95% ethanol. If there is precipitate, filter the solution.

- *1N Na_2CO_3*

Dissolve 53 g of Na_2CO_3 in distilled water and make up to 1litre.

- *1N NaOH*

Dissolve 40 g of NaOH in distilled water and make up to 1 litre and standardize with standard 1N HCl

11.3. Procedure

- *Neutralization of sample by 1N HCl*

Transfer 1 g of ground 0.26 mm sieved sample into a 500 mL conical flask. Add 25 mL standard 1N HCl. Swirl the suspension to mix thoroughly and then heat nearly to boiling. Keep the flask on a steam bath for about 5-45 minutes to complete the reaction. Then add 100 mL of distilled water and boil the solution exactly for 1 minute and then cool to room temperature.

- *Back titration*

Add five drops of phenolphthalein into the above conical flask containing sample and unreacted acid. Back titrate with 1N NaOH till pink colour persists.

11.4. Calculation

% $CaCO_3$ equivalence

$$\text{(Neutralizing Value)} = \frac{\text{mL of reacted acid x normality x 100 x Mol.Wt. of } CaCO_3}{\text{mass of sample(g)x meq. of base in 1 mole of } CaCO_3}$$

$$= \frac{\text{(mL of 1N HCl added – mL of NaOH(TV))x 1 x 100 x 100}}{\text{1 g x 2000}}$$