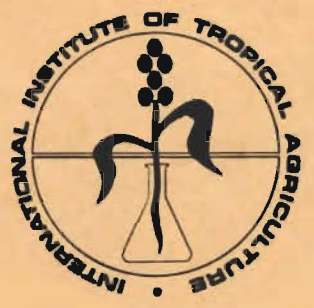
SELECTED METHODS FOR SOIL AND PLANT ANALYSIS

A. S. R. JUO
(Editor)



Manual Series No. 1
Second Edition

International Institute of Tropical Agriculture
Ibadan, Nigeria

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FOREWORD

This laboratory manual of selected methods for soil and plant analysis has been compiled for use by research assistants, technicians and student trainees working in soils laboratories at the International Institute of Tropical Agriculture (IITA). It is intended to be a working manual and therefore detailed descriptions of methodology and instrumentation are not given because they can be found in the original publications listed in the reference section.

The effective CEC method has been adopted by the IITA soils laboratories as a standard routine procedure for soil cation exchange capacity determinations. Although conventional procedures for total N determinations in soil and plant materials are given in the text, in the IITA laboratories total N content of the Kjeldahl digests is determined colorimetrically using a Technicon Auto-analyzer.

For routine soil analysis, we have recently acquired the relatively inexpensive soil testing equipment manufactured by the Custom Laboratory Equipment Company of Raleigh, North Carolina, U.S.A., which enables us to process a large number of soil samples for Bray P, pH and extractable cations.

A.S.R. Juo
Soil Chemist

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SOIL pH DETERMINATION

I *Apparatus:*

1. Glass-electrode pH meter

II *Reagents:*

1. 0.01M calcium chloride
2. Distilled water
3. 1N potassium chloride

III *Procedure:*

(A) Soil pH in water (1:1 soil/water ratio).

1. Weigh 20 g of air-dry soil passed (2 mm sieve) into a 50 ml beaker. Add 20 ml of distilled water and allow to stand for 30 minutes and stir occasionally with a glass rod.
2. Insert the electrodes of the pH meter into the partly settled suspension and measure the pH. Do not stir the suspension during measurement.
3. Report result as "soil pH measured in water."

(B) Soil pH in 0.01M $CaCl_2$.

1. Prepare a 1:2 (soil 0.01M $CaCl_2$) suspension (10 g of soil and 20 ml of solution).
2. Let the suspension stand for about 30 minutes and stir occasionally with a glass rod.
3. Measure pH and report result as "soil pH measured in 0.01M $CaCl_2$."

(C) Soil pH in 1N KCl (1:1 soil to solution ratio, 30 min).

IV *References:*

1. Bates R. G. 1954 – Electrometric pH Determinations.
2. Clark W. M. 1923 – The Determination of Hydrogen Ions.
3. ASA Monograph No. 9 – Methods of Soil Analysis.

HYDROMETER METHOD OF MECHANICAL ANALYSIS

I *Apparatus and Reagents:*

1. Multimix machine with baffled "milkshake" cups
2. Glass cylinders of approximately one liter capacity for containing soil suspension during settling
3. Special hydrometers for measuring density of soil suspension
4. Thermometers for measuring temperature of the suspension
5. Sodium hexametaphosphate dispersing agent
6. A 300 mesh sieve

II *Procedure:*

Weigh 51.0 g of air-dry soil which has been passed through a 2 mm sieve and transfer to a "milkshake" mix cup. (A 51.0 g air-dry sample represents approximately 50.0 g of oven-dry soil). If the soil is estimated to contain 75% or more sand, 101.0 g of soil are used. Add 50 cc. of 5.0% sodium hexametaphosphate along with 100 cc. of distilled water. Mix with a stirring rod and let sample set for 30 minutes.

Stir the soil suspension for 15 minutes with the multimix machine. Transfer the suspension from the cup to the glass cylinder. With the hydrometer in the suspension, add distilled water to the lower blue line. The volume will then be 1130 cc. Use upper line (1250 cc) when 100 grams are used. Remove hydrometer.

Cover top of cylinder with the hand and invert several times until all soil is in suspension. Place cylinder on flat surface and note time. Immediately place soil hydrometer into suspension. Slide slowly into suspension until hydrometer is floating. The first reading on the hydrometer is taken at 40 seconds after the cylinder is set down. Remove hydrometer and record temperature of suspension with a thermometer.

After the first hydrometer reading let the suspension stand for 3 hours and take a second reading. Also take the temperature of the suspension. The first reading measures the percentage of silt and clay in suspension. The second reading indicates the percentage of 2 micron (total) clay in the suspension.

Results are corrected to a temperature of 68° Fahrenheit. For every degree over 68° add 0.2 to hydrometer reading before computation and for under 68° subtract 0.2 from hydrometer reading (see sample calculation). Avoid extremes such as 50° or 100°. Also subtract 2.0 from every hydrometer reading to compensate for the added dispersing agent.

A check on (or a substitute for) the 40 second reading can be made by sieving the entire suspension through a 300 mesh sieve to remove sand. Dry the sand in an oven at 100° C. Sift to remove any remaining silt and weigh. Multiply weight by 2 and this is the percentage of sand in the soil.

Sample Calculation:

Given: 1a. Hydrometer reading at 40 seconds, $H_1 = 18$

1b. Temperature at 40 seconds, $T_1 = 75°$ F

2a. Hydrometer reading at 3 hours, $H_2 = 8$

2b. Temperature at 3 hours, T_2 = 63°F.

3. Temperature correction to be added to hydrometer reading = 0.2 (T-68), where T = degrees Fahrenheit.

4. Salt correction to be added to hydrometer reading = −2.0

Calculations:

A. SAND = $100.0 - [H_1 + 0.2 (T_1 - 68) - 2.0]\ 2 = 65.2\%$

B. CLAY = $[H_2 + 0.2 (T_2 - 68) - 2.0]\ 2 = 10.0\%$

C. SILT = 100.0 − (% sand + % silt) = 24.8%

Report in table form the percentages by weight of sand, silt and clay for the soils used. Show individual and arithmetic averages for each soil.

III *References:*

1. Bouyoucos, G.H. A recalibration of the hydrometer for making mechanical analysis of soils. Agron. Jour. 43:434–438. 1951.

2. Day. P.R. Experimental confirmation of hydrometer theory. Soil Sci. 75: 181 – 186. 1953.

3. Davidson, D.T. Mechanical analysis of soils. Iowa Engineering Experiment Station Report No. 21. 1954–1955.

DETERMINATION OF ORGANIC CARBON IN SOIL
(Walkey-Black Method)

I *Apparatus:*

Burettes, 50 ml or 25 ml.

II *Reagents:*

1. Potassium dichromate ($K_2Cr_2O_7$) 1N – Dissolve 49.04 g of $K_2Cr_2O_7$ in distilled water and dilute to 1 liter
2. H_2SO_4, conc. – If chloride (Cl) is present in the soil add Ag_2SO_4 to the acid at the rate of 15 g per liter
3. O-phosphoric acid (H_3PO_4), conc.
4. O-phenanthroline-ferrous complex 0.025 M (Ferroin). When Ferroin indicator is not available it can be prepared as follows:

   Dissolve 14.85 g of O-phenanthroline monohydrate and
   6.95 g of $FeSO_4 7H_2O$ in water and dilute to 1 liter

5. Barium diphenyl amine sulfonate (0.16%) – Optional. Can be used in place of O-phenanthroline-ferrous complex
6. Ferrous sulphate (0.5N) – Dissolve 140 g of $FeSO_4.7H_2O$ in water; add 15ml conc. H_2SO_4 cool and dilute to 1 liter. (Standardize this reagent daily, or each time before using for organic C determination, by titrating against 10 ml 1N $K_2Cr_2O_7$)

III. *Procedure:*

1. Take a representative sample and grind to pass through 0.5 mm sieve.
2. Weigh out soil samples in duplicate and transfer to 250 ml Erlenmeyer flask. (1.00 g should be used if the organic C content is between 1 and 3%; 2.00 g if it is less than 1%).
3. Pipette 10 ml of 1N $K_2Cr_2O_7$ solution accurately into each flask and swirl gently to disperse the soil.
4. Add rapidly 20 ml conc. H_2SO_4 using an automatic pipette, directing the stream into the suspension. Immediately swirl the flask gently until soil and reagents are mixed, then swirl more vigorously for one minute. Rotate beaker again and allow the flask to stand on a sheet of asbestos for about 30 minutes.
5. Add 100 ml of distilled water after standing for 30 minutes.

8. Calculate the results according to the following formula:

$$\% \text{ Organic C in Soil (air-dry basis)} = \frac{(\text{me } K_2Cr_2O_7 - \text{me } FeSO_4) \times 0.003 \times 100 \times (f)}{\text{g of air-dry soil}}$$

Correction factor, f = 1.33

me = Normality of solution x ml of solution used

% Organic matter in soil = % Org. C x 1.729

9. % Organic C may also be expressed on oven-dry basis after correction for moisture content in air-dry soil.

IV References:

1. Methods of Soil Analysis Part 2 --ASA Monograph No. 9 1965.
2. Walkley A. and Black C.A. (1934) Soil Sci. 37:29 – 38.
3. Piper C.S. (1942) Soil and Plant Analysis.

6. Add 3 - 4 drops of indicator and titrate with 0.5N ferrous sulphate solution. As this end point is approached, the solution takes on a greenish cast and then changes to dark green. At this point, add the ferrous sulfate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background).

7. Make the blank titration in the same manner, but without soil (step 3, 4, 5 and 6), to standardize the dichromate, $Cr_2O_7^{2-}$.

DETERMINATION OF EXCHANGEABLE ACIDITY IN SOIL (Titration Method)

I *Apparatus:*

Burettes, 50 or 25 ml.

Centrifuge tubes, 45 or 50 ml.

II *Reagents:*

(A) KCl, 1N

(B) 0.05N NaOH standard

(C) 0.05N HCl standard

(D) NaF (Sodium Fluoride) solution: Dissolve 40 g of NaF in 1 liter of water

(E) Phenolphthalein indicator: Dissolve 0.1 g of the dry indicator powder in 100 g of 95% ethanol

III *Procedure:*

Extraction with N KCl

1. Weigh 5 g of air-dry soil (passed 2 mm sieve) into a 45 ml centrifuge tube and add 30 mls of 1 N KCl. Cover the centrifuge tightly with a rubber stopper and shake for 1 hour on a reciprocal shaker.

2. Centrifuge the content at 2,000 rpm for 15 minutes. Carefully decant the clear supernatant into a 100 ml volumetric flask.

3. Add another 30 ml of 1N KCl to the same soil sample and shake for 30 minutes, then repeat step 2 and transfer the clear supernatant into the same volumetric flask.

4. Repeat step 3 for the third time and again combine the clear supernatant into the same volumetric flask. Make up the volume to mark with 1N KCl.

Titration for H and Al

1. Pipette 25 mls of KCl extract into a 250 ml Erlenmeyer flask (Pipette 50 ml if the soil pH value is above 5.0), add approximately 100 ml of distilled water.

2. Add 5 drops of phenolphthalein indicator and titrate the solution with 0.05N NaOH to a permanent pink end point with alternate stirring and standing. If needed, add a few more drops of indicator to replace that adsorbed by the precipitate of $Al(OH)_3$.

3. The amount of base used is equivalent to the total amount of acidity (H + Al) in the aliquot taken.

4. To the same flask, add 1 drop of 0.05N HCl to bring the solution back to the colorless condition, and add 10 ml of NaF solution. While stirring the solution constantly, titrate the solution with 0.05N HCl until the color of the solution disappears. Add 1 or 2 drops of indicator. If the color appears, continue addition of 0.05N HCl until the color disappears and does not return within 2 minutes. The milliequivalents of acid used are equal to the amount of exchangeable Al.

5. Subtract this value from the milliequivalent of total acidity from the base titration to obtain the milliequivalent of exchangeable H.

6 Express the exchangeable H and Al in me per 100 g of soil.

IV *References:*

McLean, E.O. (1965) Aluminum. In C.A. Black (ed). Methods of Soil Analysis. Part 2. pp. 986–994. Agronomy No. 9.

Note: Al in KCl extract can also be determined colorimetrically using the aluminon method.

DETERMINATION OF EXCHANGEABLE Ca, Mg, K, Na, Mn, AND EFFECTIVE CEC IN SOIL

I *Apparatus:*

1. Centrifuge
2. 100 ml volumetric flask
3. Flame photometer
4. Atomic absorption spectrophotometer

II *Reagents:*

1. Acetic acid, glacial and NH_4OH, conc.
2. Ammonium Acetate solution, 1N. pH7.0 — Add 58 ml of glacial acetic acid to about 600 ml of distilled water in a 2 liter beaker. Add 70 ml concentrated NH_4OH (specific gravity 0.90). The NH_4OH is best added under a fume hood through a long stemmed glass funnel so that it is introduced into the bottom of the acid solution. Cool this solution and adjust to pH7.0 with acetic acid or NH_4OH using a pH meter. Transfer the solution into a 1 liter volumetric flask and dilute to volume. Mix it and store it in a Pyrex reagent bottle.

III *Procedure:*

1. To 5 g of soil sample, add 30 ml of 1N NH_4OAC and shake on a mechanical shaker for 2 hours.
2. Centrifuge (2,000 r.p.m for 5—10 min). Carefully decant the clear supernatant into a 100 ml volumetric flask.
3. Add another 30 ml of NH_4OAC solution and shake for 30 minutes; centrifuge and transfer the supernatant into the same volumetric flask.
4. Repeat step 3 once more and transfer the supernatant again into the same volumetric flask.
5. Make up to mark with the NH_4OAC solution.
6. Determine K, Na and Ca on a flame photometer. Determine Mg and Mn on an atomic absorption spectrometer.
7. Effective CEC is thus calculated by the sum of exchangeable bases (Ca, Mg, K, Na, Mn and exchangeable Al and H, expressed in me/100 g.

Note: For soils containing an appreciable amount of soluble salts, appropriate correction should be made.

IV *References:*

1. Jackson, M.L. 1958 — Soil Chemical Analysis.
2. A. S. A. Monograph, No. 9 Part 2 — Methods of Soil Analysis.

DETERMINATION OF AVAILABLE PHOSPHORUS IN SOIL (Bray No. 1 Method)

I *Apparatus:*

1. Centrifuge
2. Mechanical shaker
3. 20 ml test-tube
4. B & L Spectronic–20 Electrophotometer

II *Reagents:*

1. Ammonium Fluoride (NH_4F), 1N: Dissolve 37 g of NH_4F in distilled water and dilute to 1 liter. (Store in polyethylene bottle).
2. HCl, 0.5N: Dilute 20.2 ml of Conc HCl to 500 ml with distilled water under fume hood.
3. Extracting solution:- Add 15 ml of 1.0N NH_4F and 25 ml of 0.5N HCl to 460 ml distilled water.
4. Stannous Chloride ($SnCl_2.2H_2O$) stock solution:- Dissolve 10 g of $SnCl_2.2H_2O$ in 25 ml of concentrated HCl. Store in a dark polyethylene bottle in refrigerator. Prepare a fresh solution every 6 weeks.
5. Ammonium Molybdate $(NH_4)\ Mo_7O_{24}.4H_2O$:- Dissolve 15 g of ammonium Molybdate in 350 ml 10N HCl in a litre volumetric flask. Cool to room temperature and dilute to 1 liter with distilled water.
6. Stannous Chloride ($SnCl_2.2H_2O$) dilute solution:- Mix 1 ml of $SnCl_2$ stock solution with 333 ml distilled water. Make fresh solution every 2 hours as needed.

III *Procedure:*

1. Weigh 1 g of air-dried soil sample (passed 2 mm sieve) into a 15 ml centrifuge tube and add 7 ml of the extracting solution.
2. Shake for 1 minute on a mechanical shaker and centrifuge the suspension at 2,000 rpm for 15 minutes.
3. Pipet 2 ml of the clear supernatant into a 20 ml test tube.
4. Add 5 ml distilled water and 2 ml of ammonium molybdate solution.
5. Mix content properly and add 1 ml of $SnCl_2 \cdot 2H_2O$ dilute solution and mix again.
6. After 5 minutes, but not later than 20 minutes, measure % transmittance on the spectronic-20 electrophotometer at 660 mu wave length.
7. Prepare standard curve within the range of 0.1 μgP/ml. (or ppm P).
8. Plot the optical density (O.D.) of standard solution against the μgP/ml (or ppm P) and calculate the content of extractable P in soil.

IV *References*

1. Bray R.H. and Kurtz, L.T. 1945 – Determination of total, organic and available forms of P in soil. Soil Sci. 59: 39–45.
2. Jackson, M.L. 1958 – Soil Chemical Analysis

OLSEN'S TEST FOR SOIL AVAILABLE PHOSPHORUS

I *Apparatus:*

1. Bausch & Lomb Spectronic-70 Electrophotocolorimeter.
2. Mechanical shaker.
3. 25 ml volumetric flasks or 35 ml pyrex test tubes marked for 25 ml.

II *Reagents:*

1. Olsen's extracting solution:
   Sodium bicarbonate ($NaHCO_3$) solution, 0.5M: Adjust the pH of this solution to 8.5 with 1M NaOH. Add mineral oil to avoid exposure of the solution to the air. Prepare a fresh solution before use if it has been standing over one month in a glass container. Store the solution in a polyethylene container for periods longer than one month, but check the pH of the solution each month.
2. Carbon black: Use carbon black G (Fisher Scientific Company, Cat. No. C-179) as received.

III *Procedure:*

1. Add 2 g of soil, 1 teaspoon of carbon black and 40 ml of the extracting solution to a 125 ml Erlenmeyer flask. Shake the flask for 30 minutes on a mechanical shaker.

   Filter the suspension through the Whatman No. 40 paper. Add more carbon black if necessary to obtain a clear filtrate. Shake the flask immediately before pouring the suspension into the funnel. Store the solution for P determination using the colorimetric method as given in a separate section (Ascorbic acid method).

IV *References:*

1. Watanabe, F.S. and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soils. SSSA Proc. 29: 677–678.
2. Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A.Dean. 1954. Estimation of available P in soils by extraction with $NaHCO_3$. USDA Cir. 939.

TOTAL NITROGEN IN SOIL
(Regular Macro-Kjeldahl Method)

I *Apparatus;*

1. Macro-Kjeldahl digestion-distillation apparatus
2. Macro-Kjeldahl flasks, 500 ml and 750 ml

II *Reagents:*

1. Mercury catalyst tablets: If the ready-made mercury catalyst tablets are not available, the catalyst mixture can be prepared as follows:

   Mix 190 g of K_2SO_4 and 5 g of HgO and grind the mixture in a mortar.

2. Mixed boric acid-indicator solution – Dissolve 80 g boric acid (H_3BO_3) in 3800 ml of distilled or deionized water by heating on a hot plate at low heat. Cool the solution, then add 80 ml of the mixed indicator solution (prepared separately by dissolving 0.099 g of bromocresol green and 0.066 g of methyl red in 100 ml of 95% ethanol). Add 0.1 N NaOH through a burette until the solution becomes reddish-purple in color (pH 5.0). Dilute the solution to 4 liters with distilled or deionized water. Mix the solution thoroughly before use.

3. Sodium hydroxide, NaOH, approximately 10 N – Weigh 2.1 kg of NaOH pellets in a heavy-walled 5 liter pyrex glass bottle or flask, add 2 liters of distilled water and swirl the flask until the alkali is dissolved. Cool the solution with a stopper in the neck of the flask to prevent absorption of atmospheric CO_2, and allow it to stand for several days to permit any Na_2CO_3 present to settle. Siphon the clear supernatant liquid into a large pyrex bottle which contains about 1 liter of CO_2-free water and is marked to indicate a volume of 5 liters, and make the solution to 5 liters by adding CO_2-free water. Swirl the bottle vigorously to mix the contents, and fit the neck with some arrangement which permits the alkali to be stored and dispensed with protection from atmospheric CO_2.

4. H_2SO_4, conc. AR.

5. HCl (or H_2SO_4) 0.01 N standard

6. K_2SO_4, AR.

III *Procedure:*

1. Weigh 5 to 10 g of soil sample containing about 10 mg of N (air-dried, grind to pass 0.5 mm sieve) in a dry 500 ml macro-Kjeldahl flask, add 20 ml of distilled water. Swirl the flask for a few minutes, then allow it to stand for 30 minutes.

2. Add 1 tablet of mercury catalyst (or 1 g of the K_2SO_4 – HgO mixture catalyst) and 10 g of K_2SO_4. Then add 30 ml of conc. H_2SO_4 through an automatic pipet.

3. Heat the flask cautiously at low heat on the digestion stand. When the water has been removed and frothing has ceased, increase the heat until the digest clears. Then boil the mixture for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about half the way up the neck of the flask.

4. Allow the flask to cool and slowly add about 100 ml of water to the flask.

5. Carefully transfer the digest into another clean macro-Kjeldahl flask (750 ml). Retain all sand particles in the original digestion flask because sand can cause severe bumping during Kjeldahl distillation. Wash the sand residue with 50 ml of distilled water four times and transfer the aliquot into the same flask.

6. Add 50 ml H_3BO_3 indicator solution into a 500 ml Erlenmeyer flask which is then placed under the condenser of the distillation apparatus. The end of the condenser is about 4 cm above the surface of the H_3BO_3 solution.

7. Attach the 750 ml Kjeldahl flask to the distillation apparatus. Pour about 150 ml of 10N NaOH through the distillation flask by opening the funnel stopcock. Commence distillation

8. Keep condenser cool (below 30C) by allowing sufficient cold water to flow through and regulate heat to minimize frothing and prevent suck-back.

9. Collect 150 ml distillate and then stop distillation.

10. Determine the NH_4-N in the distillate by titrating with 0.01N standard HCl (or H_2SO_4) using a 25 ml burette graduated at 0.1 ml intervals. The color change at the end point is from green to pink.

11. Calculate the % N content in soil.

IV *Alternate Methods:*

1. Total N (NH_4-N) in Kjeldahl Digest can also be determined colorimetrically using a Technicon auto-analyzer thus eliminating the distillation process.

2. Digestion can also be carried out using a Tecator Model 20 Soil Digestor rather than Macro-Kjeldahl apparatus. *(Supplier's Address: Tecator, Fack S-26301, Hoganas, Sweden).*

V *References:*

1. ASA Monograph No.9. Methods of Soil Analysis. Part II, 1965.

2. Jackson, M.L. 1962. Soil Chemical Analysis.

DETERMINATION OF NITRATE IN SOIL

I *Apparatus:*

B & L Spectronic-20 Electrophotometer

II *Reagents:*

1. Brucine, 2.5 per cent. Dissolve 2.5 g of brucine or brucine sulfate in 100 ml of glacial acetic acid. Store in the dark. This reagent should be handled cautiously as it is very toxic.
2. Sulfuric acid, conc., sp. gr. 1.84.
3. Standard nitrate nitrogen solution, 50 ppm. Dissolve 0.1805 g of KNO_3 in 500 ml of the extracting solution. Add 0.5 ml of chloroform as a preservative.
4. Dilute nitrate standard solutions. Carry through the procedure, simultaneously with each set of soil extracts, 1 ml aliquot of each of the standards. Measure the transmittance of these solutions and construct a calibration curve by plotting on semilog graph paper the transmittancy against concentration or mark the colorimeter scale to read directly in pounds of NO_3-N per acre.

III *Extraction:*

Extracting solution: Dissolve 100 g Na-acetate $NaC_2H_3O_2$ in about 500 ml of distilled water and add 30 ml of 99.58 acetic acid. Dilute with distilled water to 1 liter. This solution is identical with that proposed by Morgan.

Extraction: Transfer 5.0 g of soil into a shaking bottle and add ¼ teaspoon (about 0.25 g) of activated carbon and 20 ml of extracting solution. Shake for 1 minute and filter.

IV *Procedure:*

Transfer a 1 ml aliquot of the soil extract to a vial and begin mixing. Add 0.5 ml of the brucine reagent and then add rapidly 2 ml of sulfuric acid. Mix for about 30 seconds and allow the sample to stand for 5 min.

Mix again, add 2 ml distilled water and continue mixing for about 30 seconds. When working with a large number of samples, the first sample will have stood the necessary length of time by the time the sulfuric acid has been added to the last sample, whereupon continue immediately with the addition of water. Let tubes set in cold water for about 5 minutes or air-cool for 15 minutes; measure the transmittancy at 470 mu.

V *Comments:*

This test is simple, rapid and remarkably free from interferences. None of the ions commonly found in soil extracts are likely to be present in sufficient amounts to interfere with this test. Nitrate is partly included with the nitrate nitrogen in the final results. The addition of the 2 ml of water serves to stabilize the yellow color.

VI *References:*

Greweling, T. and M. Peech. Chemical Soil Tests. Cornell Univ. Bul. 30: 23–24.

DETERMINATIONS OF Mg, Mn, Zn, Cu, AND Ca IN PLANT DIGEST AND SOIL EXTRACTS
(Atomic Absorption Spectrometry)

I *Apparatus:*

Perkin-Elmer Model 403 Atomic Absorption Spectrometer. Follow operation instructions for setting up the instrument for analyzing specific element.

II *Standard Solutions and Optimum range for each element. (Linear relationship on digital readout).*

1. Mg standards – 0.1, 0.5, 1.0, 1.5 and 2.0 ppm Mg in aqueous solution.
2. Zn standards – 0.5, 1,2 and 3 ppm Zn in aqueous solution.
3. Cu standards – 2, 10, 15 and 20 ppm Cu in aqueous solution.
4. Ca standards – 2, 5, 8 and 10 ppm Ca in 100 ml volumetric flasks containing 10 ml of 10% H_2SO_4 and 20 ml of lanthanum stock solution. Lanthanum stock solution: Weigh 58.65 of La_2O_3 and wet with 50 ml of distilled water. Add 250 ml of concentrated HCl very slowly under a fume hood until the material is dissolved. Dilute to 1 liter with distilled water. This solution contains 5% lanthanum in 25%HCl. Addition of La to sample solutions from plant tissue digest is necessary to eliminate Al and P interferences.
5. Mn standards – 0.5, 2, 5 and 10 ppm Mn.

Note : Standard solution should contain the same amount of reagent as in the soil extracts or plant digest to be measured.

III *Dilution and pretreatment of sample solutions:*

1. Ca determination – Further dilution of 20 times of sample solution from soil and plant extracts is usually needed. The diluted solution should also contain 1% lanthanum solution to eliminate interference from aluminum and phosphate in the solution.
2. Mg determination – 20 times dilution is needed for plant digests. Ten to twenty times dilution is usually needed for NH_4OAc soil extracts containing high amount of Mg (e.g. soil in Western Nigeria derived from gneisses and granites). Normally no dilution is needed for NH_4OAc extracts of acid tropical soils. In cases where the content of aluminum and phosphate is high in the sample solution, addition of lanthanum may be needed.
3. Zn, Cu and Mn determinations – No dilution is needed.

IV *Procedures:*

1. Set up the instrument according to the instructions provided for the PE 403 Atomic Absorption Spectrometer.
2. Standardize the CONCENTRATION readout by using the standard solutions of the element tested.
3. Read the concentration of the element in sample solution.

Note: (1) Do not leave flame unattended. ~~(2) Do not aspire water with flame off.~~

(2) Do not aspire water with flame off.

(3) Filter your sample solution if it is not clear. Any small particles in the solution will plug up the burner-atomizer.

V *References:*

Analytical Procedure for Atomic Absorption Spectrometry. Perkin-Elmer Co. 1968.

DETERMINATIONS OF K, Na AND Ca IN PLANT TISSUE DIGEST AND SOIL EXTRACT (Flame Photometry)

I *Apparatus:*

Flame photometer, EEL

II *Reagent:*

1. K standard, 100 ppm in aqueous solution
2. Na standard, 100 ppm in aqueous solution
3. Ca standard, 100 ppm (see separate sheet for preparation of stock standard solution).

III *Range of standard solutions:*

1. K –0, 2, 4, 6, 8, 10 ppm K

   or 0, 5, 10, 15, 20 ppm K

   or 0, 10, 20, 30, 40 ppm K

   depends upon the concentration of K present in sample solution. Use high concentration range (0 to 40 ppm K) for plant tissue samples.

2. Na –0, 2, 4, 6, 8, 10 ppm Na

   Ca –0, 5, 10, 15, 20 ppm Ca

   or 0, 10, 20, 30, 40 ppm Ca.

Note: (1) When K or Ca contents in sample are high, further dilution of sample is preferred.

(2) Flame photometry is preferred to atomic absorption spectrometry for Ca determination.

IV *Procedure:*

1. Set up the flame photometer according to the instruction provided.
2. Calibrate the instrument readout using the standard solutions. Set meter at zero while aspiring distilled water or blank solution. Set meter reading at 100% E while aspiring the top concentration of your standards. Record the % E reading of all the intermediate standard solutions. Plot your standard curve linear graph paper.
3. Aspire your sample solution and record the reading (% E). (Check 0 and 100% E reading with your 0 and top standard after every 10 to 20 sample determinations).
4. Read the concentration of the element in sample solution from the standard curve.
5. Calculate K, Na or Ca contents in plant tissue, or in soil.

Note: Standard solutions should contain the same amount of reagent as in the soil extracts or plant digests to be measured on the flame photometer.

V *Reference:*

1. Method of Soil Analysis. ASA Monograph No. 9. 1965.
2. Method of Analysis, A. O. A. C. 11th edition.

COLORIMETRIC DETERMINATION OF PHOSPHORUS IN SOIL EXTRACTS (Ascorbic Acid Method)

I *Apparatus:*

B & L Spectronic-70 Electrophotocolorimeter

II *Reagent A*

1. Dissolve 12 g of ammonium molybdate $[(NH_4)_6MO_7O_{24}]$ in 250 ml distilled water.
2. Dissole 0.2908 g of Potassium antimony tartarate $(KSbOC_4H_4O_6)$ in 100 ml of distilled water.
3. Prepare 5N H_2SO_4 by diluting approx. 148 ml of conc. H_2SO_4 in about 1000 ml of dis-water.
4. Mix solutions (1), (2) and (3) together in a 2 liter volumetric flask and make up to volume with distilled water.

Reagent B

Dissolve 1.056 g of ascorbic acid to every 200 ml of Reagent A.

Note: Reagent B must be freshly prepared from Reagent A each time before use.

Standard phosphorus stock solution:

Weigh 0.4393 g of monobasic potassium phosphate (KH_2PO_4) into a 1 liter volumetric flask. Add 500 ml of distilled water and shake the contents until the salt dissolves. Dilute the solution to 1 liter with distilled water. Add 5 drops of toluene to diminish microbial activity. This solution contains 0.1 mg of P per ml (100 ppm P).

Working P standard solutions:

Pipette 5 ml of the 100 ppm P stock solution into a 100 ml volumetric flask and make up to volume with distilled water. This solution contains 5 ppm P (or 5 μg P/ml). Store the working standard solution in a brown bottle in a regrigerator. It should not be kept for more than one month.

III *Procedure:*

1. Pipette 2 to 5 ml aliquot of the soil extract (Bray No. 1 or Olsen extracts) into a 25 ml volumetric flask or a marked test tube. Add approximately 10 ml of distilled water.
2. Add 4 ml of Reagent B and make up to volume with distilled water. *(Note:* Reagent B and water can be added simultaneously to a set of marked test tubes when a dilutor-dispenser apparatus (Custom Laboratory Equipment Co., Raleigh, N.C. USA) is used.
3. Allow the color to develop for 15 minutes and determine P content in solution on a Spectronic-70 spectrophotometer at 882 μm. *(Note:* The blue color is stable within 24 hours).
4. Prepare a set of standard P solutions containing 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm P. The blank and P standards should contain the same volume of the extracting solutions used for soil P test (e.g. Bray No. 1 or Olsen).

IV *References:*

1. Murphy, J., and J.P. Rikey. 1962. A modified single solution method for the determination of phosphate in natural water. Annal. Chim. Acta 27: 31–36.

2. Watanabe, F.S. and S.R. Olsen. 1965. Test of an ascorbic acid method for determining P in water and $NaHCO_3$ extracts from soils SSSA Proc. 29: 677–678.

DETERMINATION OF WATER-SOLUBLE AND CITRIC ACID-SOLUBLE IN SOIL

I *Apparatus:*

1. Bausch & Lomb Spectronic-20 (or-70) Electrophotometer
2. Centrifuge, Sorvall GLC-1 (Use Sorvall model SS-1 superspeed centrifuge, when the suspension is highly dispersed)
3. Mechanical shaker, reciprocating
4. Polyethylene bottles, 120 ml
5. Volumetric flasks, 100 ml
6. Ni-crucible
7. Meker burner

II *Reagents:*

1. Distilled water – store in polyethylene bottle
2. 1% citric acid – dissolve 10 g of citric acid crystals in 990 ml of distilled water and store the solution in a polyethylene bottle.
3. Ammonium molybdate solution– dissolve 7.5 g of ammonium molybdatein 75 ml of water (warm up to 60°C if necessary). Add 10 ml of 18 $\underline{N}$ H_2SO_4 and dilute the solution to 100 ml with distilled water in a volumetric flask. Store the solution in a polyethylene bottle.
4. Tartaric acid solution, 10% – dissolve 50 g of tartaric acid in 450 ml of water and store the solution in a plastic bottle. Prepare a fresh solution when an appreciable amount of sediment forms.
5. Reducing solution – dissolve 0.7 g of sodium sulfite in 10 ml of water. Add 0.15 g of 1-amino-2-naphthol-4-sulfonic acid, and stir the mixture until the salts dissolve. Dissolve 9 g of sodium bisulfite in 90 ml of water and mix it with the solution above. Store the solution in a plastic bottle.
6. Standard Si solution, 50 ppm Si –digest clear quatz crystals in concentrated HCl for an hour under a fume hood. Rinse them with water, dry and grind them to a fine powder in an agate mortar. Transfer the quatz powder to a Ni-crucible, heat it to redness for a brief period, cool, and transfer it to a screw-cap vial. Fuse 0.1070 g of the above quartz powder with 1 g of NaOH in a Ni-crucible on a Meker burner. The resulting fusion product is then dissolved completely in 50 ml of water. Transfer it to a 1 liter volumetric flask. Add about 400 ml of water plus 20 ml of 6$\underline{N}$ HCl, and then add water to make up to volume.
7. H_2SO_4, 18$\underline{N}$ –Dilute 50 ml of conc. H_2SO_4 (Analar, about 36$\underline{N}$) to 100 ml with distilled water.
8. HCl, 6$\underline{N}$ –Dilute 50 ml of conc. HCl (Analar, about 12$\underline{N}$) to 100 ml with distilled water under a fume hood.

III *Procedure:*

(A) **Extraction:**

1. Weigh 2 g of soil sample (air-dry, passed 2 mm sieve) into a 120 ml polyethylene bottle. Add 100 ml of distilled water (or 100 ml of 1% citric acid).

2. Shake the bottle on a reciprocating shaker for 2 hours. Allow to stand ovemight. Then shake it again for 1 hour.

3. Centrifuge for 10–15 min. at 2,000 rpm. Filter the clear supernatant through Whatman No. 42 paper into a polyethylene bottle and store for Si determination. For most tropical soils, the highly dispersed soil-water suspension may have to be centrifuged at a higher speed using Sorvall model SS-1 Superspeed centrifuge until a clear supernatant is obtained.

(B) **Colorimetric method for Si determination.**

1. Pipet 10 to 15 ml of the sample solution into a 100-ml volumetric flask. Treat a 10 ml aliquot of the reference blank solution (distilled water or 1% citric acid) in the same manner as the sample solution.

2. Add 1 ml of the ammonium molybdate reagent, swirling the contents during addition of the reagent. Mix well, allow to stand for 10 minutes.

3. Add 4 ml of the tartaric acid reagent while swirling the flask and mix well (1 to 2 min). Add 1 ml of the reducing solution while swirling the flask and then add distilled water to ~~me~~ make up to 100 ml. Mix the contents well and allow to stand for 30 minutes.

4. Determine the percent transmission at 650 mu, setting the reference blank solution at 100%.

5. Calculate the concentration of silicon (ppm Si) in soil on air-dry basis. The Si content in soil should be expressed on oven-dry basis, particularly when one wishes to know the soluble Si content in fresh soil samples from the field.

6. Standard curve for Si: Pipet 0, 1, 2, 4, 6 and 8 ml of the standard Si solution (50 ppm Si) into 100 ml volumetric flasks (in case of citric acid extraction add sufficient 1% citric acid solution to each flask so that the volume of Si standard plus citric acid solution totals 10 ml). Develop the color as described above. The 0 standard is the same as the reference blank solution described in (B)-1. Convert the %T to O.D. (optical density) and plot O.D. versus Si concentration on a linear graph paper.

IV *References:*

1. Acquaye, D.K. and Tinsley, J. 1965. In Experimental Pedology, Hallworth and Crowford, ed. Butterworth, London, pp. 126–148.

2. Kilmer, V.J. 1965. In ASA Monograph No. 9, Madison, Wis. pp. 959–962.

3. Shapiro, L. and Brannock, W.W. 1956. U.S. Geol. Surv. Bull. 1036–C.

4. Corey, R.B. and Jackson, M.L. 1953. Anal. Chem. 25: 624–628.

DETERMINATION OF FREE IRON AND ALUMINUM OXIDES IN SOIL (Dithionite-Citrate-Bicarbonate Method)

I *Apparatus:*

1. Water Bath
2. Centrifuge tubes, pyrex glass, 45 ml
3. Centrifuge, Sorvall-GLC
4. Thermometer, 0–150°C
5. Bausch & Lomb Spectronic-70 Electrophotocolorimeter

II *Reagent:*

1. 0.3M sodium citrate – Dissolve 88 g of reagent grade tribasic sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in approximately 600 ml of distilled water and make up to 1 liter.
2. 1M $NaHCO_3$ –Dissolve 84 g of reagent grade $NaHCO_3$ in approximately 600 ml of distilled water and make up to volume of 1 liter.
3. Sodium dithionite ($Na_2S_2O_4$), reagent grade –One gram as measured by a calibrated spoon for each treatment of a sample.
4. Saturated NaCl solution.
5. Acetone (100%).
6. 1N NaCl solution.

III *Procedure:*

1. Weigh 0.2 g of soil sample (ground, passed 100 mesh sieve) into a 50 ml glass centrifuge tube and add 25 ml of 0.3 M Na-citrate solution and 3 ml of 1M $NaHCO_3$.
2. Place this tube in a water bath and the temperature of the bath brought to 75 to 80°C (not more !), then 1 g of solid Na dithionite is added by means of a calibrated spoon and the mixture is stirred constantly with a glass rod for one minute and then occasionally for 5 minutes.
3. A second 1 g portion of Na-dithionite is added with stirring as before. A third 1 g portion is added with stirring at the end of the second 5 minute period. (To avoid precipitation of FeS, do not heat water bath above 80°C).

   If a brown or red color in the sample persists, the Na dithionite treatment is repeated once more.
4. At the end of the 15-minute digestion period, add 5 ml of saturated NaCl solution and 5 ml of acetone (100%) to the tube to promote flocculation. (Keep acetone away from any flame or hot plate). The suspension is then mixed and centrifuged for 5 minutes at 1600 to 220 rpm.
5. The clear supernatant is then carefully decanted into a 100 ml volumetric flask. Wash the residue twice with two 20 ml portions of 1N NaCl, centrifuge and combine the solution into the same 100 ml volumetric flask. Make up the solution to volume with distilled water. The solution is then kept for determination of Fe (and/or Al).

6. The Fe content in solution is determined colorimerically using the phenanthroline procedure given separately. Express Fe content in terms of % Fe_2O_3 in soil. The Fe_2O_3 content determined in such a manner is called "total free Fe oxides" or DCB-extractable Fe O_3.

7. The Al content in the solution can also be determined colorimetrically using the Aluminon method given in a separate section. The Al determined in such a manner is called DCB-extractable Al.

8. Transfer the soil residue into a small glass or plastic vial using a wash bottle. The soil residue is thus stored in water for mineralogical analysis. A pure white color of the residue should generally not be expected as many soils contain greenish, bluish, cream colored or black minerals.

IV References:

1. Jackson, M.L. Soil Chemical Analysis –Advanced course. 1969 Edition. Dept. of Soil Science, University of Wisconsin.

2. Mehra, O.P. and Jackson, M.L. (1960) Iron oxide removal from soils and clays by a dithionate-citrate system buffered with sodium bicarbonate. 7th National Conf. on Clays & Clay Minerals. pp. 317–327.

COLORIMETRIC DETERMINATION OF Fe IN SOIL EXTRACTS (Orthophenanthroline Method)

I *Pretreatment of soil extracts:*

1. Dithionate – citrate – $NaHCO_3$ soil extracts: No pre-treatment is needed
2. NH_4OAc soil extracts: No pre-treatment is needed
3. NH_4 Oxalate soil extracts:

   (a) Pipette 5 ml of the NH_4-oxate extract into a 50 ml pyrex beaker and evaporate to dryness on an electric hot plate at low heat. Add 3 ml of conc. HNO_3 and 1 ml of conc. H_2SO_4 to oxidize the oxalate and organic matter and heat the solution on the hot plate at low heat to dryness under a fume hood.

   (b) Add 3 ml of 1N H_2SO_4 and warm the beaker to dissolve the residue. Add a small amount of distilled water through a wash bottle to wash the side of the beaker. Transfer the solution into a 100 ml voulmetric flask, using distilled water to rinse the beaker thoroughly. Make up the volumetric flask to volume with distilled water.

II *Apparatus:*

B & L Spectron-70 or Spectron-20
Spectrophotometer.

III *Reagents:*

1. Orthophenanthroline reagent – Dissolve 0.3 g of orthophenanthroline monohydrate in distilled water by heating the mixture to 80°C. Cool the solution and add distilled water to make a volume of 100 ml.
2. Hydroxylamine hydrochloride, 10% – Add 90 ml of distilled water to 10 g of hydroxylamine hydrochloride.
3. Sodium citrate dihydrate, 10% – Add 90 ml of distilled water to 10 g of Na-citrate dihydrate.
4. Standard solution of Fe, 100 ppm – Dissolve 0.100 g of pure elemental Fe wire or, 0.7022 g of fresh $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 100 ml of 3.6N H_2SO_4, warm the mixture if necessary for complete solution and dilute the solution to 1 liter.
5. Standard Fe solution, 5 ppm. – Add 10 ml of 36N H_2SO_4 to 50 ml of 100 ppm Fe standard solution and dilute the solution to 1 liter.

IV *Colorimetric procedure:*

1. Pipette 5 to 10 ml of aliquot containing 25 to 400 mg of Fe into a 100 ml volumetric flask. Add 2 ml of 10% hydroxylamine and mix the solution.
2. Add 20 ml of 10% Na-citrate solution and shake the flask.
3. Add 2 ml of the orthophenanthroline reagent and make up to volume. Mix the solution well and allow the color to develop for 24 hours.
4. Determine the % transmittance of the solution at 5100 A on a spectronic – 70 Spectrophotometer.

5. Standard curve of Fe

Pipette 0.5, 15, 25, 35 and 45 ml of a 5 ppm Fe standard solution in a series of 100 ml volumetric flasks. Add the same amounts of the color-developing reagents as described in the above paragraphs. A bright orange color should develop. The solutions contain 0,0.25 0.75, 1.25, 1.75 and 2.25 ppm Fe, respectively. Determine the % T of the solutions at 5100 A after 24 hours.

V *References:*

1. Jackson, M.L. Soil Chemical Analysis – Advanced course. 1969 Edition., U. of Wisconsin.
2. Mehra, O.P. and Jackson, M.L. (1960). 7th Nat'l Conf. of Clays and Clay Minerals pp. 317-327.
3. ASA Monograph No. 9. Method of Soil Analysis. 1965.

COLORIMETRIC DETERMINATION OF Al IN SOIL EXTRACTS (Modified Aluminon Method)

I *Pre-treatment of soil extracts:*

1. Diothionate-citrate-$NaHCO_3$ soil extract:

   The content of citrate and other organic matter should be decomposed before colorimetric determinations. Use the same pre-treatment as used for the destruction of oxalate as described in pre-treatment procedure for Fe determinations.

2. NH_4 –Oxalate soil extract: Use the same pre-treatment procedure as for Fe-determinations.

3. NH_4OAC extracts: No pre-treatment is needed.

4. KCl extracts: No pre-treatment is needed.

II *Apparatus:*

1. B & L Spectronic-70 or Spectronic-20 Spectrophotometer

2. Steam-bath

3. Volumetric flask, 50 ml

III *Reagents:*

(A) Aluminon-acetate buffer – Dilute 120 ml glacial acetic acid to about 800 ml with distilled water, add 24 g NaOH, mix and dissolve 0.35 aluminon in the resulting solution. Dilute to 1 liter and mix. (The pH of this solution should be 4.2. This solution is stable for at least six months).

(B) Thioglycollic acid solution – Dilute 1 ml of the purified thioglycollic acid product to 100 ml with distilled water.

(C) Standard solution of Al, 500 ppm – Dissolve 0.500 g of electrically prepared metallic AL sheet or wire in 15 ml of 6 N HCl in a liter volumetric flask. Dilute to volume with distilled water and mix thoroughly.

(D) Standard solution of Al, 5 ppm – Pipette 10 ml reagent (C) into a 1 liter volumetric flask and dilute to volume with distilled water.

IV *Colorimetric Procedure:*

1. Pipette a suitable aliquot (1 to 5 ml) of the pre-treated sample solution containing 10 to 60 μg Al into a 50 ml volumetric flask and dilute the contents to about 25 ml

2. Add 2 ml of thioglycollic acid solution and mix the contents

3. Add 1 ml of 1N HCl to the flask and heat the flask on a steam bath at 80°C to 90°C for 30 minutes. Cool the contents for 1 to 2 hours.

4. Add exactly 10 ml of the aluminon-acetate buffer and mix the contents. (The final pH of the solution should be within 3.7 to 4.0). If the pH is above 4.0, additional 1N HCl is needed to bring the pH value within 3.7 to 4.0

5. Dilute the contents to volume with distilled water and mix thoroughly

6. Determine the % transmittance on an electrophotometer at 5300 A (530 μm)

7. Standard curve of Al: Pipette 0, 2, 4, 6, 8, 10 and 12 ml of the 5 ppm Al standard solution into a series of 50 ml volumetric flasks and dilute to about 25 ml with distilled water. Add 2 ml of thioglycollic acid, 2ml of 1N HCl. Heat at 80°C to 90°C for 30 minutes on a steam bath in the same manner as for sample solutions, before color determination.

V *References:*

1. ASA monograph, No. 9. Method of Soil Analysis. pp. 982–990.

2. Hsu, P. H. (1964). Soil Sci. 96: 230–238.

3. Chenery, E.M. (1948). Analyst 73: 501–502.

4. Chenery, E.M. (1955). Plant & Soil 6: 174–200.

5. Corey, R. B. and Jackson, M. L. (1953) Anal Chem. 25: 624–628.

CATION EXCHANGE CAPACITY BY NEUTRAL POTASSIUM ACETATE (OR SODIUM ACETATE) SATURATION AND NEUTRAL ACETATE DISPLACEMENT

I *Apparatus:*

Buchner funnel (6 cm I.D.) and flask (250 ml)

Vacuum pump

Volumetric flask (100 ml)

Flame photometer

Centrifuge tube (45 ml)

Conductivity meter

II *Reagents:*

(A) 1N Potassium Acetate pH 7.0
Dissolve 98.15 g of CH_3, cook in approximately 900 ml of distilled water and adjust the pH of the solution to 7.0 with acetic acid and transfer the solution to a 1000 ml volumetric flask and make up to volume with distilled water.

(B) 1N Ammonium Acetate pH 7.0
Mix 68 ml of ammonium hydroxide (NH_4OH) Sp. gr. 0.90 and 57 ml of 99.5% acetic acid (CH_3COOH) under a fume hood. Add approximately 800 ml of distilled water. Adjust the pH of the solution to 7.0 with CH_3COOH and NH_4OH. Transfer the solution to a 1 liter volumetric flask and make up to volume with distilled water.

Optionally, Reagent (B) can also be prepared from Reagent Grade NH_4OAc salt and adjusted to pH 7.0 with NH_4OH or HOAc.

(C) Ethanol 95% U.S.P.

(D) NH_4OH AR, Sp. gr. 0.90.

(E) CH_3COOH AR, 99.5%

III *Procedure:*

1. Weigh 5 or 10 g of air-dried (2 mm) soil into a 45 ml centrifuge tube (or a 125 ml Erlenmeyer flask) and add 30 ml potassium acetate (pH 7.0) solution. Cover the tube tightly with a rubber stopper and shake for one hour on a mechanical shaker.

2. Transfer completely using a small amount of distilled water from a wash bottle for rinsing the content in the centrifuge tube into a Buchner funnel fitted with a moist Whatman No. 42 filter paper. Use gentle suction for filtration if needed. Leach with 60 ml of KOAc, adding about a 15 ml portion at a time. Remove the flask and discard leachate.

3. Connect a clean flask to the funnel. Wash the soil in the Buchner funnel with 95% Ethanol. Add about 30 ml at a time to the soil. Test the electrical conductivity of the leachate. Continue washing until the electrical conductivity of the leachate gives less than 50 μmhos/cm. Optionally decrease the volume of Ethanol by about 5 ml each washing.

4. Remove Buchner flask and replace with a clean one. Displace the exchangeable potassium from the sample by leaching five times with 20 ml portions of N NH_4OAc (pH.7.0) solution measured with 20 ml pipette. Transfer leachate to a 125 ml Erlenmeyer flask.

   Store the solution for determination of potassium on a flame photometer.

Note: Apply gentle suction so that leaching can be completed within one hour to avoid loss of solution due to evaporation).

5. CEC, me/100 g soil = me K/100 g soil.

   Use Na-acetate instead of K-acetate for soils containing K-fixing minerals, e.g. vermiculite.

IV *References:*

1. Soil Survey Laboratory Methods and Procedures for collecting soil samples. Soil Conservation Service, U.S.D.A. Soil Survey Investigations Report No. 1 Washington D.C. 1972.
2. Black C.A. (ed.) 1967. Methods of Soil Analysis Vol. II ASA Monograph No. 9.

DETERMINATION OF EXCHANGE ACIDITY by $BaCl_2$-TEA Method

I *Reagents:*

1. Extracting solution, 0.5N $BaCl_2$-0.055N trienthanolamine, adjusted to pH 8.0: Dissolve 1100g of $BaCl_2.2H_2O$ in CO_2-free water in a 5 gallon pyrex bottle. Add 500 ml of 2N triethanolamine and 72 ml of 6N HCl, and dilute the solution to 18 liters with CO_2-free water. Mix the solution well, and adjust the pH to 8.00 + 0.02 with HCl or triethanolamine. This solution is 0.03N with respect to the free base. Prevent absorption of CO_2 by the solution by providing a siphon and connecting a soda-lime tube to the air inlet. In making up the above extracting solution, dilute cp. concentrated triethanolamine, which is about 7.5N, to 2N and standardize it against standard acid using the mixed indicator solution listed below.

2. Mixed indicator solution: Dissolve 0.22 g of bromocresol green and 0.075 g of methyl red in 96 ml of 95% ethanol containing 3.5 ml of 0.1N NaOH.

3. Standard hydrochloric acid (HCl), 0.2N.

II *Procedure:*

To 10 g of soil in a 125 ml Erlenmeyer flask, add 100 ml of the extracting solution, swirl the contents to cause thorough mixing, stopper the flask, and allow it to stand overnight. Transfer the contents of the flask to a pyrex Buchner funnel, size No. 40, fitted with 4.25 cm. Whatman No. 32 filter paper, rinse the flask with the extracting solution, and continue leaching the soil by adding small portions of the extracting solution until about 225 ml of the leachate has been collected. Allow each portion of the added extracting soultion to drain before adding the next portion, but prevent the soil from cracking upon prolonged drying. Tranfer the leachate quantitatively to a 250 ml volumetric flask and make up to volume with the extracting solution. Pour the leachate into a 500 ml. Erlenmeyer flask, add 5 drops of the mixed indicator solution, and titrate with 0.2N HCl to a pink end point (pH5.1). Rinse the volumetric flask with some of the titrated solution and complete the titration. Titrate 250 ml of the original extracting solution to precisely the same end point, using the same amount of the mixed indicator.

III *Calculations:*

Calculate the exchange acidity (EA) in me per 100 g of soil, using the following equation: $EA = (B-S)\ 10N$ in which B is equal to ml of acid required to titrate 250 ml of the extracting solution, S is equal to ml of acid required to titrate the soil, and N is the normality of the acid.

Indicate result reported on oven-dry basis.

IV *Comments:*

With some soils, the end point will fade (pH increase) upon standing owing to slow dissolution of $Al(OH)_3$, but this color fading should be ignored. The small amount of aluminate dissolved in the soil extract will be converted to $Al(OH)_3$, upon completion

of the titration, and obviously introduces no error in the determination of exchange acidity. Upon standing, however, this precipitated Al $(OH)_3$ will continue to react with the free acid, and any further addition of acid to restore the end point would thus lead to a small negative error.

V *References:*

1. Black, C.A. (ed). 1967. Methods of Soil Analysis. Am. Soc. Agronomy Monograph No. 9 pp. 905–913. Madison, Wisconsin, U.S.A.

2. Peech, M. et al. (1947). Method of soil analysis for soil fertility investigations. U.S.D.A. Circ. 757.

3. Peech, M. et al. (1962). A critical study of $BaCl_2$–TEA and the NH_4OAc methods for determining the exchangeable hydrogen content of soils, Soil Sci.Soc.Am.Proc. 26:37–40.

4. USDA (1972). Soil Survey Laboratory methods. Soil Survey Investigations Report No. 1. (Price US $0.65). Write to Superintendent of Documents, U.S.Government Printing Office, Washington D.C. 20402. Stock No. 0107–0298.

EXTRACTABLE SULFATE — S IN SOILS (KH_2PO_4 Extraction Method)

I *Apparatus:*

1. Centrifuge tubes, 45 ml or Erlenmeyer flasks, 50 ml
2. Centrifuge, or Whatman No. 42 filter paper

II *Reagents:*

1. Extracting solution: KH_2PO_4 solution containing 500 ppm P.

III *Procedure:*

1. Weigh out 5 g of soil sample (air-dry, passed 2 mm sieve) into a centrifuge tube or an Erlenmeyer flask. Add 25 ml of the extracting solution.
2. Shake for 30 minutes on a mechanical shaker. Centrifuge or filter the suspension through a Whatman No. 42 filter paper.
3. Determine the SO_4–S content in solution by the turbidity method given in another section of this manual.

IV *References:*

1. Ensminger, L. E. 1954. Some factors affecting the adsorption of sulfates by Alabama soils. Soil Sci. Soc. Am. Proc. 18:259–264.

Fox, R. L., R. A. Olson, and H. F. Roades. 1964. Evaluating the S status of soils by plant and soil tests. Soil Sci. Soc. Am. Proc. 28:243–246

EASILY REDUCIBLE Mn IN SOILS

I *Apparatus:*

1. Centrifuge (i.e. Sorvall GLC–1)
2. Centrifuge tubes, 45 ml
3. Atomic absorption spectrophotometer (i.e. Perkin – Elmer 403)

II *Reagents:*

1. Neutral 1N NH_4OAc containing 0.2 percent hydroquinone. Use AR grade ammonia and glacial acetic acid to prepare the NH_4OAc solution.
2. Standard Mn solution, 1000 ppm (stock): Dissolve 2.88 g of dry, pure $KMnO_4$ in about 250 ml of distilled water in a 1 liter beaker. Add 20 ml of 18N H_2SO_4 (AR grade). Heat the solution to boiling, add solid Na_2SO_3 until the color of permanganate disappears (avoid large excess of Na_2SO_3), and boil off the SO_2 under a fume hood. Cool the solution, transfer it to a 1 liter volumetric flask, and dilute it to mark with H_2O. Store the solution in a reagent bottle.
3. Working standard, 10 ppm: Dilute 10 ml of the 1000 ppm Mn solution to 1 liter with distilled H_2O. This contains 10 μg/ml of Mn. Prepare fresh working standard periodically when necessary.

III *Procedure:*

1. Weigh out 10 g of soil sample (air-dry, passed 2 mm sieve) into a shaking bottle or 250 ml Erlenmeyer flask. Add 100 ml of reagent 1.
2. Shake the content on a mechanical shaker for 30 minutes, then intermittently for at least 6 hours.
3. Centrifuge or filter the suspension.
4. Determine the Mn content in the extract by atomic absorption spectrophotometry.

IV *References:*

1. Sherman, G. D., J. S. McHargue and W. S. Hodgkins. 1942. Determination of active Mn in soil. Soil Sci. 54:253–257.
2. Black, C. A. (ed.) Methods of Soil Analysis Part II. pp 1011–1018. Agronomy 9. ASA, Madison, Wis.
3. Randall, G. W., E. E. Shulte and R.B. Corey. 1976. Correlation of plant Mn with extractable Mn and soil factors. Soil Sci. Soc. Am. J. 40:282 – 286. (A useful reference for selecting soil test for available Mn).

AVAILABLE Zn IN SOILS BY DILUTE HCl EXTRACTION

I *Apparatus:*

1. General centrifuge, Sorvall Model GLC – 1
2. Polypropylene or polyethylene centrifuge tubes with screw cap, 45 ml (avoid rubber stopper)
3. Polyethylene vials, 25 ml with snap caps
4. Atomic Absorption Spectrophotometer (i.e. Perkin – Elmer Model 403)

II *Reagents:*

1. HCl, 0.1N: Use Zn – free water and analytical grade concentrated HCl to prepare this reagent.
2. Zn – free water: Re-distill distilled water using an all-pyrex still, or pass distilled water through a demineralizer.
3. Standard Zn solution (stock), 100 ppm Zn: Place exactly 0.1000 g of pure Zn metal powder (30 mesh) in a 1 liter volumetric flask. Add 50 ml of Zn – free water and 1 ml of conc. H_2SO_4 (AR). When the Zn has dissolved, make the solution to volume with Zn-free water. For working standard (1 ppm), dilute 10 ml of the stock solution to 1 liter.

III *Procedure:*

1. Weigh out 2.00 g of soil (air-dry, passed 2 mm polyethylene sieve) into a polyethylene centrifuge tube. Add 20 ml of 0. 1N HCl. Close the centrifuge tube (Do not use rubber stoppers).
2. Shake for 45 minutes on a reciprocal shaker. Centrifuge the content for 10 to 15 minutes at 2000 rpm, or at a higher speed until a clear supernatant is obtained.
3. Decant the clear supernatant into a polyethylene vial and store for Zn analysis.
4. Determine Zn content in the solution on an atomic absorption spectrophotometer.

IV *References:*

1. Tucker, T. C. and L. T. Kurtz. 1955. A comparison of several chemical methods with bio-assay procedure for extracting Zn from soils. Soil Sci. Soc. Am. proc. 19:477 –481.
2. Black, C. A. (ed.) Methods of Soil Analysis, Part II. pp. 1090 – 1100. Agronomy No. 9 ASA, Madison, Wisconsion.
3. Osiname, O. A. 1972. Soil tests for available Zn and Cu in soils of Western Nigeria. Ph.D. thesis, University of Wisconsin, Madison, Wisconcin.

DETERMINATION OF TOTAL N IN PLANT MATERIALS (Micro-Kjeldhal Method)

I *Apparatus:*

1. Micro-Kjeldahl digestion-distillation apparatus
2. Micro-Kjeldahl flask, 50 ml, or 100 ml

II *Reagents:*

1. Concentrated H_2SO_4 in bottle with automatic burette (10 ml x 0.05 ml).
2. 40% NaOH plus 5% $Na_2S_2O_3$ solution
   (A) 4,200 g NaOH (N-free flakes) in 4 liters of deionized water in pyrex flask (in sink). Dissolve completely by frequent shaking. Stopper and allow to stand several days.
   (B) Siphon off clear supernatant into large pyrex bottle containing 825 g $Na_2S_2O_3$ $5H_2O$ dissolved in 2300 ml of deionized water. Swirl vigorously to mix. Equip with aspirator bulb and Ascarite tube to prevent absorption of CO_2 (Jackson, P. 186).
3. **Boric Acid indicator:**
   (A) Heat 1800 ml of deionized water to boiling to remove CO_2. Add 40 g boric acid, swirl to dissolve and cool in cold water bath.
   (B) Dissolve .05 g bromcresol green in 50 ml ethanol and 0.5 g methyl red in another 50 ml of ethanol.
   (C) Transfer 50 ml of the bromcresol green solution and 10 ml of the methyl red to a 2 liter volumetric flask Add the 1800 ml of cooled boric acid solution and make up to volume with deionized water.
   (D) Store in stoppered florence flask.
4. **Potassium sulfate - catalyst mixture:**
   Mix, by grinding in mortar, 60 g K_2SO_4 and 6.5 g HgO (red). Store in capped wide-mouth jar.
5. **Salicylic acid-sulfuric acid solution:**
   10 g salicylic acid dissolved in 300 ml conc. H_2SO_4. Store in glass-stoppered flask. For use, transfer to bottle with automatic burette (10 ml x 0.05 ml).
6. 30% H_2O_2 in brown dropping bottle with glass dropper
7. .01 $\underline{N}$ H_2SO_4:
   (A) Make up $\underline{N}/10$ H_2SO_4: 3 ml conc. H_2SO_4 made up to 1, 1 with deionized water.
   (B) Make up $\underline{N}/100$ H_2SO_4: 200 ml N/10 H_2SO_4 made up to 2, 1 with deionized water.
   (C) Titrate $\underline{N}/100$ H_2SO_4 against primary standard base (.05 $\underline{N}$ THAM; equivalent weight = 121.136).

(D) Calculate normality of standardized H_2SO_4 to three decimal places.

(E) Transfer to plastic reagent bottle attached to 5 ml automatic microburette.

8. Powdered pumice (stored in capped wide-mouth jar).

9. Cigarette papers (stored in covered petri dish).

10. $Na_2S_2O_3 \cdot 5H_2O$ crystals powdered by grinding in mortar. (Store in capped wide-mouth jar).

III *Procedure (excluding nitrate):*

1. Grind plant sample to pass 40 mesh screen

2. Weigh duplicate samples containing about 1 mg N (25 to 50 mg) on a single cigarette paper and fold in for quantitative transfer to 50 ml Kjeldahl flask. Add 2 ml distilled water and let stand 30 minutes.

3. With sample in flask, add

   .02 g powdered pumice (spatula tip)
   1.33 g K_2SO_4 catalyst mixture
   1.5 ml conc. H_2SO_4

4. Heat cautiously on digestion rack until frothing stops. (Exhaust fumes to drain through aspirator). Then increase heat to gentle boil so that H_2SO_4 condenses about 1/3 way up neck of flask. If necessary, use a few drops of 30% H_2O_2 to wash isolated sample particles down neck of flask.

5. After digest clears (about ½ hour), continue boiling for ½ hour longer.

6. Allow to cool.

7. Add (slowly with swirling) 10 ml of deionized water. Continue swirling until undissolved materials are in suspension.

8. Flush out distillation apparatus for 5 minutes with steam to clean and bring it up to temperature. *(Teflon stopcocks* should be loosened during warm-up to avoid freezing and possible splitting of barrels due to expansion of the Teflon).

9. Place 50 ml receiver flask containing 5 ml boric acid-indicator solution under condenser of distillation apparatus so tip is about 4 cm above solution.

10. Attach flask with digested and diluted sample to steam jet arm of distillation apparatus.

11. Add 10 ml of 50% NaOH-5% $Na_2S_2O_3$ solution through funnel stopcock. When about 1 ml of NaOH is left in funnel, rinse quickly with about 15 ml of water, leaving about 1 or 2 ml in funnel after closing stopper.

12. Immediately commence distillation by closing steam by-pass then opening inlet stopcock on steam jet arm of distillation apparatus.

13. When distillate reaches 35-ml mark on receiver flask, stop distillation by closing inlet stopcock first, then opening steam by-pass (unless steam is being by-passed through second distillation unit).

Note: Steam flow should be adjusted so that distillate reaches the 35 ml mark 3 minutes after first drops appear at tip of condenser.

14. Rinse condenser tip with deionized water.

15. Titrate to first pink color with .01 N H_2SO_4.

16. Calculate:

$$\% N = \frac{(T - B) \times N \times 1400}{S}$$

where T = sample titration (ml)
B = blank titration (ml)
(Blank determination should include one cigarette paper)
N = normality of H_2SO_4 (to 3 decimal places)
S = sample weight (mg)

IV *Procedure to include nitrate:*

Same as above except Step #3.
Step #3: With sample in Kjeldahl flask:

(A) Add 3 ml salicylic acid-sulfuric acid mixture and allow to stand with occasional swirling for 2 hours or overnight.

(B) Through a dry thistle tube funnel, add 0.5 g powdered $Na_2S_2O_3 \cdot 5H_2O$ crystals.

(C) Heat cautiously on digestion rack until frothing stops.

(D) Cool.

(E) Add: .02 g powdered pumice (spatula tip)
1.33 g K_2SO_4-catalyst mixture

(F) Add (slowly with swirling) 20 ml of deionized water

(G) Proceed as in Steps #4 through 16 above

Note: In Step 4, heat should be adjusted to remove water as quickly as possible without loss of sample through frothing or violent boiling.

V *Alternate Methods:*

1. Total N (NH_4-N) in Kjeldahl digest can also be determined colorimetrically using a Technicon autoanalyzer thus eliminating the distillation process.

2. Plant tissue digestion can also be carried out using a Tecator Model DS-40 Digestor rather than the Micro-Kjeldahl apparatus. (Supplier's address: Tecator, Fack S-26301, Hoganas, Sweden).

VI *References:*

1. Methods of Soil Analysis, Agron. Monograph No. 9, Part 2, pp. 1149–1178, 1196. 1965.

2. Aminco Reprint No. 104: 1–4. 1959.

3. Jackson, Soil Chemistry, pp. 186. 1958.

4. A.O.A.C. Methods of Analysis, pp. 12. 1955.

PERCHLORIC ACID DIGESTION (WET-OXIDATION) OF PLANT MATERIALS FOR P, Ca, Mg, Fe, Zn AND OTHER ELEMENTS

I *Apparatus:*

1. Pyrex Erlenmeyer flask, 125 ml.
2. Whatman No. 42 filter paper (9 cm)

II *Reagents:*

1. Perchloric acid 60% AR.
2. HNO_3 conc. AR.
3. H_2SO_4 conc. AR.

III *Procedure:*

1. Weigh 0.5 to 1.0 g of plant material (oven-dry, 60°C, and ground) into a 125ml Erlenmeyer flask which has been previously washed with acid and distilled water.
2. Add 4 ml of perchloric acid, 25 ml concentrated HNO_3 and 2 ml concentrated H_2SO_4.
3. Mix the contents and heat gently at low to medium heat on a hot plate under perchloric acid fume hood.
4. Continue heating until dense white fumes appear. (If any traces of carbon remain, allow the flask to cool and add 1–2 ml of conc. HNO_3 and digest again to the fuming stage.
5. Finally, heat strongly (medium to high heat) for ½ min.
6. Allow to cool, then add 40–50 ml. distilled water; boil for ½ min. on the same plate at medium heat.
7. Cool and filter the solution completely with a wash bottle into a 100 ml pyrex volumetric flask. Make up to mark with distilled water (use Whatman No. 42 filter paper, 9 cm).
8. Store the solution for P and Fe determinations (colorimetry), Zn, Ca and Mg determinations (atomic absorption spectrometry).

IV *References:*

1. Piper – Soil and Plant Analysis.
2. Method of Analysis, A.O.A.C., 11th edition.
3. Analytical Methods for Atomic Absorption Spectrometry, Perkin-Elmer Co. 1968.

DETERMINATIONS OF Ca,Mg,K AND P IN PLANT TISSUE (Dry Ashing)

I *Apparatus and Reagents:*

1. Muffle furnace
2. Porcelain crucible (Coor)
3. HNO_3, 1N
4. HCl, 1N
5. HCl, 0.1N

II *Procedure:*

1. Weigh 0.5 g of finely ground and oven-dried (60°C) plant material into a 30 ml porcelain crucible.
2. Ignite the sample in a muffle furnace for 6–8 hours or overnight at a temperature between 450°C and 500°C (not exceeding 500°C).

   Greyish white ashes should be obtained; otherwise indicating incomplete ignition. Weigh another sample and repeat the ignition. Incomplete ignition is usually caused by placing too many samples in the furnace, or too fast heating rate.
3. Cool sample on top of asbestos sheet and add 5 ml 1N HNO_3 solution.
4. Evaporate to dryness on a steam bath or a hot plate at low heat.
5. Return sample to the furnace and heat at 400°C for 10–15 minutes until a perfectly white or greyish white ash is obtained.
6. Cool the sample on top of asbestos sheet, add 10 ml 1N HCl and filter the solution into a 50 ml volumetric flask.
7. Wash the crucible and the filter paper with additional 10 ml portion of 0.1N HCl three times and make up to volume with 0.1N HCl solution.
8. Store the filtrate for K, Ca, Mg, Na and P determinations.

III *References:*

1. Methods of Analysis, 11th edition, A.O.A.C.

COLORIMETRIC DETERMINATION OF PHOSPHORUS IN PLANT TISSUE
(Vanado-Molybdate Method)

I *Reagents:*

1. Vanado-Molybdate reagent – Dissolve 20 g NH_4 – Molybdate, $(NH_4)_6 Mo_7O_{24}\cdot 4H_2O$, in 200 ml hot water and cool. Dissolve separately 1 g of NH_4-metavanadate in 120 ml hot water, cool and add 140 ml conc. HNO_3 under a fume hood. Gradually add molybdate solution to the vanadate solution and dilute to 1 liter.
2. P standard solution, stock, 100 ppm P. – Dissolve 0.4394 g dry anhydrous KH_2PO_4 in distilled water and dilute to 1 liter. Store the solution in a dark pyrex glass bottle at a cool place.
3. P standard solution, 25 ppm P – Dilute the 100 ppm P stock solution 4 times. Fresh solution should be prepared periodically to insure high accuracy.

II *Procedure:*

1. Pipet 5 ml of sample solution (from wet digestion or dry ashing) into 100 ml volumetric flask and add 45 ml distilled water.
2. Within 5 mins, add 20 ml of vanado-molybdate reagent and dilute to volume. Mix and let stand for 10 minutes.
3. Determine % transmittance at 400 mu.
4. Standard curve for P: Pipet 0, 2, 4, 5, 10, 15 and 20 ml of the 25 ppm P standard solution into a series of 100 ml volumetric flasks and the color is developed according to the same procedure as stated in steps 1, 2 and 3.
5. Plot O. D. (optical density) against concentration.
6. Calculate % P content in plant sample.

III *Reference:*

1. Methods of analysis – A.O.A.C., 11th edition.

DIGESTION OF PLANT MATERIAL USING ALUMINUM DIGESTION BLOCK

I *Apparatus:*

Tecator Model 40 Digestor -- Manufacturer's address:
TECATOR Fack S--26301, Hoganas, Sweden

II *Reagent:*

Nitric acid, concentrated, AR.
Perchloric acid, concentrated, AR.
Hydrochloric acid, concentrated, AR.

III *Procedures:*

1. Weigh 100 to 250 mg plant tissue samples (ground and dried in oven at 65°C) into a 70 ml pyrex digestion tube. Add 5 ml of the HNO_3-$HClO_4$ agent (2:1 by volume) to each tube under a fume hood. Allow to stand overnight at room temperature under a fume hood.

2. Place the tubes into the aluminum digestion block located inside the fume hood and set the temperature control of the digestor installed outside the fume hood at 150°C. Digest for 1½ hours. Increase the temperature to 230°C and digest for another 30 minutes (white fuming stage).

3. Reduce the digestor temperature back to 150°C. Add 1 ml of the HCl reagent (1 volume of con HCl and 1 volume of water) to each tube and heat the content at 150°C for about 30 minutes.

4. Switch off the digestor. Remove the tubes from the digestion block and add about 30 ml of distilled water to each tube within a few minutes. Do not allow the concentrated digest to cool to room temperature in order to prevent the formation of insoluble precipitates, e.g. patassium perchlorate.

5. Add more water to the tube to make up to volume (50 ml) and mix the content. The solution is ready for determinations of P, Ca, Mg, K, Na, Zn, Cu, Fe and Mn.

Note: For sulfur analysis, it is necessary to place a reflux funnel on top of each tube during digestion (step 2 and 3). The digesting time at 230°C should also be increased from 30 minutes to 2 hours.

NITRIC-PERCHLORIC ACID DIGESTION OF PLANT MATERIAL FOR SULFUR ANALYSIS (Turbidimetric Method)

1. Weigh 0.25 grams of plant material into a dry digestion tube.
2. Add 5 ml of 2:1 nitric-perchloric acid.
3. Insert a small glass funnel to act as a reflux condenser and leave a few hours at about 50°C or overnight with no heat.
4. Place tubes in heating block and digest for 1 hour at 150°C.
5. Raise temperature to 235°C. Note any "cold spots" in the heating block and rotate tubes so that the nitric acid is driven off as uniformly as possible.
6. Note time when all tubes have reached the dense white fume stage. This stage is not reached until essentially all of the nitric acid has been driven off.
7. Continue digestion for 2 hours.
8. Remove tubes from digestion block, cool to about 100°C and add 1 ml 1:1 HCl to dispel the last traces of oxides of nitrogen.
9. Heat to white fuming stage again.
10. Remove from digestion block. Cool just enough to handle. Add water to 50 ml volume. Mix thoroughly and make certain that sparingly soluble perchloric salts are dissolved.
11. Let stand until silica has settled. Pipette aliquot for analysis (usually 10 ml) from the top of the solution leaving the silica sediment undisturbed.

TURBIDIMETRIC DETERMINATION OF SULFUR IN PLANT DIGEST, SOIL EXTRACT AND WATER

I *Apparatus:*

1. Bausch & Lomb Spectronic – 70 Electrophotocolorimeter.
2. Volumetric flasks, 25 ml.

II *Reagents:*

1. Gelatin (Difco Bacto Gelatin)
2. Barium chloride ($BaCl_2 2H_2O$), AR.
3. Gelatin-$BaCl_2$ reagent:

   Dissolve 0.6 g of gelatin in 200 ml hot (60–70°C) distilled water and allow the solution to stand in a refrigerator (4°C) for 16 hours. After 16 hours, bring the semi-gelatinous fluid to room temperature, add 2 g of reagent grade barium chloride and mix the content until $BaCl_2$ is dissolved. Store the solution in a refrigerator and allow the reagent to stand at room temperature for at least 2 hours before use.

4. Standard SO_4–S stock solution (100 ppm): Dissolve 0.5434 of reagent grade anhydrous potassium sulfate (K_2SO_4) in distilled water in a 1 liter volumetric flask and make up to volume to give a final concentration of 100 ppm SO_4–S, or 100 μg S per ml. Store in a reagent bottle.
5. Working S standard solution (25 ppm): Pipette 25 ml of the 100 ppm stock solution and dilute to 100 ml with distilled water. This standard solution contains 25 μg S per ml.

III *Procedure:*

1. Pipette 10 ml of the sample aliquot into a 25 ml volumetric flask. Add distilled water to bring the volume to approximately 20 ml.
2. Add 1 ml of the gelatin – $BaCl_2$ reagent. Make up to volume with distilled water. Mix the content thoroughly. Allow the content to stand for 30 minutes.
3. Determine the % and O.D. at 420 μm within 30 to 60 minutes on a B & L Spectronic–70 electrocolorimeter. Shake the content in the flask before pouring into the photo-test tube.
4. Prepare a set of standard S solutions containing 0, 25, 50, 75, 100, 125 μg SO_4–S per 25 ml from the working standard solution. The standard solution should, of course, contain 1 ml of gelatin-$BaCl_2$ reagent and 10 ml of the blank digest or extracting solution.

IV *Reference:*

1. Tabatabai, M.A. 1974. Determination of sulfate in water samples. Sulfur Institute Journal 10: 11–13.

Note: Difco Bacto gelatin can be purchased from Difco Laboratory, Inc. Detroit, Michigan, U.S.A.

OXALATE EXTRACTABLE OR "AMORPHOUS" FE AND AL IN SOILS

(Modified Tamm's Method)

I *Apparatus:*

1. Centrifuge, preferrably Sorvall SS-3
2. Centrifuge tube, polyethylene, 15 ml.
3. Mechanical shaker, reciprocal.

II *Reagents:*

1. Ammonium oxalate, 0.2M acidified to pH 3.0

III *Procedures:*

1. Further grind a carefully divided subsample of air-dried, less than 2 mm soil samples to pass a 100-mesh sieve completely.
2. Weigh 200 to 400 mg of the ground soil into a 15ml centrifuge tube. Add 10ml of the 0.2M acidified NH_4- Oxalate solution. Stopper the tube tightly.
3. Place the tubes horizontally inside a cardboard or wooden box and close the box in order to maintain the sample in darkness.
4. The box was then secured on a reciprocal shaker and shake the content for 4 hours.
5. Centrifuge the content at 2000 rpm or higher for 15 minutes until a clear supernatant is obtained. Carefully decant and store the supernatant in a clean polyethylene or glass vials for Fe and Al determinations.
6. The content of Fe and Al in the extracts may be determined colorimetrically or by atomic absorption spectrophotometry. Pretreatment by digesting an aliquot of the extract with HNO_3 and H_2SO_4 is required (See separate section of the manual for Fe and Al determinations.).

IV *References:*

1. Schwertmann, U. 1964. The differentiation of iron oxide in soils by a photochemical extraction with acid ammonium oxalate (in German) Z. pflanzenahr., Dung., Bodenkd., 105 : 194–202.
2. McKeague, J.A. and J.H. Day, 1966. Dithionite and oxalate Fe and Al as aids for in differentiating various classes of soils. Can. J. Soil Sci. 47: 95–99.
3. Schwertmann, U. 1973. Use of oxalate for Fe extraction from soils. Can. J. Soil Sci. 53: 244–246.
4. Juo, A.S.R., F.R. Moormann and H.O. Maduakor, 1974. Forms and pedogenetic distribution of extractable Fe and Al in selected soils of Nigeria. Geoderma, 11: 167–179.

APPENDIX 1
LABORATORY SAFETY

I **Handling of hazardous chemicals** All volatile, flammable, toxic and corrosive reagents and solvents should be handled under a well-ventilated fume hood:

1. *Explosion hazard* (Very volatile and flammable).

   Petroleum ether

   Diethyl ether

   Acetone

   They should also be kept away from flame and electric hot plates

2. *Toxicity*

   Benzene

   Carbon tetrachloride

   Acetonitrile

   Accidental spillage on hands or clothing should be avoided.

3. *Corrosion*

   Conc. HCl

   Conc. HNO_3

   Conc. ammonia

   glacial acetic acid etc.

   Spillage should be washed off with large amounts of water immediately.

II *Chemical Waste Disposal*

Hazardous liquid and solid wastes should be stored in a labelled container and carried to a designated area for disposal. These include the following categories:

1. Kjeldahl digests (Se, Hg).
2. Solutions, soil or plant extracts containing lead (Pb), mercury (Hg), selenium (Se), chromium (Cr), zinc (Zn), cyanide and arsenate.
3. Pesticides, herbicides and fumigants.

APPENDIX II
CONCENTRATED LIQUID ANALYTICAL REAGENTS

1. Acetic acid, glacial. No less than 99.5% by weight.
2. Acetic acid, 6 N: Dilute 350 ml of glacial acetic acid with water to 1 liter under a fume hood or ventilation hood.
3. Ammonium hydroxide, concentrated, 15 N, Sp. gr. 0.90, contains approximately 28% NH_3 by weight.
4. Ammonium hydroxide, 6 N, Sp. gr. 0.96, contains approximately 10% NH_3 by weight. Preparation: Add 3 volumes of water to 2 volumes of conc. NH_4OH under a fume hood.
5. Hydrochloric acid, concentrated, 12 N, Sp. gr. 1.19, contains approximately 37% HCl by weight.
6. Hydrochloric acid, 6 N, — Add conc. HCl to an equal volume of water under a fume hood.
7. Hydrochloric acid, HF. 48% solution.
8. Nitric acid, concentrated, 16 N, Sp. gr. 1.42, contains approximately 70% HNO_3 by weight.
9. Nitric acid, 6 N — Dilute 380 ml of conc. HNO_3 with water to 1 liter under a fume hood.
10. Perchloric acid HC10, 9 N, Sp. gr. 1.53, contains approximately 60% by weight.
11. Orthophosphoric acid, 85% solution.
12. Sulfuric acid, concentrated, 36 N, Sp. gr. 1.84, contains approximately 93% H_2SO_4 by weight.
13. Sulfuric acid, 18 N — Slowly pour concentrated H_2SO_4 into an equal volume of cold
14. Sulfuric acid, 6 N — Pour slowly 167 of concentrated H_2SO_4 into 700 ml of cold water and dilute to 1 liter.

APPENDIX III
Optical Density (D) Table for Values of Per Cent Light Transmitted (T)

$D = 2 - \log_{10} T$, where $T = \frac{1}{10} \times 100$

T	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0		3.000	2.699	2.523	2.398	2.301	2.222	2.155	2.097	2.046
1	2.000	1.959	1.921	1.886	1.854	1.824	1.796	1.770	1.745	1.721
2	1.699	1.678	1.658	1.638	1.620	1.602	1.585	1.569	1.553	1.538
3	1.523	1.509	1.495	1.481	1.469	1.456	1.444	1.432	1.420	1.409
4	1.398	1.387	1.377	1.367	1.357	1.347	1.337	1.328	1.319	1.310
5	1.301	1.292	1.284	1.276	1.268	1.260	1.252	1.244	1.237	1.229
6	1.222	1.215	1.208	1.201	1.194	1.187	1.180	1.174	1.167	1.161
7	1.155	1.149	1.143	1.137	1.131	1.125	1.119	1.114	1.108	1.102
8	1.097	1.092	1.086	1.081	1.076	1.071	1.066	1.060	1.056	1.051
9	1.046	1.041	1.036	1.032	1.027	1.022	1.018	1.013	1.009	1.004
10	1.000	0.996	0.991	0.987	0.983	0.979	0.975	0.971	1.967	0.963
11	959	955	951	947	943	939	936	932	928	924
12	921	917	914	910	907	903	900	896	893	889
13	886	883	879	876	873	870	866	863	860	857
14	854	851	848	845	842	839	836	833	830	827
15	824	821	818	815	812	810	807	804	801	799
16	796	793	790	788	785	783	780	777	775	772
17	770	767	764	762	759	757	754	752	750	747
18	745	742	740	738	735	733	730	728	726	724
19	721	719	717	714	712	710	708	706	703	701
20	699	697	695	693	690	688	686	684	682	680
21	678	676	674	672	670	668	666	664	662	660
22	658	656	654	652	650	648	646	644	642	640
23	638	636	635	633	631	629	627	625	623	622
24	620	618	616	614	613	611	609	607	606	604
25	602	600	599	597	595	593	592	590	588	587
26	585	583	582	580	578	577	575	573	572	570
27	569	567	565	564	562	561	559	558	556	554
28	553	551	550	548	547	545	544	542	541	539
29	538	536	535	533	532	530	529	527	526	524
30	523	521	520	519	517	516	514	513	511	510
31	509	507	506	504	503	502	500	409	498	496
32	495	493	492	491	489	488	487	485	484	483
33	481	480	479	478	476	475	474	472	471	470
34	469	467	466	465	463	462	461	460	458	457

35	456	455	453	452	451	450	449	447	446	445
36	444	442	441	440	439	438	437	435	334	433
37	432	431	429	428	427	426	425	424	423	421
38	420	419	418	417	416	415	413	412	411	410
39	409	408	407	406	405	403	402	401	400	399
40	398	397	396	395	394	393	391	390	389	388
41	387	386	385	384	383	382	381	380	379	378
42	377	376	375	374	373	372	371	370	369	368
43	367	366	365	364	363	362	361	360	359	358
44	357	356	355	354	353	352	351	350	349	348
45	347	346	345	344	343	342	341	340	339	338
46	337	336	335	334	333	333	332	331	330	329
47	328	327	326	325	324	323	322	321	321	320
48	319	318	317	316	315	314	313	312	312	311
49	310	309	308	307	306	305	305	304	303	302
50	301	300	299	298	298	297	296	295	294	293
51	292	292	291	290	289	288	287	287	286	285
52	284	283	282	281	281	280	279	278	277	277
53	276	275	274	273	272	272	271	270	269	268
54	268	267	266	265	264	264	263	262	261	260
55	260	259	258	257	256	256	255	254	253	253
56	252	251	250	249	249	248	247	246	246	245
57	244	243	243	242	241	240	240	239	238	237
58	237	236	235	234	234	233	232	231	231	230
59	229	228	226	227	226	225	225	224	223	223
60	222	221	220	220	219	218	218	217	216	215
61	215	214	213	213	212	211	210	210	209	208
62	208	207	206	206	205	204	203	203	202	201
63	201	200	199	199	198	197	197	196	195	194
64	194	193	192	192	191	190	190	189	188	188
65	187	186	186	185	184	184	183	182	182	181
66	180	180	179	178	178	177	177	176	175	175
67	174	173	173	172	171	171	170	169	169	168
68	167	167	166	166	165	164	164	163	162	162
69	161	161	160	159	159	158	157	157	156	156
70	155	154	154	153	152	152	151	151	150	149
71	149	148	148	147	146	146	145	144	144	143
72	143	142	141	141	140	140	139	138	138	137
73	137	136	135	135	134	134	133	133	132	131
74	131	130	130	129	128	128	127	127	126	126
75	125	124	124	123	123	122	121	121	120	120
76	119	119	118	117	117	116	116	115	115	114
77	114	113	112	112	111	111	110	110	109	108

78	108	107	107	106	106	105	105	104	103	103
79	102	102	101	101	100	100	099	099	098	097
80	097	096	096	095	095	094	094	093	093	092
81	092	091	090	090	089	089	088	088	087	087
82	086	086	085	085	084	084	083	082	082	081
83	081	080	080	079	079	078	078	077	077	076
84	076	075	075	074	074	073	073	072	072	071
85	071	070	070	069	069	068	068	067	067	066
86	066	065	064	064	063	063	062	062	061	061
87	060	060	059	059	058	058	057	057	057	056
88	056	055	055	054	054	053	053	052	052	051
89	051	050	050	049	049	048	048	047	047	046
90	046	045	045	044	044	043	043	042	042	041
91	041	040	040	040	039	039	038	038	037	037
92	036	036	035	035	034	034	033	033	032	032
93	032	031	031	030	030	029	029	028	028	027
94	027	026	026	025	025	025	024	024	023	023
95	022	022	021	021	020	020	020	019	019	018
96	018	017	017	016	016	015	015	015	014	014
97	013	013	012	012	011	011	011	010	010	009
98	009	008	007	007	007	007	006	006	005	005
99	004	004	003	003	003	002	002	001	001	000

APPENDIX IV
FIELD SAMPLING TECHNIQUES FOR SOIL TESTING

I *Experimental plots*

Take a composite sample of 20 to 40 borings per plot at 0–15 cm depth. Use a soil auger or a soil tube with 2 to 3 cm diameter. Sampling of subsurface soil (15–30 cm) is optional depending upon the objectives of the experiment.

II *Fields larger than 1/2 ha.*

The minimum number of samples of a given area required can be calculated provided a measure of soil variability is known. Generally, the micro-variability of the coarse-textured Ultisols and Alfisols in the humid tropics is high. It is recommended that a composite surface soil (0–15 cm) sample should consist of 50 to 200 borings in a given area in order to represent the mean fertility level of the area.

III *Sample preparation*

1. Mix the composite sample thoroughly. Allow to air-dry under shade.
2. Grind gently with porcelain mortar and a wooden pestle.
3. Sieve the soil to pass a 2mm brass sieve. (Use plastic ~~sivee~~ sieve for micronutrient analysis).
4. Mix the soil again. Take a subsample and store for chemical and mechanical analysis.

Note: (i) For soil profile samples, the % gravel content (greater than 2 mm portion) should also be recorded.

(ii) For soil organic matter and total N analysis, the less than 2 mm soil sample should be further ground with a porcelain mortar and pestle and completely passed through a 0.5 mm brass sieve.

IV *References*

1. Walsh, L.M. and Beaton, J.D. (ed.) 1973. –Soil Testing and Plant Analysis. (Revised Edition) Soil Sci. Soc. Am. Inc., Madison, Wis., U.S.A.
2. USDA 1972.
Soil Survey Laboratory Methods and Procedures for Collecting Soil Samples. Soil Survey Investigation Report No. 1, U.S. Gov't Printing Office, Washington, D.C., U.S.A.

APPENDIX V
FOLIAR SAMPLING TECHNIQUES FOR CHEMICAL ANALYSIS FOR FERTILIZER GUIDANCE PURPOSE

I RICE *(Oryza sativa)*

1. Take first 2 leaf blades from top of plant at time of flowering when head has more or less completely emerged.

2. Select permanent sampling locations of ½ or more hectare size. Sample along two diagonal (X) patterns, taking leaf samples from random plants distributed along the diagonals in area selected. Sample should consist of 200 or more leaves from the area. For smaller area, take 50 – 100 leaves.

II MAIZE *(Zea mays)*

1. Take the entire leaf at the ear node at
   (a) full tasselling stage, or
   (b) at silking stage.

2. Choose 1 or more permanent sampling places ½ or more hectare in size. Sample either along X-diagonals or along rows, sampling no less than 50 plants in the area selected. For small experimental plot, sample 15 to 25 plants per plot.

III SOYBEAN *(Glycine soja)* and COWPEA *(Vigna unguiculata)*

1. Take the first fully expanded trifoliar leaves (without petioles) after flowering with young pods at top and full-length pods at base of the plant.

2. Take leaves from 5 percent or more of plants in an experimental plot or in a selected area of a large field. Random samples of 50 to 100 leaves should be taken from an area of ½ ha. For small experimental plots, take 20 to 30 leaves per plot.

IV CASSAVA *(Manihot esculenta)*

1. Take the first fully expanded leaf (without petioles) of
   (a) 3 month old plants or
   (b) 6 month old plants or
   (c) at flowering stage.

2. Take 50 to 100 leaves in random in a selected area of ½ or more hecatre. For small plots, e.g. 10 m x 6 m, take 20 to 30 leaves per plot. Sample either in diagonal or along rows.

Note: Techniques for cassava tissue sampling given above are tentative. Presently, no reliable information is available.

V *Sample preparation*

1. Dry the plant tissue samples in a ventilated oven at 65° C for 24 hours or more. Grind the sample with a Wiley mill or micro-hammer mill.

2. Store the sample in a clean glass or plastic vial, or clean plastic bag for chemical analysis.

3. Dry the sample in an oven again at 65° C before weighing for chemical analysis.

4. For micronutrient analysis, caution should be taken to avoid contamination from sample containers such as paper bags, envelopes, rubber stoppers etc.

References:

1. Chapman, H.D. (ed.) 1966. Diagnostic criteria for plants and soils. Univ. of California Press.

2. Walsh, L.M. and Beaton, J.D. (ed.)1973. Soil Testing and Plant Analysis. (Revised Edition). Soil Sci. Soc. Am. Inc., Madison, Wis. U.S.A.

3. Nijholt, J. A. 1935. Opname van voedingstoffen uit den bodem by cassava. Landbouw 10:9.

APPENDIX VI
Solubility Rules

1. The nitrates, chlorates and acetates of all metals are soluble in water.
2. All common sodium, potassium and ammonium salts are soluble in water.
3. (a) The chlorides, bromides and iodides of all metals except lead, silver and mercury (1) are soluble in water.

   (b) HgI , BiI and CuI are insoluble in water. Water-insoluble chlorides, bromides and iodides are also insoluble in dilute acids.

4. The sulfates of all metals except lead, mercury (1), barium, strontium and calcium are soluble in water. Silver sulfate is only soluble in water. Water insoluble sulfates are also insoluble in dilute acids.
5. The carbonates, phosphates, borates, sulfites, chromates and arsenates of all cations except sodium, potassium and ammonium are insoluble in water, but are soluble in dilute acids. However, magnesium chromate is soluble in water; and magnesium sulfite is very slightly soluble in water.
6. The sulfides of all cations except barium, strontium, calcium, magnesium, sodium, potassium and ammonium are insoluble in water.
7. The hydroxides of sodium, potassium and ammonium are very soluble in water. The hydroxides of calcium, barium and strontium are moderately soluble in water.

   The oxides and hydroxides of all other common cations are insolube in water.