APPENDICES

APPENDIX – I

ESTIMATION OF LIGNIN

(GOERING AND VANSOEST, 1975)

Principle

Refluxing the sample material with acid detergent solution removes the water soluble and materials other than the fibrous component. The left out material is weighed after filtration, dried, treated with 72% H_2SO_4 and filtered, dried and ashed. The loss of weight on ignition gives the acid detergent lignin.

Reagents

- Acid Detergent Solution

  Dissolve 20 g of acetyl trimethyl ammonium bromide in one litre of 1 N sulphuric acid.
- 72% H_2SO_4 (W/V)
- Acetone
- Round Bottom Flask and Refluxing Set
- Muffle Furnace
- Sintered Glass Crucible – G2

Procedure

A. Acid Detergent Fibre (ADF)

- 1gm of powdered sample and 100ml of acid detergent solution was placed in a round bottom flask and boiled for 5 – 10 minutes. The heat was reduced to avoid foaming as boiling begins. Refluxing was done for 1 hour after the onset of boiling. Boiling was adjusted to slow, even level.
- The container was removed, swirled and filtered the contents through a preweighed sintered glass crucible (G2) by suction and washed with hot water twice.
- Then, washed with acetone and break up the lumps. Acetone washing was repeated until the filtrate was colourless.

- Dried at 100^{o}C for overnight.
- Weighed after cooling in a desiccator.
- ADF content was expressed in percentage i.e., W/S x 100, Where W is the weight of the fibre and S is the weight of the sample.

B. Determination of Acid Detergent Lignin (ADL)

- ADF was transferred to a 100 ml beaker with 25 - 50 ml of 72% sulphuric acid. 1g of asbestos was added to it. It was allowed to stand for 3 hrs with an intermittent stirring with a glass rod.
- The acid was diluted with distilled water and filtered with preweighed Whatman No. 1 filter paper. The glass rod and the residue were washed several times to get rid of the acid.
- The filter paper was dried at 100^{o}C and weighed after cooling in a desiccator.
- The filter paper was transferred to a preweighed silica crucible and ashed the filter paper with the content in a muffle furnace at 550 ^{o}C for about 3 hrs.
- The crucible was cooled in a desiccator and weighed. The ash content was calculated.
- 1 g asbestos was taken as blank and then added 72% H_2SO_4 and followed the steps from 2 - 5.

Calculation

$$\text{ADL (\%)} = \frac{\text{Weight 72\% } H_2SO_4 \text{ washed fibre (Test – Asbestos blank)} - \text{Ash (Test – Asbestos blank)} \times 100}{\text{Weight of sample}}$$

APPENDIX – II

ESTIMATION OF CELLULOSE

(UPDEGROFF, 1969)

Principle

Cellulose undergoes acetolysis with acetic/nitric reagent forming acetylated cello dextrins which get dissolved and hydrolyzed to formed glucose molecules upon treatment with 67% H_2SO_4. This glucose molecule is dehydrated to form hydroxyl methyl furfural which forms green coloured product with anthrone and the colour intensity is measured at 630 nm.

Reagents

- Acetic/Nitric reagent: 150 ml of 80% acetic acid was mixed with 15 ml of concentrated nitric acid.
- Anthrone reagent: 200 mg of anthrone was dissolved in 100 ml concentrated sulphuric acid and chilled for two hrs before use.
- 67% sulphuric acid.

Procedure

A quantity of 0.1 gm of sample was taken in a test tube, to which 3 ml of acetic/nitric reagent was added and mixed well and kept in a water bath for 30 minutes. It was cooled and centrifuged for 15 - 20 minutes after which the supernatant was discarded. The residue was washed with distilled water and 10 ml of 67% sulphuric acid was added and allowed to stand for 1 hr. 1ml of the solution was taken and diluted to 100ml. From the above diluted solution, 1ml was taken, to which 10ml of anthrone reagent was added and kept in a boiling water bath for 10 minutes. It was then, cooled and the absorbance was measured at 630 nm. A blank was set with anthrone reagent and distilled water. The amount of cellulose present in the sample was calculated using a standard graph corresponding to 40 - 200 μg of cellulose.

APPENDIX - III

ESTIMATION OF ORGANIC CARBON

WET CHROMIC ACID OXIDATION METHOD

(WALKEY AND BLACK, 1934)

Principle

Organic carbon present in organic matter is oxidised by chromic acid in the presence of conc. H_2SO_4. Potassium dichromate on reaction of H_2SO_4 provides nascent oxygenwhich combines with carbon and form CO_2. The H_2SO_4 enables easy digestion of organic matter by rendering heat of dilution. Only a certain quantity of chromic acid is used for oxidation. The excess chromic acid left unused by the organic matter is determine by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator.

Reagents

- 1 N potassium dichromate: Exactly 49.04 g of $K_2Cr_2O_7$ was dissolve in one litre of distilled water.
- Diphenylamine indicator: 0.5 g diphenylamine was dissolved in 20 ml of water and 100 ml of Conc. H_2SO_4 was added.
- 0.5 N ferrous sulphate or ferrous ammonium sulphate: 139.0 g of ferrous sulphate or 196 g of ferrous ammonium sulphate was dissolved in 800 ml of distilled water. 20 ml of Conc. H_2SO_4 was added and the volume was made up to one litre.
- Conc. H_2SO_4
- Phosphoric acid (Orthophosphoric acid 85%).

Procedure

Exactly 0.5gm of soil (passed through 0.2 mm sieve) was weighed and transferred to 500 ml conical flask. 10ml of 1N $K_2Cr_2O_7$ was added and mixed well by swirling the flask. Added 20ml of conc. H_2SO_4 mixed by gentle rotation for one minute to ensure complete contact of the reagent with the soil. Allowed the contents to stand for 20-30 minutes. Kept the flask on asbestos sheet to avoid burning of table due to intense heat. Added 200ml of water after 30 minutes. Then added 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. Titrated the solution with 0.5N ferrous ammonium sulphate. As the titration proceeds the dull green colour shifted to the turbid blue and at the end point

bright green colour developed. Conducted simultaneously a blank titration (without soil) and the volume of 0.5N ferrous ammonium sulphate consumed was noted.

CALCULATION

Weight of soil taken = 0.5gm

Volume of 1N $K_2Cr_2O_7$ = 10ml

Volume of 0.5N ferrous ammonium sulphate used for blank titration = X ml (Sample T. V)

Volume of 0.5N ferrous ammonium sulphate used for blank titration = Y ml (Sample T. V)

Xml of $FeSO_4$ reduces 10ml of 1N $K_2Cr_2O_7$

Therefore Yml of $FeSO_4$ reduces Y/X* 10ml

Hence actual quantity of 1N $K_2Cr_2O_7$ used for oxidation of organic matter = 10 - (10* Y/X)

1ml of 1N $K_2Cr_2O_7$ = 0.003gm of 'C'

Therefore 10 – (10*Y/X) ml of 1N $K_2Cr_2O_7$ = 10 - (10*Y/X)*0.003

This is present in 0.5gm of soil

Therefore in 100gm = 10 - (10*Y/X)*0.003*100/0.5

Organic matter (surface soil) = organic carbon * 1.724

Organic matter (sub surface soil) = organic carbon * 2.5

APPENDIX - IV

ESTIMATION OF TOTAL NITROGEN
MICROKJELDHAL METHOD
(HUMPHRIES, 1956)

Principle

A known weight of the powdered sample was treated with diacid mixture so as to oxidize the organic matter and bring the mineral elements into solution.

Reagents

- Diacid mixture: 4:1 (w/w) ratio of concentrated sulphuric acid and concentrated perchloric acid.
- Mixed indicator: 0.5g bromocresol green and 1g of methyl red were dissolved in 100ml of 90% ethyl alcohol.
- 40% sodium hydroxide solution.
- 2% boric acid.
- Concentrated sulphuric acid (0.02 N).

Procedure

- A quantity of 0.2gm of dried, sieved and homogenized sample was taken in a micro kjeldhal digestion flask (50ml capacity), to which, 12ml of diacid was added.
- Complete digestion was ensured by adding one drop of perchloric acid and the contents turns colourless like water.
- The volume was made upto 100ml with distilled water.
- 10ml aliquot was pipette out into a Wagnor- Parnas distillation apparatus and 10ml of 2% boric acid with mixed indicator was kept in a beaker at the delivery end of the distillation apparatus.
- To the distillation apparatus, 10ml of 40% sodium hydroxide was added and steam distilled. The distillate was collected until no more ammonia was evolved.
- The contents of the beaker were titrated against 0.02 N sulphuric acid until a red colour was appeared.

Total nitrogen content of the sample was determined by the formula.

$$\text{Total nitrogen (\%)} = \frac{0.00028 \times T.V \times 100 \times 100}{10 \times 0.2}$$

Where,

T.V	=	Titre value.
0.00028	=	1ml of 0.02 N sulphuric acid utilized.
10	=	Volume of extract taken for distillation (ml).
0.2	=	Weight of sample (gm).
100	=	Total volume (ml).

APPENDIX - V

ESTIMATION OF TOTAL PHOSPHORUS (JACKSON, 1973)

Princple

Phosphorus is precipitated as ammonium phosphomolybdate in nitric acid medium. The precipitate is filtered, washed free of acid, dissolved in a known excess of standard alkali and the excess alkali is determined by back titration with a standard acid using phenolphthalein indicator.

Reagent

- Hydrochloric acid – 1:1
- Nitric acid – 1:1
- Nitric acid – 1:1
- Conc. ammonium hydroxide
- Conc. nitric acid
- Solid ammonium nitrate
- Ammonium molybdate solution – 20 percent
- Potassium hydroxide – 0.1619N
- Nitric acid - 0.1619N
- Phenolphthalein

Procedure

- 200 ml of HCL extract of the sample was pipette out into a 400 ml beaker and evaporated to a small bulk.
- Then, it was transferred to a silica basin using hot water and evaporated to dryness over a water bath.
- The silica basin was kept in an air oven at 105 to 110 ^{o}C for 3 hours to dehydrate the silica
- This residue was dissolved by adding a small quantity of 1:1 hydrochloric acid and evaporated to dryness over a water bath.
- The residue was again dissolved in nitric acid, adding sufficient amount of nitric acid, to dissolve the same.

- The insoluble silica was allowed to settle overnight and then filtered through No. 42 filter paper and the residue was washed in the silica basin and on the filter paper with small quantities of 1:4 nitric acid till no yellow colour was left either in the basin or in the filter paper. The filtrate was collected in a 250 ml beaker.
- The extract was made alkaline with conc. ammonium hydroxide.
- To this, 5g of solid ammonium nitrate was added and kept on a thermostat at 65 ^{o}C for 15 minutes.
- The precipitant mixture was prepared by taking 7 ml of conc. nitric acid and 3 ml of distilled water in a 100 ml beaker and 10 ml of 20 percent ammonium molybdate was added to this solution drop by drop with constant stirring.
- 10 ml of this precipitant mixture was added drop by drop to the beaker in the thermostat with constant stirring and kept in the thermostat for another half an hour at 65 ^{o}C and allowed the precipitate to settle well.
- Then, it was filtered through No.40 filter paper by decantation, pouring only the supernatant liquid to the filter paper.
- The precipitate was then washed with cold distilled water till the filtrate runs free of acid.
- The filter paper was then transferred with the precipitate to the same beaker in which precipitation was done and enough water was added to make the filter paper into a pulp.
- Now, 0.1619N KOH was added from the burette, till the yellow precipitate was completely dissolved leaving a colourless solution. Then, another 5 ml of 0.1619N KOH was added to keep the alkali in fair excess quantity.
- A drop of phenolphthalein was added and the excess alkali was titrated against 0.1619N nitric acid. Disappearance of pink colour indicated the end point.

Calculation

Weight of sample taken	= W gm
Volume of HCL extract prepared	= 500 ml
Volume of HCL extract pipette out for analysis	= 200 ml
Volume of 0.1619N KOH added in excess	= a ml

Volume of 0.1619N HNO_3 used for back titration = b ml

Therefore, actual volume of 0.1619N KOH

used to dissolve the precipitate = (a-b)

1 ml of 0.1619N KOH = 0.0005gm P_2O_5

(a-b) ml of 0.1619N KOH = 0.0005 x (a-b) x gm P_2O_5

This was present in 200 ml of HCL extract

Therfore, in 500 ml = 0.0005 x (a-b) x 500/200

This was present in W gm of sample

Therefore, in 100 gm = 0.0005 x (a-b) x 500/200 x 100/W

Percentage of P_2O_5 on moisture free basis

= 0.0005 x (a-b) x 500/200 x 100/W x 100/(100 – M)

(M – Moisture content of the sample)

APPENDIX - VI

ESTIMATION OF TOTAL POTASSIUM

FLAME PHOTOMETER METHOD

(JACKSON, 1973)

Principle

Certain elements when excited in flame, emit radiation. The excitation causes one of the outer electrons of neutral atoms to jump to an outer orbit of higher energy level or the atoms may be excited sufficiently to loose an electron completely. When excited atoms return to lower energy levels, light of characteristics wavelength is emitted. The flame photometer measures this radiation intensity which is proportional to the concentration in a solution.

Preparation

1.907g of KCL was dissolved in 1 litre of distilled water (1000 ppm of K). From this, various standards were prepared ranging from 10 to 100ppm.

Procedure

- The atomizer was fixed in its place and introduced with distilled water.
- The compressor was started and the air pressure was adjusted to 10 psi.
- The gas was opened to light the burner through the window. Flow of gas was adjusted to give a central bluish cone.
- Zero was set with distilled water by using the zero adjustment knob. Then, 100 ppm K solution was introduced and adjusted to read 100 on the scale. Again distilled water was introduced and adjusted to zero. This process was repeated till the metre reading showed zero with distilled water and 100 with 100 ppm solution without zero adjustment.
- Then, various standard solutions were introduced, the readings were recorded and the standard curve was drawn.
- The filtrate was taken from sesquioxide estimation in a small vial and introduced through the atomizer. The readings were recorded and the percentage of K was calculated by using the standard curve.

Calculation

Weight of sample taken	= W gm
Volume of HCL extract prepared	= 500 ml
Volume HCL extract pipette out for sesquioxide estimation	=50 ml
Volume of sesquioxide filtrate made up to	= 250 ml
Metre reading	= G
Equivalent ppm from standard curve i.e. 1 ml of the solution contains	= A
A microgram of K	= $A/10^6$ gm of K
Therefore, in 250 ml of the solution	= $A/10^6$ x 250
This was present in 50 ml of HCL extract	
Therefore, in 500 ml	= $A/10^6$ x 250 x 500/50 gm
This was present in W gm of sample	
Therefore, in 100 gm	= $A/10^6$ x 250 x 500/50 x 100/W gm
Percentage of K on moisture free basis	= $A/10^6$ x 250 x 500/50 x 100/W x 100/(100 – M)

(M – Moisture content of sample)

APPENDIX- VII

ESTIMATION OF CALCIUM AND MAGNESIUM

VERSANATE METHOD

(JACKSON, 1973)

PRINCIPLE

Calcium and magnesium get complexed by EDTA in the order calcium first followed by magnesium. Calcium is estimated first by using murexide indicator at pH 12 in the presence of sodium hydroxide. Then calcium and magnesium is estimated using Erichrome Black – T at pH 10 in the presence of ammonium chloride and ammonium hydroxide buffer solution.

REAGENTS

- 0.02 N EDTA
- 10% sodium hydroxide
- Ammonium chloride – ammonium hydroxide buffer solution
- Murexide solution
- Erichrome Black – T indicator

PROCEDURE

Calcium alone

- Pipette out 10 ml of seaqui oxide filterate into a porcelain basin.
- Add 10% sodium hydroxide solution drop by drop to neutralise the activity (red litmus turns blue) and another 5ml excess to maintain the pH at 12.
- Add a pinch (50 mg) of murexide indicator and titrate with 0.02N EDTA till the colour changes from pinkish red to purple or violet.

Calcium and Magnesium

- Pipette out 10 ml of seaqui oxide filterate into a porcelain basin.
- Add ammonium chloride – ammonium hydroxide buffer solution drop by drop to neutralise the acidity (use red litmus paper) and 5 ml excess to maintain the pH at 10.

- Add 2 – 3 drop of Erichrome Black – T indicator solution and titrate with 0.02 N EDTA till the colour changes from purple red to sky blue.

CALCULATION

Weight of the sample taken = W gm

Volume of hydrochloric acid extract prepared =500 ml

Volume of hydrochloric acid extract pipette out for R_2O_3 estimation =50 ml

Volume of R_2O_3filterate made upto = 250 ml

Volume of R_2O_3filterate pipetted out for calcium estimation =10 ml

Volume of 0.02 N EDTA used for calcium and magnesium = a ml

Volume of 0.02 N EDTA used for calcium alone = b ml

Volume of 0.02 N EDTA used formagnesium alone = (a – b) ml

1 ml of 0.02 N EDTA =0.0004g calcium

1 ml of 0.02 N EDTA = 0.0004 g magnesium

Percentage of calcium on moisture free basis

$$= 0.0004 * b * \frac{250}{10} * \frac{500}{50} * \frac{100}{W} * \frac{100}{(100-M)}$$

Percentage of magnesium on moisture free basis

$$= 0.00024*(a - b)*\frac{250}{10} * \frac{500}{50} * \frac{100}{W} * \frac{100}{(100-M)}$$

M = Moisture basis

APPENDIX – VIII

ESTIMATION OF AVAILABLE NITROGEN IN SOIL

ALKALINE PERMANGANATE METHOD

(SUBBIAH AND ASIJIA, 1956)

Principle

A known weight of soil is mixed with excess of alkaline permanganate and distilled organic matter present in soil is oxidised by the nascent oxygen liberated by $KMnO_4$ in the presence of NaOH and thus ammonia is released. This released ammonia is absorbed in a known volume of boric acid (2%) containing double indicator and converted to ammonium borate. This ammonium borate is titrated against standard H_2SO_4.

Reagents

- 0.32% $KMnO_4$ solution (3.2 gm of $KMnO_4$ dissolved in one litre of distilled water).
- 2.5% NaOH solution (25 gm of NaOH dissolved in one litre of distilled water).
- 2% boric acid (20 gm of boric acid dissolved in one litre of distilled water).
- N/50 H_2SO_4 (30 ml of Conc. H_2SO_4 is diluted to one litre with distilled water and standardized by titration with N/10 Na_2CO_3. This gives N/10 H_2SO_4. From this N/50 H_2SO_4 is prepared by dilution.
- Double indicator bromocresol green (0.5 gm) and methyl red (0.1 gm) dissolved in 100 ml and ethyl alcohol.

Procedure

Weighed 20 gm of soil and tranferred into a distillation flask. Added 30 ml of distilled water to moist the soil and 1 ml of liquid paraffin. Added few pieces of glassbeads to avoid frothing. Added 100 ml of freshly prepared 0.32% $KMnO_4$ and 100 ml 2.5% NaOH to the soil in the distillation flask. A 100 ml beaker containing approximately 20 ml of 2% boric acid with double indicator was kept below the delivery end of the condensor in the distillation set. Distilled the contents and the liberated ammonia was collected in boric acid. Distillation continued until the release of ammonia. Titrate the ammonia collected in boric acid with N/50 H_2SO_4.

Calculation

Weight of the soil taken	= 20gm
Volume of N/50 H_2SO_4	= X ml (titre value)
1 ml of N/10 H_2SO_4	= 0.0014 gm N
Therfore 1 ml of N/50 H_2SO_4	= 0.00028 gm N
X ml of N/50 H_2SO_4	= 0.00028 * X gm N
This is present in 20 gm of soil	
Therefore N present in Kg/Ha	= 0.00028 (X/20)*10^6

APPENDIX - IX

ESTIMATION OF AVAILABLE PHOSPHORUS IN SOIL CALORIMETRY METHOD (BRAY 1 METHOD – JACKSON, 1973)

PRINCIPLE

The combination of HCL and NH_4F extracts acid soluble forms of phosphorus such as mono calcium phosphate. The flouride ion has the special property of complexing Al^{+++} and Fe^{+++} in acid solution with consequent release of phosphorus held in the soil by these ions. The phosphorus so released into the soil solution in estimated colorimetrically as available phosphorus.

REAGENTS

- NH_4F solution (1N): 37g of NH_4F was dissolved in 1 litre of distilled water.
- HCL (0.05N): 20.2 ml conc. HCL diluted 500 ml with distilled 500 ml with distill water.
- Bray No. 1 extractant [0.03 NH_4F and 0.02 N HCL]: 15 ml of 1N NH_4F and 25 ml of 0.5N HCL are mixed and the volume was upto 500 ml with distilled water.
- Ascorbic acid.

PROCEDURE

Weighed 5gm of soil and transfer to a 100 ml polythene shaking bottle. Added 50 ml of Bray 1 extractant. Shake the contents in a reciprocatory mechanical shaker for one minutes. Filtered the contents through whatman No. 40 filter paper. Simultaneously conducted a blank. Pipetted out 5 ml of filterate into 25 ml volumetric flask. Added 4 ml of reagent B as in Olsen's method and made up the volume to 25 ml. The intensity of the colour developed was measured in a photoelectric calorimeter using filter (660 nm).

CALCULATION

Weight of soil taken	= 5gm
Volumn of NaHCO3	= 50 ml
Volume of extractant solution used for Phosphorus estimation (aliqreo)	= 5 ml

Calorimeter reading	= T
Concentration of phosphorus read from	
standard graph for the reading T	= X ppm
	= X mg/ml
	= $X/10^6$ gm/ml
Therfore in 25 ml of solution	= $X/10^6$*25gm
This is present in 50 ml of the extractant solution and 5 g of soil	
Therefore available P_2O_5 in kg/ha	= X *25 *50 *2 *10^6
	10^6 *5 *5

APPENDIX – X

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL FLAME PHOTOMETRY METHOD (STANDFORD AND ENGLISH, 1949)

PRINCIPLE

The potassium ions in the exchange site are replaced with NH4+ and K+ is released. The concentration of K ions in the solution is then determined using flame photometer.

REAGENTS

1 N Ammonium acetate (Neutral in pH): Dissolved 77 g of AR grade ammonium acetate in 1000 ml distilled water. pH adjusted to 7.0.

PROCEDURE

Transferred 5g of soil into a polythene shaking bottle. Added 25 ml of 1 N ammonium acetate and contents shaked in a mechanical reciprocating shaker for 5 minutes. Contents filtered through whatman No. 40 filter paper. Filterates were fed into the flame photometer and the readings recorded. Using standard curve available potassium content was calculated.

CALCULATION

Weight of the soil taken	= 5 gm
Volume of the extractant used	= 25 ml
Flame photometer reading	= T
Concentration of K in the standard curve	= X ppm
	= X mg/ml
	= $X/10^6$ gm/ml
Therefore in 25 ml solution	= $X/10^6$*25gm
This is present in 5gm of soil	
Therefore available K in soil in kg/ha	= $X/10^6$*25*2*10^6/5

APPENDIX - XI

ESTIMATION OF AVAILABLE SULPHUR IN SOIL

(TABATABAI, 1982)

Principle

Different reagents have been prepared for extracting plant available sulphur from soil. This includes water and 0.15% $CaCl_2$, 500 ppm $CaH_2(PO_4)$ or KH_2PO_4 and acidic solutions in form of ammonium acetate (0.5 N) + acetic acid (0.25 N) and Bray No.1. Generally PO_4^{2-} solutions extract more sulphur (sulphate form) from the soil extracted with H_2O or salt solutions because phosphate ions displaces the absorbed SO_4^{2-}which is known to be readily available in plant.

Reagents

- 0.15% $CaCl_2$ solution: Dissolved 1.5gm of $CaCl_2$ in about 500ml distilled water & made up the volume to 1 litre.
- Gum acacia solution: Dissolved 0.25gm of chemically pure gum acacia in hot water and filtered the solution through Whatman No. 42 filter paper.
- Then cooled the filtrate and diluted to 100ml.
- Barium Chloride ($BaCl_2$): Ground analytical grade $BaCl_2$ to pass through 1mm sieve.
- Concentrated standard sulphate solution (100mg S/l): Dissolved 0.5434 g oven dried AR K_2SO_4 in distilled water & made up to 1 liter.

Procedure

Extraction by TABATABAI, 1982

10gm of air dried processed soil was shaken with 50 ml 0.15% $CaCl_2$ solution in 250 ml conical flask for 30 minutes. The extract was filtered through Whatman No.42 filter paper and estimated the SO_4^{2-} content by turbidimetric procedure.

Preparation of standard curve

- Pipetted out 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 2.5ml of standard sulphate solution in separate 25 ml volumetric flask and added 10 ml of extracting solution (0.15% $CaCl_2$) Fresh standard was prepared each time when a batch of sample is analyzed.

- 1g of $BaCl_2$ crystal was added to each flask and swirled to dissolve.
- 1ml of 0.25% gum acacia solution was added and made up to 25ml with distilled water and then shaken well
- Within 5-30 minutes of development of turbidity, absorbance was read in spectrophotometer at 440 nm.
- Standard curve was drawn with absorbance in Y axis and concentration on X axis

Turbidimetric estimation of sulphur by Massoumi and Cornfield, 1963

- Pipetted out 10 ml of soil extract into 25ml volumetric flask.
- Dissolved with 1g of $BaCl_2$ crystal.
- 1ml of 0.25% gum acacia solution was added and made up the volume with distilled water and shaken well and the absorbance was read at 440nm on a spectrophotometer.

Calculation

Weight of soil taken = 10 gm
Volume of extractant = 50ml
Volume of soil extract taken = 10ml
Volume made up = 25ml

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

$$= \frac{\text{Absorbance from the sample}}{\text{Slope of standard curve}} \times 12.5$$

$$= \frac{\text{Reading}}{0.025} \times 12.5$$

APPENDIX – XII

ESTIMATION OF AVAILABLE MICRONUTRIENTS IN SOIL (LINDSAY AND NORWELL, 1978)

The soils were extracted with DTPA extract. The extractant was prepared by mixing 0.005M diethylene triamine penta acetic acid (DTPA) with 0.1 triethanolamine (TEA) and 0.01M calcium chloride in the ratio of 1:2 taken for 2 hours. This extract was used for all the cationic micronutrient estimations.

Available copper

The DTPA extract was aspirated into the atomic absorption spectrophotometer, varian (AA120) wavelength of 2347.5 nm to estimate the available copper content.

Available Zinc

The DTPA extract was aspirated into the AA120 at the wavelength of 2138.6 nm to estimate the available zinc.

Available Manganese

The DTPA extract was aspirated into the AA120 at the wavelength of 2794.8 nm to estimate the available manganese.

Available iron

The DTPA extract was aspirated into the AA120 at the wavelength of 2483.3 nm to estimate the available iron.