**SOIL AND PLANT STANDARD OPERATION PROCEDURES**

**Determination of available ammonium in soil.**

**1. Title:** Standard operating procedure Calorimetric determination of Ammonium in soil

**Introduction**

Nitrogen (N) is a major element essential for plant growth because it is a constituent of all proteins and nucleic acid. The bulk of soil nitrogen resides in the organic matter, but this N is continuously being mineralized into ammonium (NH4+) and nitrates (NO3-) ions the form assimilated by plants.

**2. Scope:** The procedure covers the extraction and analysis ofAmmonium available in all types of soil.

**3. Purpose:** The purpose of this procedure is to determineavailable Ammonium to make informed decisions and recommendations on the need and rates of N- bearing fertilizers and organic inputs for soil fertility improvement.

**4. Terms, Acronyms and Definitions:**

1. Colorimetry – determination of analyte concentration through intensity of colour development using spectroscopy
2. UV-Vis Spectrophotometer: Ultraviolet-visible spectrophotometer. The absorption or reflectance in the visible range directly affects the perceived [colour of the chemicals](http://en.wikipedia.org/wiki/Color_of_chemicals) involved and directly proportional to the concentration of the chemical

**5. Apparatus/ Equipment**

1. Mechanical shaker
2. Analytical balance
3. UV-VIS spectrophotometer
4. Vortex shaker
5. Funnels
6. Test tubes
7. Automatic pipettes (0.2 ml- 1ml)
8. Filter papers grade no.542/42/2
9. 250mls plastic shaking bottles
10. Cuvettes

6. Reagents

1. Sodium hydroxide (NaOH)
2. Sodium hypochlorite ( NaOCl)
3. Sodium nitroprusside Na2{(FeCN)5NO}.2H2O
4. Sodium salicylate (C7H5O3.Na)
5. Sodium tart rate (Na2C2H4O6)
6. Sodium citrate (Na3C6H5O7)
7. Potassium Sulphate (K2SO4)
8. Distilled water

**6.1 Preparation of reagents and stock solution**

1. Reagent N1:

Dissolve 34 g of sodium salicylate, 25g of sodium citrate and 25g of sodium tart rate together in about 750ml water. Add 0.12g sodium nitroprusside and make up to 1 litre with distilled water. The sample filtrate solution above is strongly acid.

1. Reagent N2

Dissolve 30g sodium hydroxide in about 750 ml distilled water. Allow to cool. Add 10 ml sodium hypochlorite mix well and make up to 1litre.

***N.B. Remark.*** Reagent N1 and N2 should be made at least 24 hours before use and stored in the dark.

1. Stock solution 1000 µg N/litre:

Dissolve 4.714 g of ammonium sulphate ((NH4)2 SO4) in1000 ml volumetric flasks make up to the mark with distilled water.

1. Standard solution 0.01mg/litre (100µg NH4+/ml):

Dilute 50ml of the above stock solution in 500 ml with distilled water and make to the mark with distilled water.

# Ammonium working Standards.

# Into a clean set of 100-ml volumetric flasks, pipette 0, 5.0, 10.0, 15.0, 20.0 and 25.0 ml the standard solution (100µg NH4+/ml), above and make up to the mark with 0.5M potassium sulphate (K2SO4). The standard series contains 0, 0.5, 10.0, 15.0, 20.0 and 25.0 µg NH4-N/ml.

1. **Procedure**

# 7.1 Extraction of Ammonium from the soil

# Weigh 10.0 g of freshly sampled soil sample (or sample kept in a refrigerator) into a plastic shaking bottle.

# Add 100 ml of 0.5 M potassium sulphate (K2SO4) extracting solution. Stopper and shake contents for 1 hour.

# Filter through No. 542/2 or No. 42 Whatman filter paper. If analysis will not be complete in one day, store the filtrate in a refrigerator.

# Analysis should be done on freshly collected soil samples as stored samples may have accumulated nitrate as consequence of continued mineralisation.

# *Note:*

# *Potassium sulphate 0.5M is used instead of the potassium chloride because Cl- ions interfere with the calorimetric reaction.*

# *Storing the soils under refrigerator at 4oC suppressesmicrobial activity associated with N-mineralization*

Pipette 0.2 ml of the sample extract( from section 7.1 above),the blanks and the standard series into clearly labelled test tubes,

* 1. **Measurement of Ammonium Concentration.**

Pipette 0.2 ml of the sample extract(from section 7.1 above), the blanks and the standard series into clearly labelled test tubes

Add 5.0ml of the reagent N1 into each of the test tubes and allow to stand for at least 15 minutes then vortex for at least 1 minute.

1. Add 5.0ml of reagent N2 into each of the test tubes and vortex for at least 1 minute.
2. Allow solution in the test tubes to stand for 1 hour and measure the absorbance at 655nm using UV/VIS spectrophotometer.
3. The blue colour is stable for at least 10 hours.
4. Plot a calibration curve using the standard solutions
5. Read and record the concentration of NH4+-N in the sample solution.
6. Determine the actual concentration of NH4+-N the sample solution using the formula below.

## Calculation

## The concentration of ammonium-nitrogen in the oven dry soil expressed in µgNH4+-N/kg then is calculated as follows;

**NH4-N (µg kg-1) = (a-b) × v ×MCF × f ×1000**

**W**

Where a = concentration of N in the solution, b = concentration of N the blank, v = volume of the extract; w = weight of the fresh soil; MCF = moisture correction factor; f = multiplication factor.

1. **References**
2. Bremner, J.M. and Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. *Analytical Chim. Acta***32**, 485-495.
3. Page, A.L. (1982). Methods of Soil Analysis: Part 2. American Society of Agronomy.
4. Freney, J.R. and Westlaw, R. (1969). the determination of mineral nitrogen in soil with particular reference to nitrate. *Division of Plant Industry Tech. Paper No. 23*. CSIRO, Melbourne, Australia.
5. Robinson, J.B.D. (1968). A simple available soil nitrogen index. I. Laboratory and greenhouse studies. *J. Soil Science***19**, 269-279.

Standard operating procedure of determination of available Phosphorus in soil samples

1. Title: Determination of available Phosphorus in soil samples

**2. Scope:** The procedure is applicable for extraction and analysis of available Phosphorus in all soiltypes.

**3. Purpose:** The purpose of this procedure is to determine phosphorus levels for advisory services in support of improvement on soil fertility

**4. Terms, Acronyms and Definitions:**

1. UV-Vis Spectrophotometer: Ultraviolet-visible spectrophotometer. The absorption or reflectance in the visible range directly affects the perceived [colour of the chemicals](http://en.wikipedia.org/wiki/Color_of_chemicals) involved and directly proportional to the concentration of the chemical

**5. Apparatus/ Equipment**

1. Mechanical shaker
2. Analytical balance
3. 100mls plastic bottles
4. UV- vis beam spectrophotometer
5. 50mls volumetric flasks
6. Conical flasks
7. Automatic pipettes
8. Filter papers grade no. 542/42/2
9. Funnels
10. Activated charcoal
11. Asbestos mat
12. Reagent brown bottle
13. Water bath
14. pH meter
15. Polythene container

6. Reagents

1. Sodium bicarbonate (NaHCO3) (Olsen extracting solution)
2. Sodium hydroxide, (NaOH)
3. Sulphuric acid (H2SO4),
4. Ammonium molybdate (NH4)6Mo7O24.4H2O) ,
5. Antimony potassium tart rate(KSb.C4H4O6) ,
6. Ascorbic acid (C6H8O6)
7. Potassium Di hydrogen Phosphate (KH2PO4)
8. Boric Acid (H3BO3)
9. Distilled water

**6.1 Preparation of reagents and standard solutions**

1. Sodium bicarbonate, NaHCO3, 0.5 M of pH 8.5.

Dissolve 42 g of analytical reagent grade (AR) NaHCO3 in 1 litre of distilled water. Adjust pH to 8.5 with 1M sodium hydroxide solution (prepared by dissolving 40 g of AR NaOH in 1litre of distilled water). This is the Olsen's extracting solution. Store this solution in a polythene container and check the pH of the solution each month. Five litres of the extracting solution may be prepared by weighing out 210 g of NaHCO3 and buffering to pH of 8.5 with 1 M NaOH.

1. Sulphuric acid, H2SO4, 5N:

Place one litreclean beaker on asbestos mat (or in cold water in sink). Add slowly 148 ml of concentrated H2SO4 to about 500 ml of distilled water while stirring. When cool dilute to 1litre with distilled water.

1. Ammonium molybdate/antimony potassium tart rate solution.

Dissolve 12 g of ammonium molybdate (NH4)6Mo7O24.4H2O) in 250 ml of warm (50°C) distilled water. Separately dissolve 0.291g of antimony potassium tart rate (KSb.C4H4O6) in 100 ml distilled water. Add both solutions to 1000 ml of 5N H2SO4 . Mix thoroughly and dilute with distilled water to 2 litres. Transfer to a reagent bottle and store in a dark, cool place. The mixture is viable for 2 months.

1. Ascorbic acid reducing agent.

Dissolve 2.108g of ascorbic acid (C6H8O6) in 400 ml of ammonium molybdate/antimony potassium tart rate solution (above) and mix well. This must be prepared as required on the day of analysis (This amount is adequate for about 30 samples plus the P standards). The solution keeps for about 24 hours. Larger quantities of this reducing agent may be prepared depending on the output of a specific laboratory.

1. Boric acid( H3BO3) 0.8M.

Weigh out 49.4 g of AR H3BO3 powder disolve and dilute to 1 litre mark(with vigorous shaking)with distilled water.

1. Standard phosphorus stock solution, 100 ppm P:

Weigh 0.10967g of oven-dried KH2PO4; dissolve and make to 250 ml mark with distilled water (1 ml = 1 mg P). Make a series of working standards as follows: 0, 2, 4, 6, 8 and 10 ppm P working solution by diluting100ppm P standard stock.

1. **Procedure**
   1. Weigh out accurately 2.5 g of air-dried soil into a 150 or 250 ml polythene shaking bottle.
   2. Add 50 ml of the Olsen's extracting solution (0.5 M NaHCO3 pH 8.5) to each bottle.
   3. Stopper well and place on a mechanical shaker for 30 minutes.
   4. Filter the suspension after shaking through the Whatman No. 542/42/2 paper.
   5. Add charcoal if necessary to obtain a clear filtrate. This filtrate is used for the colorimetric P measurements.
   6. Pipette 5 ml of the clear filtrate into a 50-ml volumetric flask and add about 20-ml distilled water to each volumetric flask.
   7. Add 10 ml of the ascorbic acid reducing agent to each volumetric flask, beginning with the standards.Make to 50 ml mark with distilled water; stopper and shake well. Let solution to stand for 1 hour to permit full colour development. (The colour is stable for only a short while)
   8. Measure the standards and sample absorbance’s (blue colour) at 880nm wavelength using UV-vis spectrophotometer.
   9. Plot a graph of absorbance against standard concentration.
   10. Determine solution concentrations for each unknown using the formula below. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**P in sample (ppm) = C x ppm solution x df/w**

Where C = the corrected concentration of P in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

1. **References**
2. Okalebo J.R., Gathua, K.W. and Woomer P.L. (2002). Laboratory methods of soil and plant analysis: a working manual. Second Edition. TSBF. CIAT and SACRED Africa, Nairobi, Kenya.
3. Gachene, C.K.K. and Kimaru, G. (2003). Soil Fertility and Land Productivity - A guide for extension workers in the eastern Africa region. Technical Handbook No.30. Regional Land Management Unit (RELMA)/ Swedish International Development Cooperation Agency (Sida).
4. Tea Research Foundation of Kenya(TRFK), 2012,Soil Manual and Fertilizer Sampling and Analytical Methods Manual
5. Olsen, S.R. and Sommers, L.E.(1982).Phosphorus. *In*: A.L. Pageetal. (eds.) Methods ofsoil analysis, part2. Agron. Mongr.9. 2nd ed. ASA and SSSA, Madison, WI.
6. Truog, 1930. Journal Am .Soc.Agr. 22( pages 874-882)
7. Murphy and Rley, 1962.
8. Wilde, S.A., R.B. Corey, J.G. Lyer and G.K. Voigt. 1979. Soil and plant analysis for tree culture. oxfordIBH, Bombay and New Delhi. Reprinted 1985

**Standard operating procedure for determining available potassium in soil.**

**1. Title:** Determination of Available Potassium in soil

**2. Scope:** The method of extraction and analysis of available potassium is applicable in all types of soils

**3. Purpose:** The purpose of this procedure is to determine available potassium levels in soils for advisory services in support of improvement on soil fertility

**4. Terms, Acronyms and Definitions:**

1. Atomic Absorption Spectrophotometer (AAS) - it is an instrument used for quantitative determination of chemical elements using the absorption of optical radiation by free atoms in the gaseous state. The atoms make transitions to higher electronic energy levels. The analyte concentration is determined by the amount of absorption.

**5. Apparatus/ Equipment**

1. Mechanical shaker
2. Analytical balance
3. 100mls plastic bottles
4. Atomic Absorption Spectrophotometer (AAS)
5. Volumetric flasks 50mls -1000mls
6. Automatic pipettes
7. Filter papers grade no. 542/42/2
8. Funnels
9. pH meter
10. Brown bottle
11. Oven

##### 6. Reagents

1. Ammonium acetate (NH4C2H3O2)
2. Bromothymol blue indicator
3. Ammonium hydroxide
4. Acetic acid
5. Potassium Chloride (KCl)
6. Strontium chloride
7. Distilled water

##### Preparation of Reagents

1. Molar neutral ammonium acetate solution:

Dissolve 77.08 g of ammonium acetate (NH4C2H3O2) in 1 litre of distilled water. Check the pH with bromothymol blue or with a pH meter. If not neutral, add either ammonium hydroxide or acetic acid as needed to neutralize it to pH 7.0.

1. Stock Potassium solution, 100 ppm K.

Weigh 0.1907 g of potassium chloride and dry in an oven at 100°C for 2 hours. Remove from oven and cool in desiccator then dissolve and make to 1 litre with distilled water. Transfer prepared solution into a reagent bottle and store.

1. Potassium working standard series as follows: Pipette 0, 1, 2, 3, 4 and 5mls of the 100ppm potassium standard solution into well labelled 50mls volumetric flask to prepare 0, 2, 4, 6, 8 and 10ppm working standards respectively. Make to the mark with distilled water.

**7. Methodology**

* 1. Weigh 5g of air dried soil samples into 250mls plastic bottles and add 100mls of molar neutral ammonium acetate solution and shake for 30 minutes.
  2. Filter the solution using filter paper 542/42/2.
  3. Pipette 5 ml of the extracted sample filtrate (above) into a 50 ml volumetric flask.
  4. Add 5mls of 0.5% strontium chloride solution into each flask and make to the mark with distilled water.
  5. Aspirate the solutions starting with standards, blank and the sample solutions directly into atomic absorption spectrophotometer.
  6. Plot a graph of absorbance against standard concentrations. Plot a calibration curve using the standards solution
  7. Read off the amount of potassium present in the sample solution from the calibration curve prepared by plotting absorbance (or transmission) readings against potassium standards concentrations.
  8. Calculate the solution concentrations for each unknown sample using the formula below.. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= Cin subsequent calculations).

**K in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of K in the sample; ppm solution = graph reading;

df = dilution factor; w = weight of dry the sample.

**8.References**

1. Okalebo J.R., Gathua, K.W. and Woomer P.L. (2002). Laboratory methods of soil and plant analysis: a working manual. Second Edition. TSBF. CIAT and SACRED Africa, Nairobi, Kenya.
2. Tea Research Foundation of Kenya (TRFK), 2012,Soil Manual and Fertilizer Sampling and Analytical Methods Manual.
3. Department of Agriculture and Cooperation Ministry of Agriculture Government of India (2011) New Delhi

**Standard operating procedure on determination of exchangeable Calcium, magnesium, potassium, sodium, manganese and cation exchange capacity (CEC) in soil**

1. **Title**: Determination of Exchangeable Calcium, magnesium, potassium, sodium, manganese and CEC in soil
2. **Scope**: The method is applicable to all soil types.
3. **Purpose:** The purpose of this procedure is to determine exchangeable Calcium, magnesium, potassium, sodium, manganeseand cation exchange capacity for advisory services and making informed decision in application of organic and inorganic fertilizers.

**4. Terms, Acronyms and Definitions:**

1. Cation exchange capacity (CEC)- The cation exchange capacity (CEC) of a soil is defined as the total sum of exchangeable cations that it can adsorb at a specific pH. Cation exchange of exchangeable cations in reversible chemical reactions is a quality important in terms of soil fertility and nutritional studies. CEC is an inherent soil characteristic and is difficult to alter significantly. It influences the soil's ability to hold onto essential nutrients and provides a buffer against soil acidification. CEC is expressed in meq/100 g which is numerically equal to centimoles of charge per kilogram of exchanger (cmol(+)/kg).

**5. Apparatus/equipment**

1. Analytical balance
2. Centrifuge
3. Mechanical shaker
4. 100ml-1000ml volumetric flask
5. Atomic Absorption Spectrophotometer
6. pH meter
7. Reagent bottle
8. Glass funnel
9. Beaker

**6. Reagents and chemicals**

1. Glacial acetic acid
2. Concentrated Ammonium hydroxide (NH4OH)
3. Ammonia Acetate solution (NH4Ac)
4. Distilled water

**6.1 Preparation of reagents**

1. Ammonium acetate solution, 1 M pH 7.0.

Add 58ml of glacial acetic acid to about 600ml of distilled water in a 2 litre beaker. Add 70ml of concentrated ammonium hydroxide (NH4OH) (specific gravity 0.90) under a fume hood through a long stemmed glass funnel so that it is introduced into the bottom of the acid solution. Cool the solution for about 30 minutes and adjust to pH 7.0 with acetic acid or NH4OH using a pH meter.

Transfer the solution into a 1litre volumetric flask and dilute to volume. Mix it in a Pyrex reagent bottle.

**7. Procedure**

* 1. Weigh 5g of soil sample, add 30ml of 1M NH4OAc and shake on a mechanical shaker for 2 hours.
  2. Centrifuge at 2000 rpm for 5-10 minutes. Carefully decant the clear supernatant into a 100ml volumetric flask.
  3. Add another 30ml of NH4OAc solution and shake for 30 minutes. Centrifuge and transfer the supernatant into the same volumetric flask.
  4. Repeat step 7.3 and transfer the supernatant into the same volumetric.
  5. Make up to the mark with NH4OAc solution.
  6. Determine K, Na, Ca, Mg, and Mn on an atomic absorption spectrophotometer (AAS).
  7. Effective CEC is thus calculated by the sum of exchangeable bases (Ca, Mg, K and Na) and exchangeable Al and H expressed in meq/100g

Each of these elements has its individual atomic weight as found on a Periodic Table.

Calcium= 40; Potassium= 39; Sodium = 23; Magnesium= 24; Hydrogen=1;

Calculate the equivalent weight from the atomic weight by dividing the atomic weight by the number of valences we determine the equivalent weight. Calcium and magnesium have two valences or positive charges. Sodium, potassium, and hydrogen each have one positive charge. Therefore,

Calcium= 20; Potassium= 39; Sodium = 23; Magnesium= 12; Hydrogen=1;

However, the CEC is reported in meq/100 gm. The equivalent weights are reported as equivalents per gram. Therefore, the equivalent weights must be multiplied by ten to be converted to meq/100 gm. Hence: Calcium= 200; Potassium= 390; Sodium = 230; Magnesium= 120; Hydrogen=10.

Use these values to calculate the CEC form the ppm of each of these elements in the soil test.

Potassium = concentration reading/390 = a

Magnesium = concentration reading /120 = b

Calcium = concentration reading /200 = c

Sodium = concentration reading /230 = d

CEC meq/100 gm = a + b + c + d

**8. References**

1. Tea Research Foundation of Kenya (1st Edition 2012). Soil, PlantTissue and Fertilizer Sampling and Analytical Methods Manual
2. <https://www.midwestlabs.com/wp-content/uploads/2012/09/168_calculating_cation_exchange_cap_and_percent_base_saturation.pdf>

**Standard operating procedure on determination of heavy metalsCopper, Lead, Cadmium, Chromium, Arsenic and Selenium (Cu, Pb, Cd, Cr, As and Se) in soil and plant**

**1 Title:** Standard operating procedure on determination of heavy metals Copper,Lead, Cadmium, Chromium, Arsenic and Selenium(Cu, Pb, Cd, Cr, As and Se) in soil and plant samples

**2 Scope:** The method is applicable for the analysis of soil / plant samples.

**3 Purpose:** The purpose of this procedure is to determine levels ofCopper,Lead, Cadmium, Chromium, Arsenic and Selenium in soil and plant samples for toxicity levels.

**4. Terms, Acronyms and Definitions:**

1. Heavy Metal: A heavy metal is a toxic metal. [Most heavy metals](http://chemistry.about.com/od/metalsalloys/f/What-Is-A-Heavy-Metal.htm) have a high atomic number, [atomic weight](http://chemistry.about.com/od/chemistryglossary/a/atomicweightdef.htm) and a [specific gravity](http://chemistry.about.com/od/chemistryglossary/a/specgravitydef.htm) greater than 5.0 Heavy metals include some metalloids, [transition metals](http://chemistry.about.com/od/metalsalloys/f/Why-Are-Transition-Metals-Called-Transition-Metals.htm), [basic metals](http://chemistry.about.com/od/elementgroups/a/basicmetalslist.htm), lanthanides and actinides. Examples of heavy metals include lead, mercury, cadmium, sometimes chromium. Less commonly, metals [including iron](http://chemistry.about.com/od/chemistryglossary/g/Iron-Definition.htm), copper, zinc, aluminum, beryllium, cobalt, manganese and arsenic may be considered heavy metals

**5. Apparatus/equipment**

1. Digestion block
2. Volumetric flask
3. Conical flasks
4. Micro pipettes
5. Digestion tubes
6. Analytical balance
7. Hot plate/Magnetic stirrer
8. grinder
9. Atomic Absorption Spectrophotometer

**6. Reagents and chemicals**

1. Sulphuric acid – H2SO4 (93-98%)
2. Nitric acid (HNO3)
3. Hydrated Copper sulphate – CuSO4H2O (AR grade)
4. Potassium Sulphate (K2SO4) –or anhydrous Sodium Sulphate (Na2SO4) (AR grade)
5. Commercial manufactured standards of, Cu, Pb, Cd, Cr, As and Se stock solution of 1000ppm

**7. Procedure**

7.1. Weigh 0.3 g soil or ground plant sample in each digestion tube.

7.2. Add 0.5 g Copper Sulphate and 5g Potassium Sulphate into the digestion tube.

7.3. Add 20 ml concentrated sulphuric acid in each tube.

7.4. Place the digestion tubes on the digestion block and heat gently at 110°C for 1 hour until frothingceases.

7.5. Raise the temperature to 360°C and continue heating for 2 hours to attain complete oxidation.

7.6. Allow contents to cool for about 30 minutes.

7.7. Add 25 ml of distilled water and transfer the contents to 50 ml volumetric flask.

7.8. Determine Pb, Cu, Cd, Cr, As and Se in the digests first by preparing working standards as outlined below.

7.8.1 Make separate standard stocks 100 ppm from commercially manufactured Copper, Lead, Cadmium, Chromium, and Arsenic and Selenium1000 ppm stock.

7.8.2 Prepare working Copper, Lead, Cadmium, Chromium, Arsenic and Selenium standards series as follows: 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 ppm solution by diluting stock solution of 100 ppm Copper, Lead, Cadmium, Chromium, Arsenic and Selenium stock standards respectively.

7.8.3 In Lead, Cadmium, Chromium, Arsenic and Selenium working standards add a drop of concentrated HNO3.

* 1. Aspirate Copper, Lead, Cadmium, Chromium, Arsenic and Selenium standard series, blank digests and samples into the atomic absorption spectrophotometer and measure the absorbencies.

Plot a calibration curve from the readings of the standards series and determine the concentration of the unknown.

**Calculations**

Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**M in sample (ppm) = C\*ppm solution\*df/w**

Where M= concentration of Copper, Lead, Cadmium, Chromium, Arsenic or Selenium in the sample; C= the corrected concentration of M in the sample; ppm solution= graph reading; df= dilution factor; w= weight of dry sample

**8. References**

1. Tea Research Foundation of Kenya (1st Edition 2012). Soil, PlantTissue and Fertilizer Sampling and Analytical Methods Manual.
2. Methods of Analysis. Adapted from ICRAF 2001
3. Manjula V. Nathan and Yichang Sun(2006). Methods of Analysis. A Guide for Conducting Plant Analysis in Missouri. University of Missouri-Columbia.
4. Nitrogen (Total Kjeldahl) 2004.
5. Glaston M.Kenji and Paul N. Karanja (2009). Analysis of foods. A Practical Manual. First Draft Edition. Jomo Kenyatta University of Agriculture and technology.
6. Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (2002). Laboratory methods of soil and plant analysis: a working manual. Second Edition. TSBF. CIAT and SACRED Africa, Nairobi, Kenya.

**Standard operating procedure colorimetric determination of soil Nitrate in soil**

**1. Title**: Colorimetric Determination of Nitrate in soil

**2. Scope:** The procedure covers the extraction and analysis ofNitrate available in all types of soil.

**3. Purpose:** The purpose of this procedure is to determine available nitrate to make informed decision/recommendations on the rates of N- bearing fertilizers and organic inputs for soil fertility improvement.

**4. Terms, Acronyms and Definitions:**

1. Colorimetry – determination of analyte concentration through intensity of colour development using an instrument.
2. UV-Vis Spectrophotometer: Ultraviolet-visible spectrophotometer. The absorption or reflectance in the visible range directly affects the perceived [colour of the chemicals](http://en.wikipedia.org/wiki/Color_of_chemicals) involved and directly proportional to the concentration of the chemical.

**5. Apparatus/ Equipment**

1. Mechanical shaker
2. Analytical balance
3. UV- Vis spectrophotometer
4. Vortex shaker
5. Funnels
6. Test tubes
7. Automatic pipettes (0.2 ml- 1ml)
8. Filter papers grade no.542/42/2
9. 250mls plastic shaking bottles
10. Cuvettes

6. Reagents

1. Sodium hydroxide (NaOH)
2. Sodium hypochlorite ( NaOCl)
3. Sodium nitroprusside Na2{(FeCN)5NO}.2H2O
4. Sodium salicylate (C7H5O3.Na)
5. Sodium tart rate (Na2C2H4O6)
6. Sodium citrate (Na3C6H5O7)
7. Potassium Sulphate (K2SO4)
8. Potassium nitrate (KNO3)
9. Salicylic acid (C7H6O3 )
10. Sulphuric acid (H2SO4)
11. Distilled water

**6.1 Preparation of Reagents and standard solutions**

1. Sodium hydroxide, 4M.

Dissolve 160 g of sodium hydroxide with distilled water in a 1 litre volumetric flask and make to the mark.

1. Salicylic acid, 5%:

Dissolve 5g of salicylic acid in 95 ml sulphuric acid. Prepare this reagent at least one day before its use. The reagent remains stable for 7 days if stored in a cool and dark place.

1. Potassium nitrate stock solution, 1000 µg ml-1 NO3-N.

Place 7.223g of potassium nitrate that was dried at 105oC and cooled in a desiccator, into a 1000ml volumetric flask. Fill to the 1000 ml mark with distilled water.

1. Standard solution, 50 µg ml-1 NO3 N.

Dilute 25.0 ml of the potassium nitrate stock solution in a 500 ml volumetric flask and fill to the 500 ml mark with distilled water.

1. Nitrate working standards*.*

Transfer 0, 2.0, 4.0, 6.0, 8.0 and 10.0 ml of the standard solution (50 µg ml-1 NO3-N) into clean well labelled set of 100 ml volumetric flasks. These working standards contain 0, 2, 4, 6, 8, and 10 µg NO3-N ml-1. Fill each volumetric flask to the 100 ml mark with 0.5 M potassium sulphate

**7. Procedure**

# 7.1 Extraction of Nitrates from the soil

# Weigh 10.0 g of freshly sampled soil sample (or sample kept in a refrigerator) into a plastic shaking bottle.

# Add 100 ml of 0.5 M K2SO4 extracting solution. Stopper and shake contents for 1 hour.

# Filter through No. 542/2 or No. 42 Whatman filter paper. If analysis will not be complete in one day, store the filtrate in a refrigerator.

# Freshly sampled soil samples should be analysed within the same day to avoid accumulated nitrate as consequence of continued mineralization.

# *Note:*

# *Potassium sulphate 0.5M is used instead of the potassium chloride because Cl- ions interfere with the colorimetric reaction.*

# *Microbial activity, associated with N-mineralization may also be suppressed by storing the extract under refrigerator when the distillation cannot be conducted immediately.*

**7.2 Measurement of Nitrates**

1. Into clearly labelled test tubes, pipette 0.5 ml of the sample extract (from section 7.1 above) the blanks and the standard series
2. Add 1.0 ml of salicylic acid to each test tube, mix well and wait for 30 minutes.
3. Add 10 ml 4M sodium hydroxide to each test tube. Mix well and leave for 1hour for a full yellow colour development.
4. The colour is stable for one day.
5. Measure the absorbance at wavelength 419 nm using UV/VIS spectrophotometer
6. Plot a calibration curve using the standards solution
7. Read and record the concentration of NO3--N in the sample solutions and blank.
8. Determine the actual concentration of NO3--N the sample solution using the formula below.

###### 7.3 Calculation

**NO3 N (µg kg-1) = (a-b) × v ×MCF ×1000**

**W**

Where a = concentration of NO3+-N in the solution, b = concentration of NO3+-N the blank, v = volume of the extract; w = weight of the fresh soil; MCF = moisture correction factor. The aliquot taken for both the standards and the unknown are the same therefore no multiplication factor is required within the calculations.

**8. References**

1. Bremner, J.M. and Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. *Analytical Chim. Acta***32**, 485-495.
2. Page, A.L. (1882). Methods of Soil Analysis: Part 2. American Society of Agronomy.
3. Freney, J.R. and Westlaw, R. (1969). the determination of mineral nitrogen in soil with particular reference to nitrate. *Division of Plant Industry Tech. Paper No. 23*. CSIRO, Melbourne, Australia.
4. Robinson, J.B.D. (1968). A simple available soil nitrogen index. I. Laboratory and greenhouse studies. *J. Soil Science***19**, 269-279.

**Standard operating procedure on determination of total Nitrogen, Phosphorus, Calcium, Magnesium, Potassium, Manganese, Iron, Copper and Zinc( N, P, Ca, Mg, K, Mn, Fe, Cu and Zn) in plant samples**

**1. Title:** Standard operating procedure on determination of total N, P, Ca, Mg, K, Mn, Fe, Cu and Zn in plant samples

**2. Scope:** The method is applicable for the analysis of organic / plant samples.

**3. Purpose:** The purpose of this procedure is to determine levels of Nitrogen, Phosphorus, Calcium, Magnesium, Potassium, Manganese, Iron, Copper and Zinc in plant samples for nutritional value

**4. Terms, Acronyms and Definitions:**

1. UV-Vis Spectrophotometer: Ultraviolet-visible spectrophotometer. The absorption or reflectance in the visible range directly affects the perceived [color of the chemicals](http://en.wikipedia.org/wiki/Color_of_chemicals) involved and directly proportional to the concentration of the chemical
2. AR: Analytical reagent

**5. Apparatus/equipment**

1. Digestion block
2. Distillation unit
3. Conical flasks
4. Burettes
5. Pipettes
6. Digestion tubes
7. Analytical balance
8. Hot plate/Magnetic stirrer
9. Vortex mixer
10. UV-Vis Spectrophotometer
11. Atomic absorption spectrophotometer
12. Acetylene, Nitrous Oxide and air gas cylinders
13. Pestle and mortar
14. Grinder
15. Volumetric flasks

**6. Reagents and chemicals**

1. Sulphuric acid – H2SO4 (93-98%)
2. Hydrated Copper sulphate – CuSO4H2O (AR grade)
3. Potassium sulphate (K2SO4) or anhydrous Sodium Sulphate (Na2SO4) (AR grade)
4. Sodium hydroxide solution (NaOH)
5. Potassium hydrogen phthalate
6. Hydrochloric acid (HCl)
7. Methyl red indicator
8. Boric acid 1%
9. Distilled water
10. Analytical standards 1000ppm of Iron, Copper, Magnesium, Manganese and Zinc

**6.1 Preparation of reagents**

1. 40% Sodium hydroxide solution: Dissolve 400 g solid NaOH in distilled water and dilute to one litre.
2. 0.1M NaOH: Prepare 0.1M NaOH by dissolving 4.0 g NaOH in distilled water and make volume to 1 litre. Standardize against 0.1N potassium hydrogen phthalate or standard H2SO4
3. N/70 HCl: Prepare approximately N/70 acid solution and standardize against 0.1M sodium carbonate

**7. Extraction procedure**

7.1. Weigh 0.2 g ground plant sample in each digestion tube.

7.2. Add 0.5 g Copper Sulphate and 5g Potassium Sulphate into the digestion tubes

7.3. Add 20 ml concentrated sulphuric acid in each tube.

7.4. Place the digestion tubes on the digestion block and heat gently at 110°C for 1 hour until frothingceases.

7.5. Raise the temperature to 360°C and continue heating for 2 hours to attain complete oxidation.

7.6. Allow contents to cool for about 30 minutes.

7.7. After cooling add 25 ml of distilled water into the digestion tubes and transfer the contents to 50 ml volumetric flask.

7.8. Determine totalN, P, Ca, Mg, K, Mn, Fe, Cu and Znin the digests as outlined below

1. **Determination of total nitrogen**

The above plant digest is followed by either distillation-titration or by colorimetric for nitrogen determination. The choice is dependent on local facilities; but colorimetric procedures are more rapid and accurate enough.

**A.1 Distillation-titration determination of Total Nitrogen**

1. Set up a steam distillation apparatus (Markham or Hoskyn nitrogen still) and use Ammonia -free distilled water wherever possible.
2. Transfer 5 ml aliquot of digest solution (from section 7 above) to the reaction chamber of the still and add 10 ml of 40% NaOH.
3. Steam-distill immediately into 5 ml of 1% boric acid containing 4 drops of the methyl red indicator.
4. Continue distillation until 50ml of distillate is collected from the time the indicator turns green.
5. Remove the distillate and titrate with N/70 HCl until the indicator changes from green through grey to a definite pink. Note volume in ml of the standard Hydrochloric acid required.
6. Pass steam through the apparatus for 30 min. Check the steam blank by collecting 50-ml distillate and titrate with N/70 HCl as given below.
7. Occasionally check that the distillation recovery is satisfactory by taking an aliquot (e.g. 5.0-ml) of the standard ammonium sulphate solution in place of the sample

**Calculation:**

**% Nitrogen = (ml N/70 HCL acid for sample – ml of N/70 HCL acid for blank) × Molarity of acid × 14 × 100/weight of sample (g)**

# A.2 Colorimetric Determination of Total Nitrogen

**A.2.1 Apparatus/equipment**

1. Pipettes
2. Analytical balance
3. Magnetic stirrer
4. Vortex mixer
5. Uv-Vis Spectrophotometer
6. Volumetric flasks

**A.2.2 Reagents**

1. Sodium citrate
2. Sodium hydroxide
3. Sodium hypochlorite
4. Sodium nitroprusside
5. Sodium salicylate
6. Sodium tart rate
7. Reagent N1
8. Reagent N2
9. Ammonium sulphate, (NH4)2 SO4.

# A.2.2.1 Standards and reagents preparation

1. Stock solution 100 mgN/litre (ppm): Dissolve 0.47172 g of ammonium sulphate, (NH4)2 SO4 into a 1000ml volumetric flask and make up to the mark with distilled waterand store in a refrigerator.
2. Make a series of working standards as follows: 0, 2,4,6,8 and 10ppm by diluting 100 ppm stock solution prepared in step 2 above.
3. Reagent N1: Dissolve 34 g sodium salicylate, 25 g sodium citrate and 25g sodium tart rate together in about 750-ml water. Add 0.12 g sodium nitroprusside and make up to 1 litre of distilled water. The sample digest solution above is strongly acidic.
4. Reagent N2: Dissolve 30- g sodium hydroxide in about 750 ml distilled water. Allow to cool. Add 10 ml sodium hypochlorite mix well and make up to 1-litre.

**A.2.3 Method**

1. Dilute the entire digest, the blanks to 1+9 (v/v) with distilled water. Add 5.0 ml of the reagent N1 and vortex for one minute.
2. Add 5.0 ml reagent N2 and vortex for one minute. Allow to stand for 2 hours. The blue colour developed is stable for at least 10 hours.
3. Using the UV-VIS spectrophotomer set at 660nm measure the absorbance of the standards and samples.
4. Plot a calibration curve using standards and read off the concentration of Nitrogen in the sample solution

## Calculation

## The nitrogen concentration in the sample material expressed in %N is calculated as follows:

**N % = (a-b) × v × 100**

**1000 × w × al ×1000**

Where a = concentration of Nitrogen in the solution,

b = concentration of Nitrogen in the blank,

v = total volume at the end of analysis procedure,

w = weight of the dried sample and

al = aliquot of the solution taken.

B. Determination of Phosphorus in plant samples digest using ascorbic acid

**B.1 Apparatus/ Material**

1. Beakers
2. Asbestos mat
3. Volumetric flasks
4. Reagent bottle
5. Oven
6. Analytical balance
7. Pipette

**B.2 Reagents**

1. Sulphuric acid, H2SO4,
2. Ammonium molybdate (NH4)6Mo7O24.4H2O)
3. Antimony potassium tart rate (KSb.C4H4O6)
4. Ascorbic acid (C6H8O6)
5. Potassium hydrogen phosphate KH2PO4
6. Distilled water

**B.2.1Preparation of reagents**

1. Sulphuric acid, H2SO4, 5N:

Place one litre clean beaker on asbestos mat (or in cold water in sink). To about 500 ml distilled water, add slowly with stirring, 148 ml conc. H2SO4. When cool dilute to 1 litre with distilled water.

1. Ammonium molybdate/antimony potassium tart rate solution –

Dissolve 12 g of ammonium molybdate (NH4)6Mo7O24.4H2O) in 250 ml of warm (50°C) distilled water. Separately dissolve 0.291 g antimony potassium tart rate (KSb.C4H4O6) in 100 ml distilled water. Add both solutions to 1000 ml of 5 N H2SO4 (above). Mix thoroughly and dilute with distilled water to 2 litres. Transfer to a reagent bottle. Store in a dark and cool place. The mixture keeps for 2 months.

1. Ascorbic acid reducing agent –

Dissolve 2.108 g of ascorbic acid (C6H8O6) in 400 ml of ammonium molybdate/antimony potassium tart rate solution prepared above and mix well. This must be prepared as required on the day of analysis (This is adequate for about 30 samples plus the P standards). The solution keeps for about 24 hours. Larger quantities of this reducing agent may be prepared depending on the output of a specific laboratory.

1. Standard phosphorus stock solution, 100 ppm P: Weigh 0.10967g of oven-dry KH2PO4; dissolve and make to 250 ml mark with distilled water (1 ml = 1 mg P).
2. Make a series of working standards as follows: 0, 2, 4, 6, 8 and 10 ppm P working solution by diluting100ppm P standard stock.

**B.3 Procedure for measuring phosphorus concentration**

1. Pipette 5 ml of the clear digest solution (from the extraction procedure from section 7) into a 50 ml volumetric flask.
2. Add about 20-ml of distilled water to each volumetric flask.
3. Add 10 ml of the ascorbic acid reducing agent to each flask, beginning with the standards prepared.
4. Make to the mark with 50 ml with distilled water; stopper and shake well. Let stand for 1 hour to permit full colour development.
5. Measure the standards and sample absorbance’s (blue colour) using UV-VIS spectrophotometer set at 880nm by following the steps below.
6. Plot a graph of absorbance against standard concentration.
7. Determine concentrations of Phosphorus in sample solution and blanks.

**Calculations**

Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= c in subsequent calculations).

**P in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of P in the sample;

ppm solution = graph reading;

df = dilution factor;

w = weight of dry the sample.

**C. Determination of Potassium**

##### C.1 Apparatus/ Materials

1. Beakers
2. Volumetric flasks
3. Reagent bottle
4. Oven
5. Analytical balance
6. Pipette

##### C.2 Reagents

1. Potassium chloride.
2. Working Potassium standard
3. Distilled water

**C.2.1 Preparation of reagents**

1. Stock Potassium solution, 100 ppm K. Weigh 0.1907 g dry (100°C, 2 hr) potassium chloride. Dissolve and make to 1 litre with distilled water. Store in a reagent bottle.
2. Prepare working Potassium standard series as follows: 0, 2, 4, 6, 8 and 10ppm solution by diluting stock solution of 100 ppm K.

**C.3 Procedure for measurement of potassium concentration**

1. Pipette 2 ml of the digested sample solution (above from the extraction procedure from section 7) into a 50 ml volumetric flask. Make to mark with distilled water and mix well.
2. Aspirate the sample solutions starting with standards, the sample and blank solutions directly into the flame of the flame photometer or atomic absorption spectrophotometer (wavelength at 766.5 nm).
3. Plot a calibration graph of the standard solutions.
4. Read off the amount of potassium present in the solution from the calibration curve prepared by plotting absorbance (or transmission) readings against potassium concentrations. Follow the operation instructions given for flame photometer or atomic absorption spectrophotometer.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**K in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of K in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**D. Determination of Calcium**

##### D.1 Apparatus/ Materials

1. Beakers
2. Volumetric flasks
3. Reagent bottle
4. Oven
5. Analytical balance
6. Pipette

##### D.2 Reagents

1. Calcium carbonate (CaCO3)
2. Hydrochloric acid (HCl)
3. Distilled water
4. working Calcium standard
5. Lanthanum chloride, LaCl3⋅7H2O

##### D.2.1 Preparation of Reagents

1. Stock calcium solution, 100 ppm Ca. Dissolve 0.2497 g of dry (105°C, 2 hr) calcium carbonate (CaCO3) in the minimum quantity of dilute (1N) HCl and make to 1 litre with distilled water.
2. Prepare working Calcium standard series as follows: 0, 5,10,15,20 and 30 ppm solution by diluting stock solution of 100 ppm Ca. Add 10mls of Lanthanum chloride, LaCl3⋅7H2O, 0.15% and make to the mark in a 50ml volumetric flask with distilled water.

**D.3Procedure for measurement of calcium concentration**

1. Pipette 10 ml of the digested sample solution (from section 7 extraction procedure) into a 50 ml volumetric flask.
2. Add 10 ml of 0.15% lanthanum chloride into the sample extract and make to the mark with distilled water. Shake contents well.
3. Aspirate the standard, blank and sample solutions into the flame of the flame photometer or atomic absorption spectrophotometer at wavelength 422.7 nm.
4. Plot a calibration curve of the standard series readings and read off the concentration of calcium in the sample and blank solutions

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**Ca in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Ca in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**E. Determination of Magnesium**

**E.1 Principle**

Unlike the procedures for potassium and calcium analysis where either flame photometry or atomic absorption spectrophotometer can be used, magnesium must only be analysed by atomic absorption spectrophotometer.

##### E.2 Apparatus/ Materials

1. Beakers
2. Volumetric flasks
3. Reagent bottle
4. Oven
5. Analytical balance
6. Pipette

**E.3 Reagents**

##### Spec-pure magnesium rod

##### Nitric acid (HNO3)

1. Distilled water

##### E.3.1 Preparation of Reagents

1. Stock Magnesium solution, 100 ppm Mg. Weigh accurately 0.1 g of Spec-pure magnesium rod and dissolve in about 30 ml of 1:1 nitric acid HNO3. Make to one litre with distilled water.
2. Prepare working magnesium standard series as follows: 0, 0.25, 0.5, 1, 2, 4 and 5 ppm solution by diluting stock solution of 100 ppm Mg.

**E.4 Procedure for measurement of magnesium concentration**

1. Pipette 5 ml of the digested sample (from the extraction procedure from section 7) solution into a 50 ml volumetric flask. Fill to the 50 ml mark with distilled water and mix contents well.
2. Aspirate the magnesium standard series, the blank and sample solutions into the flame of atomic absorption spectrophotometer. Measure the concentration of the magnesium in the in the standard series and sample and the blank solutions.
3. Plot a calibration curve of the standard series readings and read off the concentration of the sample and blank solution.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= in subsequent calculations).

**Mg in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Mg in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**F. Determination of Manganese**

**F.1 Principle**

Manganese is measured by atomic absorption as it absorbs radiation from an element-specific hollow cathode lamp at a wavelength of 248.3 nm

**F.2 Reagents**

##### Manganese standard

1. Distilled water

**F.2.1 Preparation of working standards**

Prepare working manganese standard series as follows: 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 ppm solution by diluting stock solution of 100 ppm Mn.

**F.3 Procedureformeasurement of manganese concentration**

Pipette 5 ml of the digested sample solution (from the extraction procedure from section 7) into a 50 ml volumetric flask. Fill to the 50 ml mark with distilled water and mix contents well.

Switch on the instrument and let it warm for at least 30 minutes.

Check to see that acetylene gas tank is not below 75 psi.

Adjust the regulators for proper readings according to the equipment specifications AIR and ACETYLENE and proceed.

Aspirate the Manganese standard series, the blank and sample solutions into the atomic absorption spectrophotometer at 248.3 nm and measure the absorbencies.

1. Plot a calibration graph of the standard solutions and read off the concentration of the sample and blank solution.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**Mn in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Mn in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**G. Determination of Copper**

**G.1 Principle**

Copper is measured by atomic absorption using an element-specific cathode lamp of wavelength of 324.7 nm.

**G.2 Reagents**

##### Copper standard

1. Distilled water

**G.2.1 Preparation of working standards**

Make standard stock 100 ppm from factory manufactured Copper 1000 ppm stock. Prepare working Copper standard series as follows: 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 ppm solution by diluting stock solution of 100 ppm Copper.

**G.3 Procedure formeasurement of copper concentration**

Aspirate 10 times diluted sample (from section 7 extraction procedure); blank digests and the standard series into the atomic absorption spectrophotometer calibrated for copper measurement at wavelength 324.7 nm and measure the absorbencies.

Plot a calibration curve from the readings of the standard series and determine the concentration of the unknown.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**Cu in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Cu in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**H. Determination of Iron**

**H.1Principle**

Iron is measured by atomic absorption as it absorbs radiation from an element-specific hollow cathode lamp at a wavelength of 248.3 nm

**H.2 Reagents**

##### Iron standard

1. Distilled water

**H.2.1 Preparation of working standards**

Make standard stock solution 100 ppm from factory manufactured Fe 1000 ppm stock. Prepare working Iron standard series as follows: 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 ppm solution by diluting stock solution of 100 ppm Fe.

**H.3 Procedure for measurement of iron concentration**

Aspirate 10 times diluted sample (from section 7 extraction procedure), blank digests and the standard series in to the atomic absorption spectrophotometer calibrated for iron measurement at wavelength 248.3 nm and measure the absorbencies.

Plot a calibration curve from the absorbencies of the standard series and determine the concentration of the unknown.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**Fe in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Fe in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**I. Determination of Zinc**

**I.1 Principle**

Zinc is measured by atomic absorption as it absorbs radiation from an element-specific hollow cathode lamp at a wavelength of 213.9 nm

**I.2 Reagents**

##### Zinc standard

1. Distilled water

**I.2.1 Preparation of working standards**

Make standard stock solution 100 ppm from factory manufactured Zn 1000 ppm stock. Prepare working Zinc standard series as follows: 0, 0.5, 1, 2, 4, and 5 ppm solution by diluting stock solution of 100 ppm Zn.

**I.3 Procedure for measurement of zinc concentration**

Aspirate 10 times diluted sample (from section 7 extraction procedure), blanks digests and the standard series in to the atomic absorption spectrophotometer calibrated for Zinc measurement at wavelength 213.9nm and measure the absorbencies.

Plot a calibration curve from the readings of the standard series and determine the concentration of the unknown.

**Calculations**

Determine solution concentrations for each unknown and the 2 blanks. Subtract the mean blank value from the unknowns; this gives a value for corrected concentration (= C in subsequent calculations).

**Zn in sample (ppm) = C\*ppm solution\*df/w**

Where C = the corrected concentration of Zn in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

**8. References**

1. Tea Research Foundation of Kenya (1st Edition 2012). Soil, PlantTissue and Fertilizer Sampling and Analytical Methods Manual.
2. Methods of Analysis. Adapted from ICRAF 2001
3. Manjula V. Nathan and Yichang Sun(2006). Methods of Analysis. A Guide for Conducting Plant Analysis in Missouri. University of Missouri-Columbia.
4. Nitrogen (Total Kjeldahl) 2004.
5. Glaston M.Kenji and Paul N. Karanja (2009). Analysis of foods. A Practical Manual. First Draft Edition. Jomo Kenyatta University of Agriculture and technology.
6. Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (2002). Laboratory methods of soil and plant analysis: working manual. Second Edition. TSBF. CIAT and SACRED Africa, Nairobi, Kenya.

**Standard operating procedure for field soil sampling and preparation in the laboratory**

**1. Title:** Standard operating procedure for field soil sampling and preparation in the laboratory

**2. Scope:** The procedure covers forest, agriculture and wetland soil samples for physical, chemical and biological analyses in the laboratory.

**3. Purpose:** The purpose of this procedure is to collect soil samples for determination of nutrient levels for advisory services for improvement of soil fertility

**4. Terms, Acronyms and Definitions:**

1. Auger: a tool with a helical bit for boring holes in the ground.
2. Munsell color chart- it is used to describe soil colour for maximum accuracy and communication. Three descriptive elements are used and are always written in a specific order: Hue Value/ Chroma e.g. 10R 5/8.

Hue describes the basis spectral color wavelength red (R), yellow (Y), green (G), blue (B), and purple (P) or intermediates yellow-red (YR), green-yellow (GY), blue-green (BG), purple-blue (PB) and red-purple (RP). The letter abbreviations are preceded by numbers 0 to 10. The intensity of the color increases as the number increases.

Value indicates the degree of lightness or darkness of the color. The scale of value ranges from 0 for pure black to 10 for pure white. Black, white and the grays between them are called neutral colors (have no hues).

Chroma is the degree of departure of a color from the neutral color of the same value. The scale is form 0-8 on the Munsell color chart. 0 indicates no strength (no color; gray) and 8 greatest strength (most color).

**5. Apparatus/ Equipment**

1. Polythene soil sampling bags
2. Labels
3. Marker pens
4. String
5. Buckets
6. Hand shovel
7. Soil auger
8. Soil pH tester
9. Munsell colour chart
10. Sub–sampler (core sampler)
11. Hoe
12. Panga
13. Spade
14. Mattock
15. Tape measure
16. Field note book
17. Core sampler
18. Cooler box
19. Mallet
20. Sieve (2 mm, 0.5mm)
21. Pestle and Mortar

**6. Soil sampling techniques**

**6.1 Soil sampling techniques using an auger**

1. Remove surface plant and debris material from a minimum of three randomly selected sites within the plot/ area.
2. Auger to the required depths depending on which type of plant found in the field i.e. crops are shallow rooted compared to trees which are deep rooted.
3. Put the samples into the bucket which has been labeled with the required depths e.g. 0-25cm, 25-50cm for the three auguring and thoroughly mix well prior to sub sampling.
4. Take a sub sample of about 1kg and discard the remaining soil within the plot.

**6.2 Soil sampling techniques using cores for undisturbed soils**

1. Clean a vertical face in a profile pit at any required depth.
2. Firmly drive a soil coring cylinder of known volume and mass fully into the face causing no disturbance and compaction to the core.
3. Cut the surrounding soil neatly across the inserted end of the cylinder.
4. Carefully lift and remove the core away, trim the ends and cap tightly.

**7. Soil reception and preparation in the laboratory**

**7.1 Soil reception in the laboratory**

1. Soil samples are received by the technician in charge.
2. The samples are inspected in order confirm the physical integrity of the packages and samples, their identity and evidence of sample leakages.
3. Samples are arranged according to plot numbers and soil depth in ascending order.
4. The samples are registered in the inward register, given a laboratory number according to the date of sampling, the date samples were received, the name of the client, plot numbers, and soil depths recorded.

**7.2 Sample preparation**

1. Soil samples are air dried at room temperature or a under a shade.
2. Soil samples are ground and mixed thoroughly using a porcelain mortar and pestle.
3. Samples are sieved using a 2 mm brass sieve and stored for chemical and physical analysis.

**8. References**

1. Okalebo J.R., Gathua, K.W. and Woomer P.L. (2002). Laboratory methods of soil and plant analysis: a working manual. Second Edition. TSBF. CIAT and SACRED Africa, Nairobi, Kenya.
2. Gachene, C.K.K. and Kimaru, G. (2003). Soil Fertility and Land Productivity - A guide for extension workers in the eastern Africa region. Technical Handbook No.30. Regional Land Management Unit (RELMA)/ Swedish International Development Cooperation Agency (Sida).
3. Tea Research Foundation of Kenya (TRFK), 2012, Soil Manual and Fertilizer Sampling and Analytical Methods Manual.

**KENYA FORESTRY RESEARCH INSTITUTE**

|  |  |  |
| --- | --- | --- |
| Tel: +254 20 2010651/2  +254 722 157 414  +254 724 259 781/2  +254 734 251 888  Email:director@kefri.org  Website:www.kefri.org |  | P. O. Box 20412  00200 Nairobi  KENYA |

Ref: …………………………..….. Date…..………………………

**ANALYSIS REPORT**

**Client:**

**Location**:

**Contacts**:

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Results** | **Remarks** | **Adequate Levels** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Recommendations**

Name ………………………………………………………… Date………………………